RESEARCH ARTICLE

Pollen morphology and variability of native and alien, including invasive, species of the genus Spiraea L. (Rosaceae) in Poland

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Abstract

The pollen morphology was studied in 25 taxa of the genus Spiraea L. The aims of this study were to describe the pollen morphology and variability and to determine whether the pollen features of alien, expansive or invasive Spiraea species differ from those of other taxa. The species of Spiraea were analysed for nine quantitative pollen traits as well as the following qualitative traits: the outline, shape and exine ornamentation. In total, 750 pollen grains were measured. Based on the pollen key exine ornamentation features, then individual Spiraea species were distinguished, while the other species formed groups of usually 2–3, up to 8 species. The most important pollen features included length, width and course of grooves and striae, presence or absence of perforations, as well as their number and diameter. The most variable taxa for all the nine biometric traits jointly were S. ×billardii, S. veitchii, S. nipponica and S. cana. The pollen of the invasive S. lomentosa differed from the other taxa studied, unlike the other invasive species (S. douglasii and S. japonica).

Introduction

The genus Spiraea L. belongs to the tribe Spiraeae, the family Rosaceae Juss., to the order Rosales and to the rosid clade [1–4]. The taxonomy introduced by Rehder [5] divides the genus Spiraea into three sections: Chamaedryon Ser., Calospira K. Koch and Spiraria Ser. However, this was later developed by Yu and Kuan [6] into four sections and 10 series.

There are 80 to 100 species of this genus throughout the world, although the exact number of Spiraea species has not been specified [3, 7]. Spiraea is by far the largest and most widespread genus in the tribe Spiraeae [3], which are native to the temperate Northern Hemisphere, with the greatest diversity in eastern Asia, mainly China, where approx 70 Spiraea species (47 endemic) are found [1, 7–9]. In Europe there are seven native species of Spiraea—S. cana, S. chamaedryfolia, S. crenata, S. decumbens, S. hypericifolia, S. media and S. salicifolia [10, 11].

Representatives of the genus Spiraea are very popular decorative plants in North America, Asia and Europe, including Poland. In the latter, many species of meadowsweet from Asia and
North America are cultivated, while some of them, e.g. *S. tomentosa, S. douglasii*, and *S. japonica* L., have become naturalized and are invasive in some European countries [12–14], posing a threat to biodiversity in these areas [15].

According to Lambdon et al. [16] and the DAISIE [17], 21 alien *Spiraea* taxa (13 species and 8 hybrids) grow in Europe, of which 18 taxa were ‘aliens in Europe’ naturalized within the continent and 12 species were naturalized neophytes within the continent. A further five species were ‘aliens of European origin’ (five naturalized and three—naturalized neophytes). The third group—‘aliens to Europe’ consisted of 16 species (13 naturalized and nine—naturalized neophytes). Mirek et al. [18] reported the occurrence of two native species (*S. salicifolia* L., and *S. media* Schmidt) in Poland and the following eight alien species and hybrids: *S. albiflora* Zabel, *S. chamaedryfolia* L. em. Jacq., *S. hypericifolia* L., *S. japonica* L. f., *S. ulmifolia* Scop., *S. ×vanhouttei* (Briot) Zabel, *S. ×arguata* Zabel and *S. ×bunalda* Burv. In turn, Tokarska-Guzik et al. [15] listed six domestic, alien meadowsweet species and cultivars in Poland. These were locally established kenophytes—*S. alba* Du Roi (North America), *S. chamaedryfolia* L. em. Jacq., (southeastern Europe and southeastern Asia) and *S. douglasii* Hook. (western North America), a domestic, invasive species—*S. tomentosa* L. (from eastern North America), and two locally established cultivars—*S. ×pseudosalicifolia* Silverside (= *S. salicifolia* L. × *S. douglasii* Hook) and *S. ×vanhouttei* (Briot) Zabel. *S. ×pseudosalicifolia* Silverside and *S. ×billardii* Hércinc, analysed by our team, are often treated synonymously and traded as the same taxon. This is quite unfortunate because *S. ×pseudosalicifolia* Silverside is a hybrid of *S. salicifolia* L. × *S. douglasii* Hook, while *S. ×billardii* Hércinc is a hybrid of *S. alba* Du Roi × *S. douglasii* Hook [19].

Many palynologists believe that palynomorphological characteristics tend to be more useful to distinguish higher ranking taxa of the family Rosaceae (subfamily, tribe or genus) rather than those of lower rank (section, series or species) [20–41]. Especially exine sculpture was found to be an important feature to distinguish species of many genera from the family Rosaceae. The most important traits of exine sculpture were the number and width of striae and grooves, as well as the number, area and size of perforations [20–34, 41]. Many scientists have emphasized that for species delimitation within the family Rosaceae the length of polar axis (P), pollen shape (P/E ratio), operculum structure as well as the presence or lack of costae colpi are very important [20–26, 28, 29, 36, 37, 41, 42].

