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All authors made substantial contributions to idea and design of the study; AMJ and DWP analyzed the data and prepared a draft of the paper; IMR collected plant material and analyzed the data; JB collaborated with statistics and results interpretation

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ORIGINAL RESEARCH PAPER

Taxonomic significance of achene morphology of selected *Rosa* taxa (Rosaceae) occurring in Poland

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Abstract
Achenes of roses were rarely studied and the studies were focused on anatomical research, mainly on pericarp structure and its development. We investigated the achene morphology by light- and scanning electron microscopy (LM and SEM) of 17 *Rosa* taxa from three sections (*R. gallica* from *Gallicanae* section, *R. pendulina* and *R. rugosa* from *Rosa* section, and *R. agrestis*, *R. canina*, *R. canina* var. *corymbifera*, *R. dumalis*, *R. dumalis* var. *caesia*, *R. inodora*, *R. jundzillii*, *R. micrantha*, *R. rubiginosa*, *R. sherardii*, *R. ×subcanina*, *R. tomentosa*, *R. villosa*, *R. zalana* from *Caninae* section). Eight quantitative and eight qualitative features were examined based on 9181 achenes, in total. Average achene size ranged from 4.37 to 5.39 mm in length and from 2.57 to 3.32 mm in width. The lowest morphological variability among the examined taxa was found in *R. canina* var. *corymbifera*, and the highest in *R. gallica*, *R. inodora*, and *R. sherardii*. The most diagnostic features of the achenes studied were suture (visible or invisible), presence or absence of hairs, hairs distribution and density, the exocarp sculpture and cuticle pattern type (we have distinguished four exocarp sculpture and three cuticle pattern types), and length. Qualitative achene features have significantly higher diagnostic value than quantitative ones. Taxonomical value of these features is quite high on the species and section level. Our study has shown that the previously mentioned morphological features of achenes can be used as valuable, additional diagnostic features in delimitation of *Rosa* taxa at the species and section level. Based on the morphological features of achenes, a determination key for all *Rosa* taxa studied was created.

Keywords
achenes; exocarp sculpture; variability; suture; cuticle pattern; SEM; LM

Introduction

Species of the *Rosa* L. genus are distributed throughout temperate and subtropical regions of the Northern Hemisphere, in Europe, Asia, Ethiopia, the Middle East, and North America [1]. The genus comprises from 100 (120) to 200 (250) species and the taxonomic treatment of this highly diverse group is complicated due to biological phenomena in reproductive biology, insufficient number of morphological and anatomical features to adequately discriminate between species and the human impact of rose breeding [2–8].

In Europe, depending on taxonomic treatment, 47 [9] or 30–60 [5] naturally growing rose species are recognized, with a majority from the *Caninae* section [5,9–11].

The structure of fruits has been the basis for traditional divisions of the family Rosaceae into subfamilies [2,12,13], however, these do not always correspond to the latest taxonomic descriptions of this family [14–16]. Potter et al. [16] recognized three subfamilies in Rosaceae: Rosoideae, including *Filipendula*, *Rubus*, and *Rosa*, Dryadoideae, and Spiraeoideae. Division of the genus *Rosa* into subgenera has also been based mainly on fruit features [3,5,9]. The fruit of *Rosa* (the "rose hip") develops from an apocarpous gynoecium enveloped in a hypanthium. According to Spjut [17] this represents a multiple fruit called a "pometum". The individual carpels represent the fruitlets and they can be addressed as achenes [7,9,12,18]. We use, according to Zieliński et al. [19], the more practical term "achenes", instead of "nutlets" as proposed by some authors (e.g., [5,20–22]).

There are only a few papers in the carpological literature on the morphology of rose achenes. Researchers tend to focus more on anatomical studies of achenes, mainly on pericarp structure or its development (e.g., [19–21,23–26]). Morphological descriptions of achenes of *Rosa* taxa are usually brief and superficial [27–29]. Extensive morphological studies on seeds and achenes of 47 taxa of the Rosaceae sensu lato were conducted by Dowidar et al. [28], but they took into account only *R. canina* and *R. gallica*. Tantawy and Naseri [29] studied taxonomic relations in the Rosoideae subfamily, based on achene structure of 29 taxa belonging to four tribes and 10 genera (including: *R. glauca*, *R. hugonis*, *R. pendulina*, *R. sempervirens*, and *R. spinosissima*). Starikova [20,21,26] published a series of papers in which she described achenes of 17 *Rosa* species. The cited author focused on the anatomy of fruits, thus the description of their basic morphological characteristics (length, width, shape, pericarp surface, color) was more or less laconic and incomplete. Also, Khrzhanovskii et al. [23] focused on the anatomical structure of pericarp of 24 *Rosa* species. The above-cited studies were carried out only on the basis of light microscopy. He et al. [27] studied the germination of *R. multiflora* var. *adenochaeta*, *R. persica*, and *R. platyacantha*, referring only to a few achene features. Bojňanský and Fargašová [30] put together the basic morphological features of achenes of 44 *Rosa* species originating from Central and Eastern Europe.

Despite numerous taxonomic studies published recently of this relatively well know genus, species relationships within *Rosa* still remain problematic mainly as a result of high intraspecific variability, polyploidy, introgression, and interspecific hybridization. Thus, the newest research trend on *Rosa* genus is focused on phylogenetic relationships among taxa based on chloroplast DNA sequences, nuclear DNA, or microsatellite analysis (e.g., [11,31–33]). However, there is still a lack in basic descriptions of morphological similarities and differences among *Rosa* species which might be helpful in classical taxonomic approach. In general, there are no studies describing morphological variation of taxa belonging to the genus *Rosa*, based on statistical analyses of achene biometric features.

In this study, achene morphology of 17 *Rosa* taxa, belonging to the three sections (*Caninae*, *Gallicanae*, *Rosa*) was analyzed (Tab. 1). Achenes of the taxa described in our study were previously analyzed by Bojňanský and Fargašová [30], however, their descriptions included in the atlas of *Rosa* achenes are very general and contain only basic characteristics (size, shape, outline, color, and surface sculpture), without any comparisons and statistical analyses.

The most common European *Rosa* species were chosen for this study, e.g., *R. canina*, *R. dumalis*, *R. agrestis*, *R. rubiginosa*, *R. sherardii*, *R. tomentosa*, or *R. villosa* [5,9,10], as well as less frequent species for which achene morphology was not previously described in detail (e.g., *R. jundzillii*, *R. gallica*, *R. micrantha*). The study also included *R. rugosa* – a species from eastern Asia – because this species is one of the most common rose species cultivated in many European countries [31]. *Rosa rugosa* is recorded in 16 European countries [34–37]. In Poland this species is considered as an invasive, naturalized species [38].

The aim of the study is a morphological analysis of achenes of 17 *Rosa* taxa to estimate the usefulness of investigated features in the taxonomy of the genus. Important, new aspects raised in this study, are the measurement of four quantitative achene features not previously described (e.g., surface area, projected area, volume, and mass) and to determine the morphological variability of achenes of the species studied.

Material and methods

The study was conducted on 17 rose taxa (14 species, two varieties, and one hybrid) which represented three sections (*Caninae*, *Gallicanae*, *Rosa*) of the genus *Rosa* (Tab. 1).

Fruit samples of 17 wild *Rosa* taxa were both collected in the field and gathered from specimens in the Herbarium of the Institute of Dendrology PAS in Kórnik (Poland) – KOR. All material originated from natural sites in Poland. The list of localities of the *Rosa* taxa studied is given in the Appendix S1.

The observations were carried out on ripe and fully developed achenes; some of achenes collected were not fully developed thus we reduced the number of plant material used in the study. Depending on the taxa, 32 to 1178 randomly selected achenes were measured (Tab. 1). In total 9181 achenes were examined in this study. The size of particular samples for a given individual ranged from 32 to 108 achenes. The number of samples from a given species depended on their availability in natural sites and herbarium sheets.