So far, the few studies on the pollen morphology of the genus *Spiraea* have been regional in character and covered at most a dozen species. Polyakova and Gataulina [38] described the pollen morphology of 12 *Spiraea* species from different regions of Siberia and the Far East. Hebda and Chinnappa [23] described some *Spiraea* species growing in Canada. In turn, Liu et al. [39] analyzed 18 Chinese *Spiraea* species, while Song et al. [40] described 17 Korean species. Wronska-Pilarek et al. [32] examined pollen morphology and intraspecific variability of invasive *S. tomentosa* in Poland. In the PalDat database, Heigl [43] and Auer [44] briefly described *S. chamaedryfolia* and *S. oblongifolia*. All authors agree that the most important pollen features of the *Spiraea* species were connected with exine ornamentation, although some researchers also mentioned other features, such as the endoaperture diameter, equatorial and polar views, and length of the polar axis (P).

The presented study aimed to describe and analyze pollen morphology and intrageneric and interspecific variability of 25 *Spiraea* taxa—two species native to Poland and Europe and 23 alien species and hybrids—which were naturally distributed in Asia, Europe and North America, whence they came to Poland. This is the first palynological review of research on this genus in Europe. This study was also aimed at determining whether the pollen features of expansive and invasive *Spiraea* species differ from those of other taxa, and if so, whether and to what extent it may increase the effectiveness of the expansion of these species. It is believed
that, due to the invasive nature of some of the studied species in Poland and Europe, any new data on its reproduction may be useful in the fight against the expansion of this species.

Material and methods

Palynological analysis

Inflorescences of the 25 Spiraea taxa under analysis (22 species, one variety and two hybrids) were collected from the herbarium belonging to the Botanical Garden of the Adam Mickiewicz University in Poznań (OB UAM) (Table 1). The plants grown there come from other botanical gardens or natural sites of individual Spiraea species (Table 1). All samples were taken from herbarium sheets prepared in 2019. A total of 24 samples came from shrubs not older than 40 years. The youngest was 12 years old, while the oldest was 39. Additionally, four samples came from shrubs that are 48, 55, 82 and 86 years old. These are plants (except for S. tomentosa L.), which are growing scattered over an area of about 22 ha. Their flowering takes place over a period of three months (from April to June/July).

In this paper the taxonomic classification of the species from the genus Spiraea under analysis was adopted from Yü and Kuan [6], since the latest studies by Potter et al. [2, 3] and APG IV [4] do not provide systematic divisions within the studied genus. The taxa under examination represented all the four sections and eight (out of 10) series of the genus Spiraea [6]. Additionally, S. ×billardii Hérincq was included—a hybrid of two species (S. alba Du Roi × S. douglasii Hook) from the Spiraea section, and S. ×cinerea Zabel ‘Grefsheim’, a hybrid of the species from the section Chamaedryon with a species from the section Glomerati Nakai.

The nomenclature of taxa was adopted from botanical studies characterizing flora of the corresponding regions of the world. For Europe it was Flora Europaea [10] and Illustrierte Flora von Mitteleuropa [11], for Central Asia and the Russian Far East—Flora of the U.S.S.R. [45], Deriewa i kustraniki SSSR [46] and Flora Sibiriae [47], while for the Far East—Flora of Japan [48] and Flora of China [49]. The names of hybrids were adopted from The European Garden Flora [50]. All plant names were compared and validated against the online databases [51, 52]. A list of the taxa analyzed together with their affiliation to particular sections and series is shown in Table 2.

In accordance with the study by Wrońska-Pilarek et al. [53], each sample consisted of 30 randomly selected, mature and correctly formed pollen grains derived from a single individual (shrub). In total, 750 pollen grains were studied.

The pollen grains were prepared for light (LM) and scanning electron microscopy (SEM) using the standard acetylation method described by Erdtmann [54, 55]. An acetylation mixture was prepared to consist of 9 parts acetic acid anhydride and one part concentrated sulfuric acid, while the acetylation process lasted 2.5 minutes. The prepared material was divided into two parts: one part was immersed in an alcohol solution of glycerine (for LM) and the other in 96% ethyl alcohol (for SEM). Morphological observations were carried out using a light microscope (Biolar 2308, Nikon HFX-DX) and a scanning electron microscope (Jeol 7001TTLS). The pollen grains were measured in the equatorial view at a magnification of 640x.