The achenes were cleaned before observations were made. The biometrical traits of *Rosa* fruits were analyzed using the WinSeedle™ 2003a software (Régent Instruments Inc., Quebec, Canada; http://www.regentinstruments.com). The following achene traits were measured: length (mm) and width (mm), surface area (mm^2), projected

Tab. 1 Number of individuals (rose shrubs) and number of achenes examined for each taxon.

Section	Taxon	No. of individulas	Total No. of achenes
Caninae	*Rosa agrestis*	2	89
Caninae	*Rosa canina*	15	970
Caninae	*Rosa canina* var. *corymbifera*	13	639
Caninae	*Rosa dumalis*	13	702
Caninae	*Rosa dumalis* var. *caesia*	13	810
Gallicanae	*Rosa gallica*	2	73
Caninae	*Rosa inodora*	13	607
Caninae	*Rosa jundzillii*	4	167
Caninae	*Rosa micrantha*	1	32
Rosa	*Rosa pendulina*	4	177
Caninae	*Rosa rubiginosa*	15	1117
Rosa	*Rosa rugosa*	2	215
Caninae	*Rosa sherardii*	15	948
Caninae	*Rosa* ×*subcanina*	11	598
Caninae	*Rosa tomentosa*	11	726
Caninae	*Rosa villosa*	14	1178
Caninae	*Rosa zalana*	2	133
Total		150	9181

area (mm^2), volume (mm^3), projected perimeter (mm), and dry mass (g). Surface area means total area of the surface of a three-dimensional object, projected area is two-dimensional area measurement of a three-dimensional object by projecting its shape on to an arbitrary plane and projected perimeter means perimeter of achene projected area. Achene length to width ratio (L/W) was also calculated. Furthermore, selected qualitative achene features, as: shape, outline, exocarp surface sculpture, cuticle pattern, suture visible or invisible, and the presence or absence of hairs and, if present, their distribution and density, were determined.

For SEM five achenes of each *Rosa* taxa, originated from different sites, were analyzed. They were mounted on aluminum stubs, sputter-coated with gold and examined with a Hitachi S3000N field emission scanning electron microscope at 5 kV in the Institute of Plant Protection in Poznań (Poland). For LM, achenes were photographed on a black background using a stereomicroscope (Nikon SMZ 800) with attached camera (Canon Power Shot G6).

The terminology for descriptions of morphological characteristics of the achenes followed Bojňanský and Fargašová [30], Stearn [39], and Andenberg [40].

For each achene feature measured, one-factor analysis of variance (ANOVA) was used to examine differences in the mean values among taxa studied. When critical differences were noted ($p < 0.05$), multiple comparisons were carried out using Tukey's test for unequal sample sizes. The same letters on figures indicate a lack of statistically significant differences between the taxa studied according to Tukey's a posteriori test. To show similarities and differences among the taxa studied, Ward's hierarchical clustering method was used to delineate groups based on all achene morphological features. Results were also analyzed using multivariate methods. Analysis of canonical variables was applied in order to present multitrait assessment of similarity of tested taxa in a lower number of dimensions with the least possible loss of information [41]. This makes it possible to illustrate variation in species in terms of all observed traits in the graphic form. Statistical analyses were performed using JMP Pro 12.1.0 (SAS Institute Inc. Cary, NC, USA; http://www.jmp.com) and GenStat 17th edition.

Results

Achenes of the *Rosa* taxa studied were bilateral, rarely three- or extremely rarely five-sided, mostly ovoid or ellipsoid in shape, with acute or ± rounded apices and obtuse or rounded bases (Fig. 1, Fig. 2).

The outline of achenes was mostly ovate and elliptical, hardly ever lanceolate, triangular with obtuse apices, or circular and rarely obovate, oblanceolate or heartlike.

Individual achene sizes ranged from 1.90 mm (*R. villosa*) to 8.43 mm (*R. dumalis*) in length and from 0.70 mm (*R. villosa*) to 5.06 mm (*R. canina*) in width. The highest range of achene length was found in *R. villosa*, and the lowest in *R. micrantha* (Fig. 3). Achene mean length was fairly stable, because the difference in the extreme values of this trait was 1.02 mm (*R. rubiginosa* vs. *R. rugosa*). The mean width was even less diverse (0.75 mm range) with the smallest in *R. zalana* and largest in *R. agrestis* (Fig. 4).

Achenes were elongated. Mean length to width ratio ranged from 1.50 in *R. gallica* to 1.94 in *R. pendulina* (Fig. 5). In our study, the shape of achenes was mostly ovoid (e.g., *R. agrestis*, *R. dumalis*, *R. micrantha*, *R. pendulina*, *R. tomentosa*, *R. zalana*) and ellipsoid (e.g., *R. agrestis*, *R. dumalis*, *R. sherardii*, *R. rubiginosa*, *R. tomentosa*, *R. villosa*), less often three-sided (*R. canina*, *R. gallica*, *R. jundzillii*, *R. micrantha*), three-sided angular (*R. agrestis*), rarely angular (*R. zalana*), obovoid (*R. rugosa*), or heart-shaped (*R. inodora*).

The average value of achene surface area ranged from 20.31 mm^2 (*R. zalana*) to 29.99 mm^2 (*R. agrestis*; Fig. 6). The mean projected area ranged from 8.45 mm^2 in *R. zalana* to 12.51 mm^2 in *R. canina* (Fig. 7). The average projected perimeter ranged from 11.70 and 11.74 mm in *R. rubiginosa* and *R. zalana* to 13.98 and 14.00 mm in *R. agrestis* and *R. rugosa*, respectively (Fig. 8). The lowest mean volume was found in fruits of *R. zalana*, and the biggest ones were observed in *R. agrestis* (Fig. 9). Masses

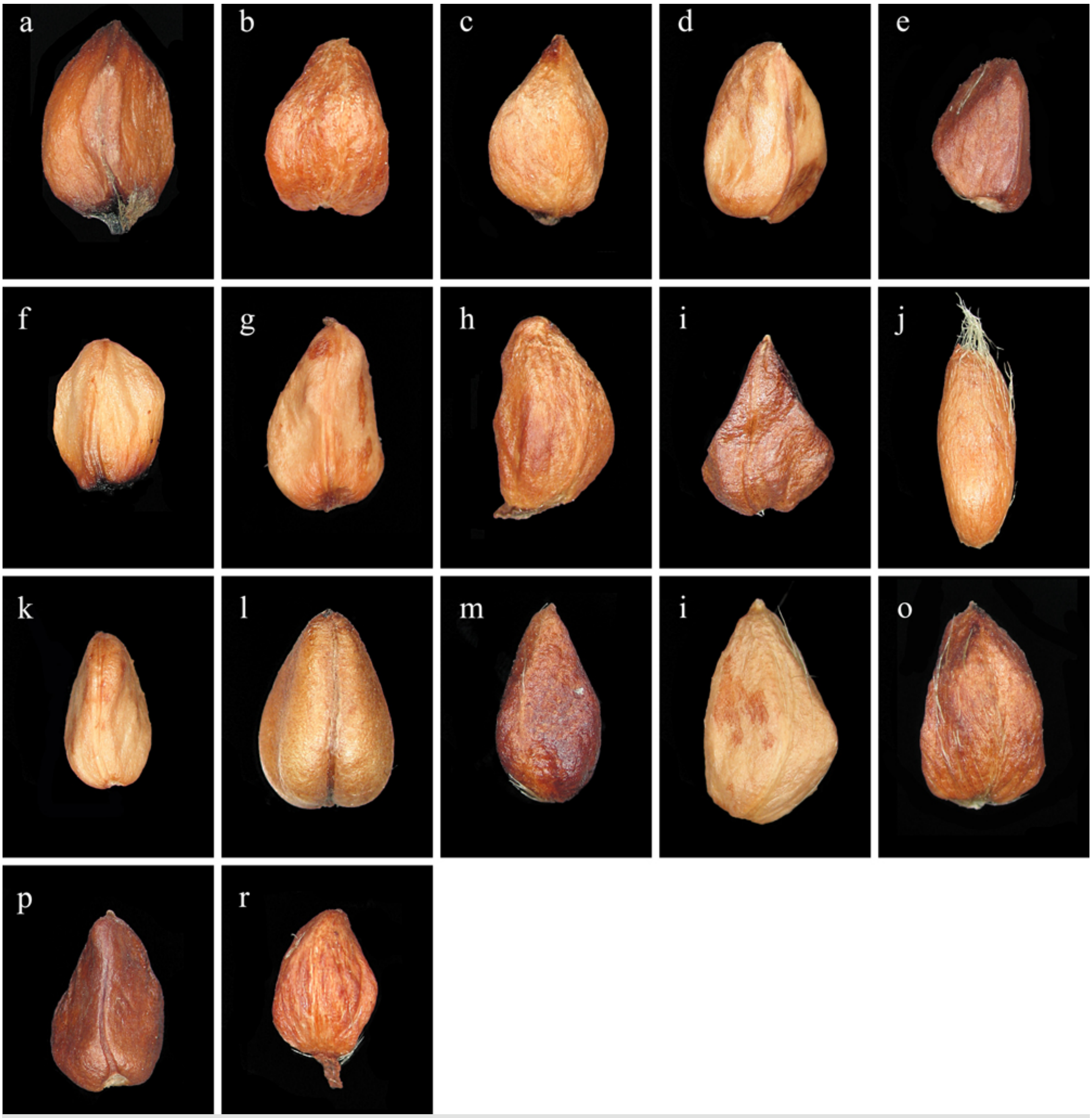

Fig. 1 LM micrographs of achenes of taxa studied. Dorsal side of achenes; achenes outline, shape, suture, and hairs visible. **a** *R. agrestis*; **b** *R. canina*; **c** *R. canina* var. *corymbifera*; **d** *R. dumalis*; **e** *R. dumalis* var. *caesia*; **f** *R. gallica*; **g** *R. inodora*; **h** *R. jundzillii*; **i** *R. micrantha*; **j** *R. pendulina*; **k** *R. rubiginosa*; **l** *R. rugosa*; **m** *R. sherardii*; **n** *R. ×subcanina*; **o** *R. tomentosa*; **p** *R. villosa*; **r** *R. zalana*.

of individual achenes of the taxa studied were also highly variable and significantly different (Fig. 10).

We found statistically significant differences ($p < 0.001$) among the *Rosa* taxa examined with regard to all the achene features measured. Coefficients of variation (CV; calculated taking into account all the *Rosa* taxa examined) for the achene features analyzed were as follows: projected perimeter – 11.5%, length – 12.7%, width – 13.8%, L/W ratio – 16.3%, projected area – 19.7%, surface area – 19.9%, mass – 25.7%, and volume – 32.3%.

The agglomeration grouping using the Ward method yielded a dendrogram (Fig. 11), which was used to divide the *Rosa* taxa examined into two groups and five subgroups. The first group was comprised of *R. agrestis*, *R. jundzillii*, and *R. rugosa* (subgroup 1), *R. canina*, *R. dumalis* var. *caesia*, *R. tomentosa*, *R. ×subcanina*, *R. villosa* (subgroup 2), *R. dumalis*, *R. inodora*, and *R. gallica* (subgroup 3), while in the second group were included six taxa, *R. canina* var. *corymbifera*, *R. micrantha*, *R. rubiginosa*

Fig. 2 LM micrographs of achenes of taxa studied. Ventral side of achenes; achenes outline, shape, suture and hairs visible. **a** *R. agrestis*; **b** *R. canina*; **c** *R. canina* var. *corymbifera*; **d** *R. dumalis*; **e** *R. dumalis* var. *caesia*; **f** *R. gallica*; **g** *R. inodora*; **h** *R. jundzillii*; **i** *R. micrantha*; **j** *R. pendulina*; **k** *R. rubiginosa*; **l** *R. rugosa*; **m** *R. sherardii*; **n** *R. ×subcanina*; **o** *R. tomentosa*; **p** *R. villosa*; **r** *R. zalana*.

(subgroup 1), *R. pendulina*, *R. sherardii*, and *R. zalana* (subgroup 2). *Rosa agrestis* and *R. zalana* showed the most distant positions on the dendrogram.

On the outer surface of the exocarp there is a distinct cuticle layer with patterns that varied among taxa. The cuticle patterns were mostly striate, very rarely striate-smooth or smooth (Fig. 12). In many taxa striae were long and extended along the achene (*R. agrestis*, *R. gallica*, *R. inodora*, *R. micrantha*, *R. rubiginosa*, *R. sherardii*, *R. ×subcanina*, *R. villosa*). *Rosa dumalis*, *R. dumalis* var. *caesia*, and *R. jundzillii* striae had a very distinctive cuticle pattern, because the striae were short, very numerous and run across the achene. Such striae were often concentrated near the stomata, distributed over the surface of the fruit. There were also taxa in which striae ran both along (longer) and across (shorter) the achene (e.g., *R. canina*, *R. canina* var. *corymbifera*, *R. tomentosa*). In two species (*R. micrantha*, *R. zalana*) at least part of the achene surface was striate (long striae), and part was also smooth. In *R. pendulina* and *R. rugosa* the cuticle pattern was smooth.

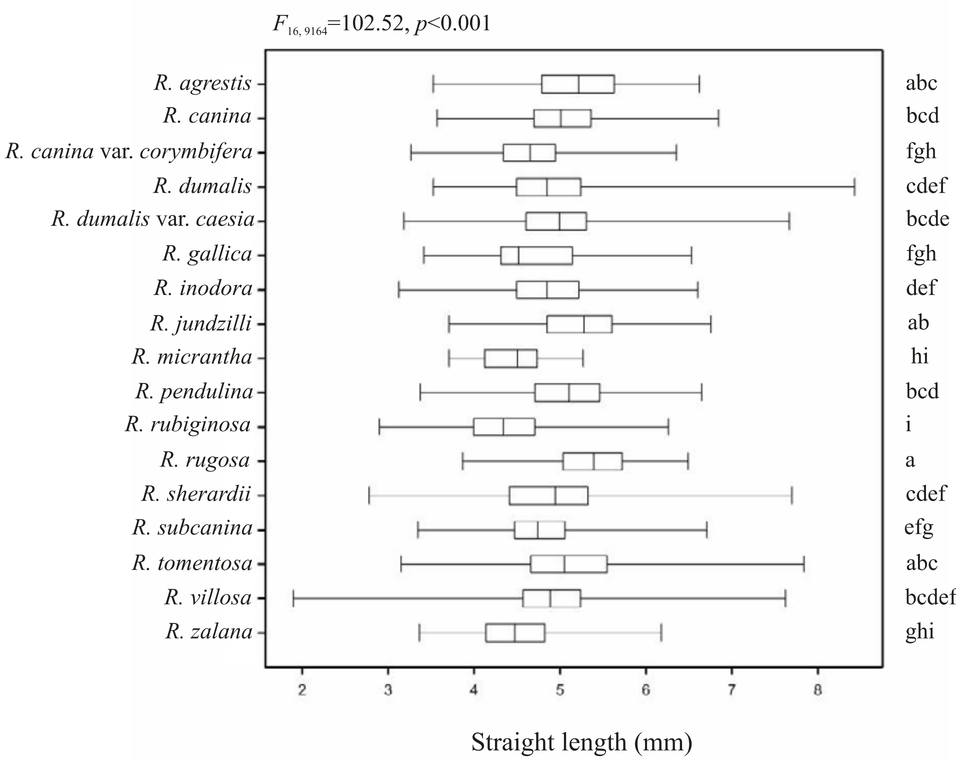

Fig. 3 Box-and-whisker diagram of achene length values for the particular *Rosa* taxa analyzed. Description: the left side of the box is determined by the lower quantile, Q1, and the right side of the by the upper quantile, Q3. The length of the box corresponds to interquartile range. The vertical line inside the box corresponds to median value. The end of the left section determines the minimum value in a set, while the right end – the maximum value of the trait. The same letters on figure indicate a lack of statistically significant differences between the taxa according to Tukey's a posteriori test.