Nine quantitative features of the pollen grains were analyzed, i.e., the length of the polar axis (P) and equatorial diameter (E), the length of the ectoaperture (Le), the thickness of the exine along the polar axis and equatorial diameter (Exp, Exe), as well as the P/E, Le/P, Exp/P and Exe/E ratios. Moreover, the following qualitative features were examined: the pollen outline and shape, and exine ornamentation (type, width and direction of grooves and striae, number and diameter of perforations).

The palynological terminology follows Punt et al. [56] and Halbritter et al. [57].
Statistical analysis

The normality of the distributions for the studied traits (length of polar axis—P, equatorial diameter—E, length of ectoaperture—Le, exine thickness along the polar axis—Exp, exine thickness along the equatorial diameter—Exe, as well as the P/E, Le/P, Exp/P and Exe/E ratios)
was tested using Shapiro-Wilk’s normality test [58]. A multivariate analysis of variance (MANOVA) was performed based on the following model using a MANOVA procedure in GenStat 18: 

\[ Y = X + E, \]

where:

- \( Y \) is \((n \times p)\)–the dimensional matrix of observations,
- \( n \) is the total number of observations,
- \( p \) is the number of traits (in this study \( p = 9 \)),
- \( X \) is \((n \times k)\)–the dimensional matrix of design,
- \( k \) is the number of species (in this study \( k = 25 \)),
- \( T \) is \((k \times p)\)–the dimensional matrix of unknown effects, and
- \( E \) is \((n \times p)\)–the dimensional matrix of residuals. Afterward, one-way analyses of variance (ANOVA) were performed to verify the null-hypothesis of a lack of a species effect in terms of the values of the nine observed traits, independently for each trait, based on the following model: 

\[ y_{ij} = \bar{y} + |i| + \Sigma_{ij}, \]

where: \( y_{ij} \) is \( j \)th observation of \( i \)th species, \( \bar{y} \) is the grand mean, \(|i|\) is the effect of \( i \)th species and \( \Sigma_{ij} \) is error observation. The minimal and maximal values of the traits as well as the arithmetic means and coefficients of variation (CV, in %) were calculated. Moreover, Fisher’s least significant differences (LSDs) were estimated at a significance level of \( \alpha = 0.001 \). The relationships between the observed traits were estimated based on the species’ means using Pearson’s correlation coefficients. The results were also analyzed using multivariate methods. A canonical variate analysis was applied to present a multi-trait assessment of the similarity of the tested species in a lower number of dimensions with the least possible loss of information [59]. This made it possible to illustrate in the graphic form any variation in the species in terms of all the observed traits. The Mahalanobis distance was suggested as a measure of “polytrait” species similarity [60], the significance of which was verified using critical value \( D_{\alpha} \) called “the least significant distance” [61]. Mahalanobis distances were calculated for the species. The differences between the analyzed species were verified by cluster analysis using the nearest neighbor method and Euclidean distances [62]. All the analyses were conducted using the GenStat 18 statistical software package.

**Results**

**Morphological description of pollen**

A description of pollen grain morphology of the *Spiraea* species under analysis is given below and illustrated in the SEM photographs (Figs 1–5). The morphological observations for the quantitative features are summarized in Tables 3–5 and S1 Table.
The pollen grains of the studied species occur as radially symmetric, tricolporate, isopolar monads (Figs 1 and 2).

According to Erdtman's pollen size classification [54], all of the analyzed pollen grains were small (10.1–25 μm). The length of the polar axis (P) was 16.46 (12.00–22.00) μm. The smallest P (12.00 μm) were found in *S. pubescens*, while the largest ones (22.00 μm) in *S. nipponica*. The average value of this feature ranged from 14.00 to 19.73 μm (S1 Fig).

The mean length of the equatorial diameter (E) was 16.00 (12.00–22.00) μm. The smallest value of this feature (12.00 μm) was found in *S. cana*, *S. douglasii*, *S. pubescens*, *S. thunbergii* and *S. ×cinerea 'Grefsheim', whereas the largest ones (22.00 μm) only in *S. hypericifolia*. The average value of this trait ranged between 13.20 and 18.27 μm (S1 Fig).

The outline in the polar view was mostly circular or more rarely elliptic, while in the equatorial view it was circular or elliptic (Figs 1 and 2).

The mean P/E ratio was 1.04, ranging from 0.78 in *S. media* to 1.43 in *S. uratensis*. The average value of this ratio was very similar and ranged from 0.81 to 0.97. The pollen shape was most frequently spheroidal (398 pollen grains– 53.1%) and prolate-spheroidal (215–28.7%).

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Fig 1. Pollen grains in polar view in *S. douglasii*, *S. elegans*, *S. thunbergii*, *S. japonica*, *S. uratensis*, A-E.

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rarely oblate-spheroidal (61–8.1%), suboblate (37–4.9%), subprolate (37–4.9%), and very rarely prolate (2–0.3%).