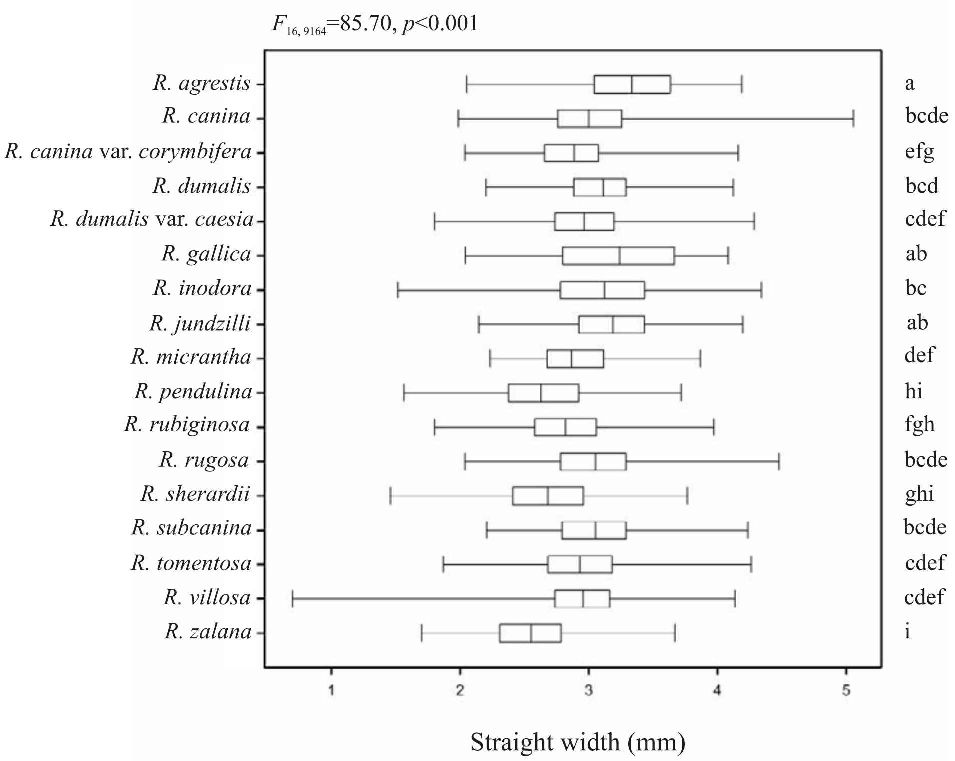

Fig. 4 Box-and-whisker diagram of achene width values for the particular *Rosa* taxa analyzed. Description as in Fig. 3.

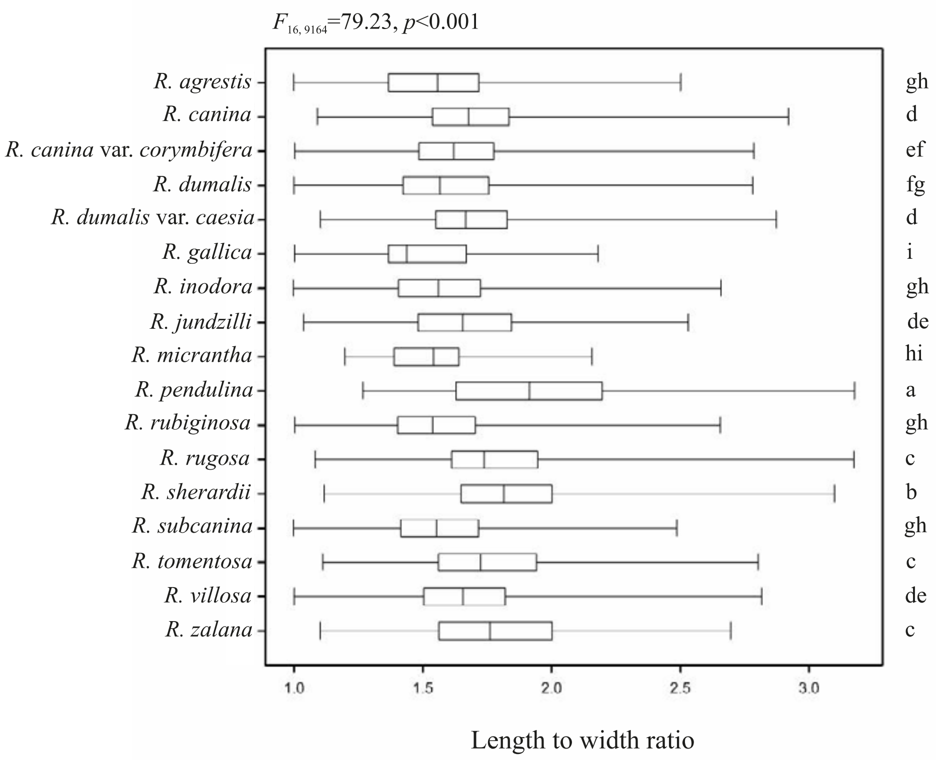

Fig. 5 Box-and-whisker diagram of achene length to width ratio values for the particular *Rosa* taxa analyzed. Description as in Fig. 3.

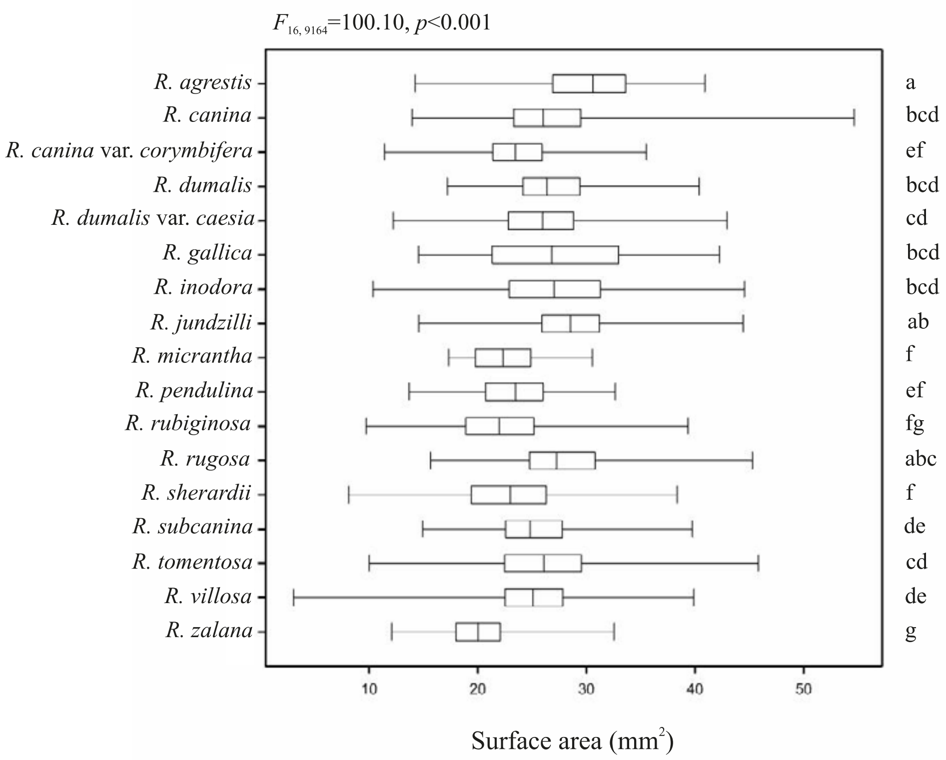

Fig. 6 Box-and-whisker diagram of achene surface area values for the particular *Rosa* taxa analyzed. Description as in Fig. 3.

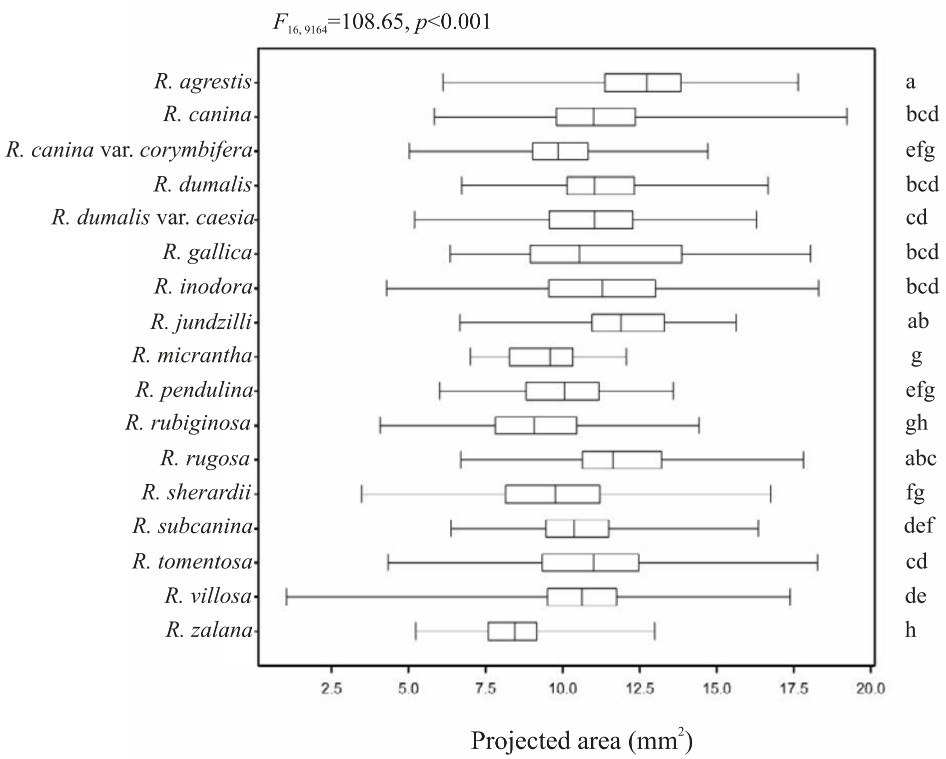

Fig. 7 Box-and-whisker diagram of achene projected area values for the particular *Rosa* taxa analyzed. Description as in Fig. 3.

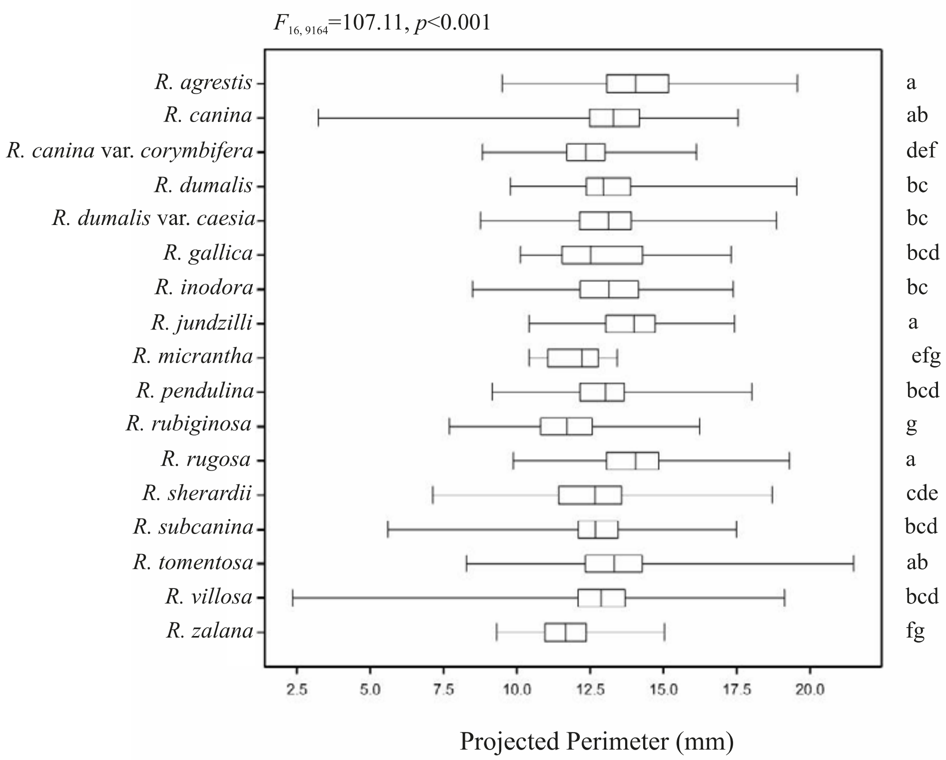

Fig. 8 Box-and-whisker diagram of achene projected perimeter values for the particular *Rosa* taxa analyzed. Description as in Fig. 3.

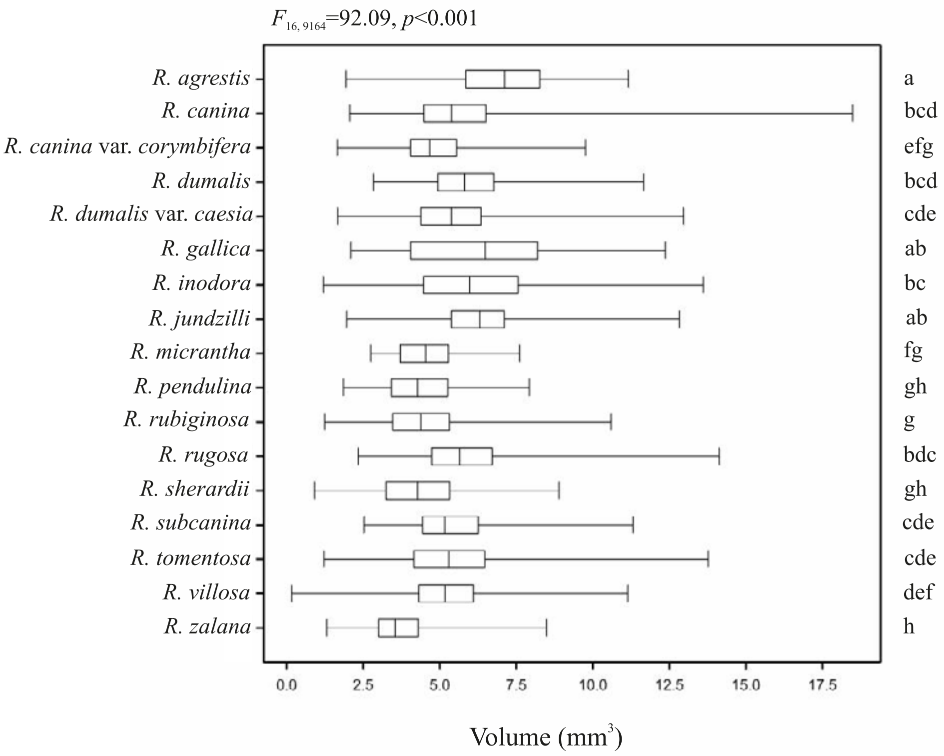

Fig. 9 Box-and-whisker diagram of achene volume values for the particular *Rosa* taxa analyzed. Description as in Fig. 3.

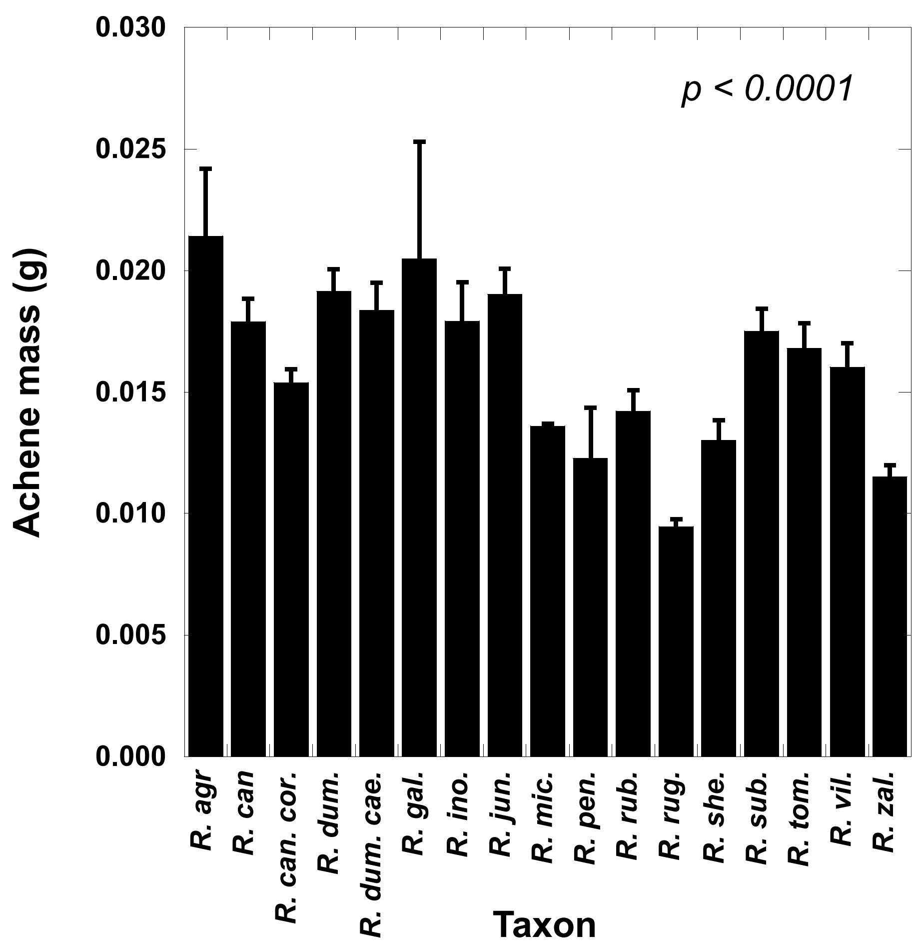

Fig. 10 Mean masses (±*SE*) of achenes for all *Rosa* taxa studied.

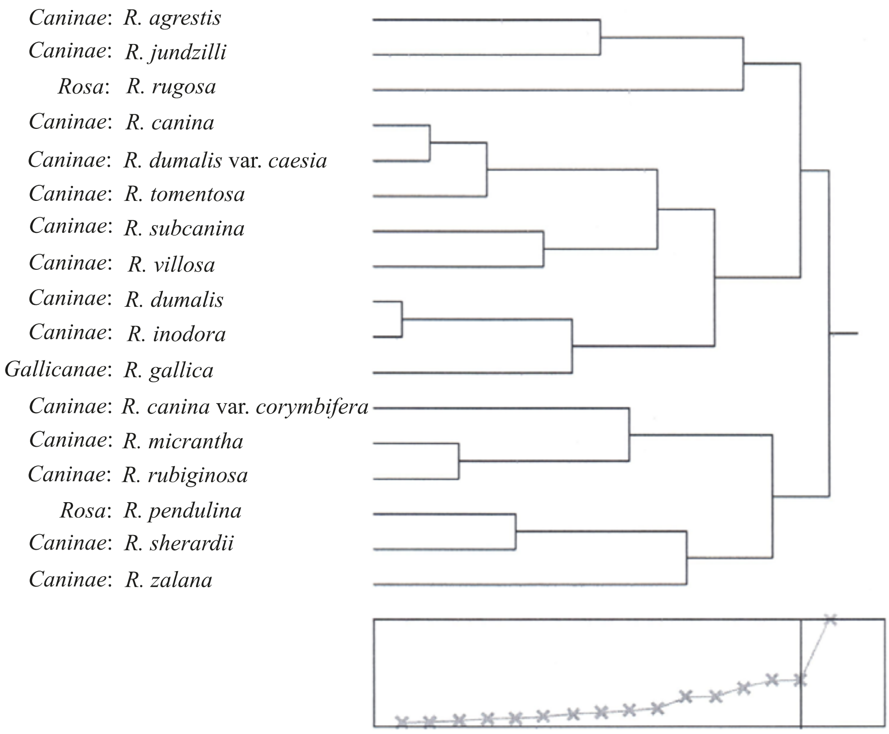

Fig. 11 Dendrogram of cluster groupings of *Rosa* taxa on the basis of achene morphological features.

SEM analyses revealed four types of exocarp surface (Fig. 12). Mostly the exocarp sculpture was scalariform, reticulate, reticulate-scalariform and very rarely smooth. The exocarp cells were elongated or isodiametric and circular, tetra-, penta-, or hexagonal-shaped. The anticlinal walls were mostly raised, straight and irregular, bent and of varying width, periclinal walls were slightly depressed. In the scalariform surface type exocarp cells were elongated (e.g., *R. tomentosa*). The walls of these cells were relatively wide and of average height in some taxa (*R. agrestis*, *R. canina*, *R. canina* var. *corymbifera*, *R. ×subcanina*). In other taxa they were narrow and slightly flat (*R. inodora*) or very flat (*R. sherardii*, *R. tomentosa*). A reticulate surface was present in *R. rugosa*, which exhibited a specific surface type, because its cells were irregularly square or rectangular with very broad, low walls. In other taxa with reticulate surfaces, the walls were wider (*R. villosa*) or narrower (*R. gallica*). Some of the rose taxa studied also had reticulate-scalariform surfaces, in this case one part of achene surface was reticulate, and remaining – scalariform. The cells were elongated and rounded. The cell walls were similar, usually with average width and height. Such reticulate-scalariform surfaces were observed in *R. dumalis*, *R. dumalis* var. *caesia*, *R. jundzillii*, and *R. rubiginosa*. Smooth surface was the rarest – this type was found in three species (*R. micrantha*, *R. pendulina*, *R. zalana*). In this case, most of the achene surface was smooth, and only in some places was a scalariform or reticulate surface observed (Fig. 12).

Rose fruits are closed with a pericarp suture, originating from the fusion of carpel margins. Many fruits of the rose taxa studied had a suture, which was more often present on the ventral side (Fig. 2). A few taxa had indistinct sutures (*R. canina* var. *corymbifera*, *R. canina* var. *corymbifera*, *R. ×subcanina*, *R. dumalis* var. *caesia*, *R. inodora*, *R. jundzillii*), however, in *R. tomentosa* and *R. zalana*, suturae were not visible (Fig. 1, Fig. 2).

Achenes of the taxa studied were slightly to densely hairy or hairless (Fig. 1, Fig. 2). Hairs were usually long and white. Hairs occurred slightly more often on the ventral side, than on the dorsal side. They appeared on the entire surface of the ventral or dorsal side of achenes (e.g., *R. dumalis*, *R. dumalis* var. *caesia*, *R. rubiginosa*, *R. tomentosa*), or concentrated only at the apices or at the bases, around the attachment scar (e.g., *R. agrestis*, *R. canina*, *R. pendulina*, *R. sherardii*). Quite dense hairy achenes were

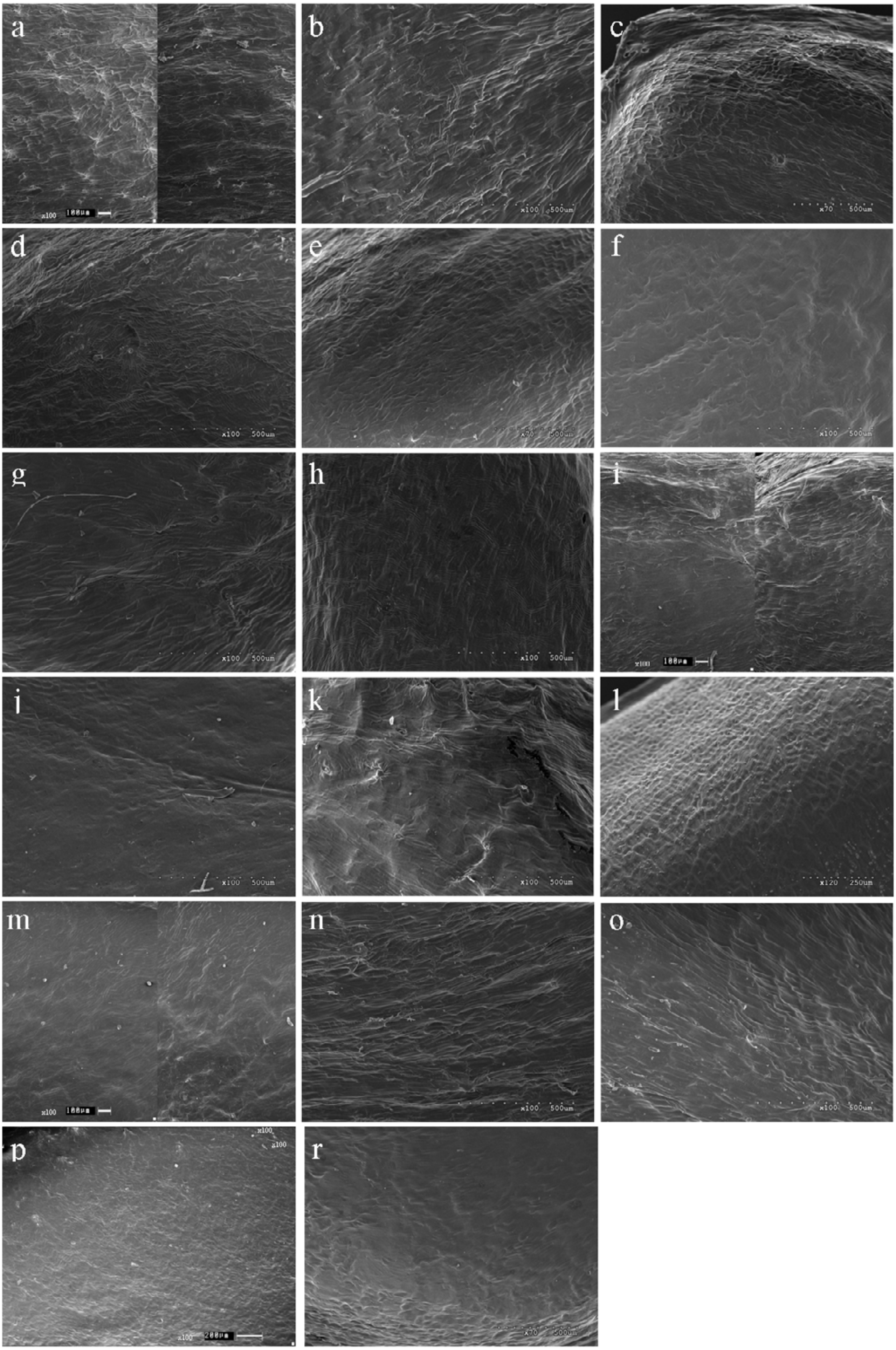
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Fig. 12 SEM micrographs of achenes of taxa studied with cuticle pattern and exocarp surface sculpture. **a** *R. agrestis*; **b** *R. canina*; **c** *R. canina* var. *corymbifera*; **d** *R. dumalis*; **e** *R. dumalis* var. *caesia*; **f** *R. gallica*; **g** *R. inodora*; **h** *R. jundzillii*; **i** *R. micrantha*; **j** *R. pendulina*; **k** *R. rubiginosa*; **l** *R. rugosa*; **m** *R. sherardii*; **n** *R. ×subcanina*; **o** *R. tomentosa*; **p** *R. villosa*; **r** *R. zalana*. Types of cuticle pattern: striate **a–h,k,m,n,p**; striate-smooth **i,r**; smooth **j,l**. Types of exocarp sculpture: scalariform **a–c,h,m–o**; reticulate **f,l,p**; reticulate-scalariform **d,e,g,k**; smooth **i,j,r**.

found in *R. dumalis*, *R. dumalis* var. *caesia*, *R. inodora*, *R. rubiginosa*, *R. tomentosa*, and *R. villosa*. Single hairs were present in *R. agrestis*, *R gallica*, or *R. ×subcanina*. Hairless fruits were found for *R. canina* var. *corymbifera*, *R. jundzillii*, *R. micrantha*, *R. rugosa*, and *R. zalana* (Fig. 1, Fig. 2).

Key to the studied *Rosa* taxa, based on the morphological characters of achene

1 Suture visible 2
1* Suture invisible 14
2 Achenes hairy 3
2* Achenes hairless 11
3 Achenes hairy on the ventral side 4
3* Achenes hairy on the dorsal side 8
4 Achenes hairy ± on the whole surface 5
4* Achenes hairy only near the attachment scar or at the apex 6
5 Exocarp surface reticulate and scalariform. Striae run mainly across achene, very numerous, short .... *R. dumalis*, *R. dumalis* var. *caesia*
5* Exocarp surface reticulate and scalariform. Striae run along the achene .... *R. inodora*, *R. rubiginosa*
6 Achenes hairy around the attachment scar .... *R. canina*
6* Achenes hairy at the apex 7
7 Exocarp surface and cuticle pattern smooth .... *R. pendulina*
7* Exocarp surface scalariform. Cuticle pattern striate, in some places smooth .... *R. sherardii*
8 Achenes densely hairy .... *R. villosa*
8* Achenes slightly hairy 9
9 Exocarp surface reticulate. The cell walls are wide .... *R. gallica*
9* Exocarp surface scalariform. The cell walls are narrow 10
10 Achenes slightly hairy ± on the whole surface .... *R. ×subcanina*
10* Achenes slightly hairy only at the apex .... *R. agrestis*
11 Exocarp surface smooth. Cuticle pattern striate .... *R. micrantha*
11* Exocarp surface reticulate or scalariform. Cuticle pattern smooth or striate .... *R. rugosa*, *R. canina* var. *corymbifera*, *R. jundzillii*
12 Exocarp surface reticulate. Cuticle pattern smooth .... *R. rugosa*
12* Exocarp surface scalariform. Cuticle pattern striate 13
13 Striae long, run along the achene .... *R. canina* var. *corymbifera*
13* Striae both long and short, the latter run across achene .... *R. jundzillii*
14 Achenes hairy .... *R. tomentosa*
14* Achenes hairless .... *R. zalana*

The multidimensional analysis of the studied traits compared *Rosa* taxa in respect of seven morphological achene features (excluding mass). The first and second canonical varieties elucidated 49.28% and 32.67%, respectively, of multivariate variability of the taxa studied (Fig. 13).

Discussion

Many of the achene morphological traits had not been analyzed yet, thus with the exception of Bojňanský and Fargašová [30], it was impossible to compare current results with those of other authors. Comparison of the current study on achene morphology of the selected *Rosa* taxa to literature data [20,21,23,25–30,40,42] revealed that our results were sometimes similar to these findings (this relates to average length

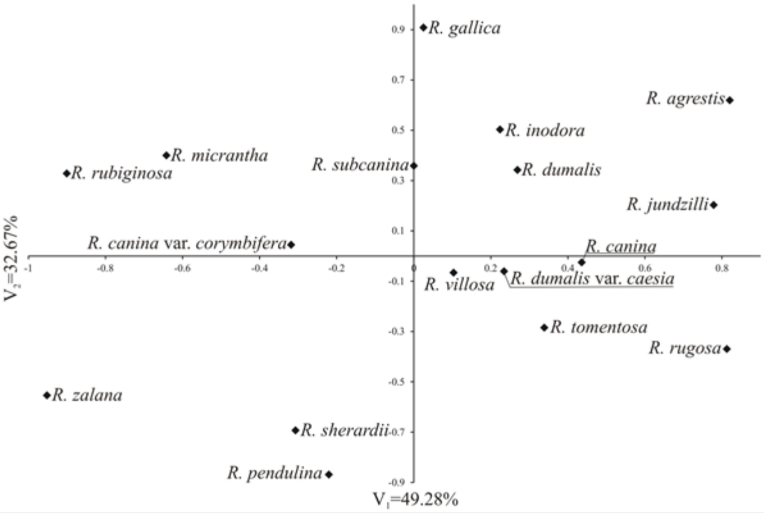

Fig. 13 Configuration of *Rosa* taxa in the space of two canonical varieties calculated for the all observed features.

and width, outline, shape and color), but in the case of several characteristics, especially for suture and hair, but also for exocarp surface types or size range, they varied considerably.

Bojňanský and Fargašová [30] emphasized the high diagnostic value of such qualitative features as the presence or absence of suture and hairs, hair distribution and density. We fully agree with this opinion, although our results sometimes differed from data provided by the authors cited. On the basis of the features mentioned above, we were able to distinguish most of the taxa studied. One controversial proposal by Bojňanský and Fargašová [30] was division of *Rosa* species into types with or without sutures. In our opinion, confirmed by anatomical studies by Zieliński et al. [19], achenes of all roses have a suture, but in some taxa it is not visible. Therefore, instead of determining achenes to be with or without suture, we used the term achenes with visible or invisible suture.

Other very important features were the cuticle pattern and exocarp sculpture. According to Zieliński et al. [19], the cuticle layer of roses is usually thick. Our SEM morphological analyses have shown that despite this, the exocarp surface was visible in all taxa studied. The cuticle pattern of rose achenes was described in any of the available papers. It is mostly striate, very rarely striate-smooth or smooth. In our opinion this is an important feature, helpful in distinguishing particular taxa (e.g., *R. gallica*, *R. pendulina*, or *R. sherardii*) or groups of species (e.g., *R. dumalis*, *R. dumalis* var. *caesia*). Our results show that achene surface sculpture was mostly scalariform, reticulate or scalariform and reticulate and very rarely smooth. These results differ from published data by Starikova [20,21,25,26], where achene surface sculpture was most often ribbed, rarely smooth or slightly angled-convex or smooth. This was due to the use of only the light microscope by the cited author. Tantawy and Naseri [29] give two surface types – glabrous and pubescent – for the five *Rosa* species studied. Our results indicate, however, that the diversity of features described is larger than these authors reported as we have found four achene surface types (scalariform, reticulate, reticulate-scalariform, and smooth). Bojňanský and Fargašová [30] have identified many types of achene surface sculpture – from smooth, through barely humpy or shallow furrowed, to waved, glabrous and finely wrinkled. The one qualitative feature with lower rank was achene shape. The taxonomic value of this feature is low because most species examined in our study had two shape types – ovoid or ellipsoid. All authors cited above have reported the same achene shapes.

Among the quantitative features, the highest taxonomic importance was the length of achenes. The average values of the length and width of achenes shown in our study were similar to values obtained by other authors [20,21,23,25–29], but ranges of these two features were much larger than those given by Bojňanský and Fargašová [30]. For example, in *R. canina* the previously reported range of length and width of achenes was 5.5–6.0 × 2.8–3.5 mm, while in our study it was 3.57–6.84 × 1.99–5.06 mm, respectively (Fig. 4, Fig. 5). This most likely results from a much larger number of achenes for *Rosa* taxa included in our study. Some quantitative features of achenes not previously studied, but analyzed in our study (e.g., surface area, projected area, volume, and mass) have not been very useful for identification of the rose taxa studied.

As can be concluded from this study, achene morphological structure reflects only slightly the consanguinity relationships between the species from the *Caninae* section described by Zieliński [10] and Henker [5]. According to Zieliński [10], *R. canina* was the "initial" species for this section. It is from here that six development lines run formed by *R. judzillii* (1), then by *R. micrantha* and *R. rubiginosa* (2), *R. agrestis* and *R. inodora* (3), *R. tomentosa*, *R. sherardii*, and *R. villosa* (4) and two single species – *R.*

dumalis (5) and *R. glauca* (6). On the dendrogram (Fig. 11), some closely related species (e.g., *R. agrestis* and *R. inodora* or *R. tomentosa*, *R. sherardii* and *R. villosa*) occupied rather distinct positions, but a close relationship was confirmed for *R. micrantha* and *R. rubiginosa*. *Rosa jundzillii* showed a separate position, but forms a subgroup with *R. agrestis* belonging to a different lineage. On the section level, neither *R. gallica* from section *Gallicanae*, nor *R. pendulina* from section *Rosa*, were distinguished (among the taxa studied) from section *Caninae*. Obtained results are not unambiguous, because of the strong polymorphic character of the *Caninae* section, forming hybrid swarm with *R. canina*, that link all taxa in that section [3,10].

Conclusions

- Qualitative achene features have significantly higher diagnostic value than quantitative ones. Taxonomical value of these features is quite high on the species and section level. Based on the above-mentioned list of achene features we were able to distinguish 13 taxa from three sections: *R. gallica* (from *Gallicanae* section), *R. pendulina*, *R. rugosa* (from *Rosa* section), and *R. agrestis*, *R. canina*, *R. canina* var. *corymbifera*, *R. jundzillii*, *R. micrantha*, *R. sherardii*, *R. ×subcanina*, *R. tomentosa*, *R. villosa*, *R. zalana* (from *Caninae* section). Therefore, achene morphology can be used as a valuable, auxiliary feature for diagnosis of these taxa.
- The most valuable diagnostic achene characteristics for separating 17 taxa of the genus *Rosa* studied are: visible or invisible suture, hair presence or absence, their distribution and density, the cuticle pattern, exocarp surface sculpture, and fruit length.
- The most significant quantitative taxonomic feature is length of achenes.
- The achene features examined are moderately variable. The least variable are projected perimeter and length while the most variable – achene mass and volume.
- The lowest variability among the examined taxa was found in *R. canina* var. *corymbifera*, and the highest in *R. gallica*, *R. inodora*, and *R. sherardii*.
- The distribution of the taxa studied on the dendrogram partly confirms the division of the genus *Rosa* into sections currently adopted in taxonomy [5]. In addition, it only slightly reflects consanguinity relationships between species from the section *Caninae* described by Zieliński [10] and Henker [5] (Fig. 11).

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Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3493/0:

Appendix S1 List of localities of the *Rosa* taxa studied.

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