The mean exine thickness was 0.98 μm (Exe)– 0.99 μm (Exp) (within the range of 0.20–2.00 μm). The relative exine thickness (Exp/P and Exe/E ratios) averaged 0.09 and 0.06 (Exp/P–from 0.01 to 0.90 and Exe/E–from 0.01 to 0.14), respectively.

Exine ornamentation was usually striate-perforate, rarely striate and in one species striate-reticulate (Figs 3–5). The striae and grooves usually ran parallel to the polar axis, but they also frequently formed loops. They were straight or forked and of varying length and width (Figs 3–5). Either the grooves were narrower than the striae, or the widths of the grooves and striae were similar and averaged from 0.1 to 0.3 μm. Circular or elliptic perforations of different diameters–small to large (from 0.1 to 1 μm)–were found at the bottom of the striae (Figs 3–5).

Pollen of the individual *Spiraea* species under analysis was classified according to the striate exine ornamentation classification proposed by Ueda and Tomita [63] and Ueda [24] into five types (I-V) and six subtypes (I A,B, II A,B and III A,B). Ueda and Tomita [63] distinguished six types (I-VI) and six subtypes (I-III, each A and B). The above classification was applied,
because it is based on the analysis of striate exine ornamentation in pollen of many species and genera from the family Rosaceae, and thus it best differentiates this type of ornamentation in the studied genus *Spiraea*. Type VI was not found in this study (Figs 3–5, Table 6). The greatest number of species (9) belonged to the IIA subtype, which was characterized by fairly distinct striae, narrow grooves and frequently numerous perforations of different diameters. Subtypes IA, IIIA, IIIB, IV and V were represented by two or three species, while type IB, by only one species (Figs 3–5, Table 6). In *S. henryi* only striate-reticulate exine ornamentation was found.

Pollen grains usually possess three apertures—the colpi. These were distributed symmetrically, elongated, and narrowed toward the poles, with granular aperture membranes. The mean length of the colpi ranged between 10.00 and 22.00 μm, with an average of 15.27 μm. The length of the colpi typically constituted 93% of the polar axis length (P) and ranged from
63 to 100%. Their width was variable and usually the greatest in the equatorial region. The endoapertures were typically located in the middle of the colpi, less frequently asymmetrically, usually singly. They were circular or elliptic in outline with irregular margins.

Key to identification of the *Spiraea* species under analysis based on pollen features.

1 Exine ornamentation striate-reticulate. ................................................................. *S. henryi*

1’ Exine ornamentation striate. ......................................................................................... 2

2 Exine ornamentation striate without perforations. ......................................................... 3

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Fig 4. Striate exine ornamentation types according to Ueda and Tomita [63]; see Table 6. A-H. A, *S. chinensis*; B, *S. ×cinerea ‘Grefsheim’; C, *S. dasyantha*; D, *S. douglasii*; E, *S. elegans*; F, *S. henryi*; G, *S. japonica*; H, *S. alba var. latifolia*. 

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Fig 5. Striate exine ornamentation types according to Ueda and Tomita [63]; see Table 6. A-I. A, *S. media*; B, *S. pubescens*; C, *S. salicifolia*; D, *S. splendens*; E, *S. thunbergii*; F, *S. trichocarpa*; G, *S. uratensis*; H, *S. veitchii*; I, *S. tomentosa*.

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Table 3. Minimal, maximal and mean values as well as coefficients of variation (CV) for length of polar axis (P), equatorial diameter (E) and length of ectoaperture (Le) in studied taxa.

| Trait | P | E | Le |
|-------|---|---|----|
| Species | Mean | Min-Max | s.d. | Mean | Min-Max | s.d. | Mean | Min-Max | s.d. |
| S. alba | 16.33 | def | 14–20 | 1.06 | 16.07 | cd | 14–20 | 1.11 | 15.27 | c | 12–20 | 1.70 |
| S. alba var. latifolia | 18.27 | ab | 16–20 | 1.02 | 17.87 | b | 16–20 | 1.04 | 17.53 | ab | 14–20 | 1.46 |
| S. betulifolia | 18.67 | a | 16–20 | 1.32 | 19.27 | a | 16–20 | 1.23 | 16.67 | b | 14–20 | 1.61 |
| S. cana | 16 | defg | 14–18 | 1.17 | 14.87 | fghij | 12–18 | 1.72 | 14.53 | cde | 12–18 | 1.57 |
| S. chamaedryfolia | 18.6 | a | 16–20 | 1.07 | 19.73 | a | 18–20 | 0.69 | 17.67 | ab | 16–20 | 1.40 |
| S. chinensis | 15.8 | defg | 14–18 | 1.10 | 15.6 | cdef | 14–18 | 0.97 | 14.93 | cd | 12–18 | 1.72 |
| S. dasyantha | 14.4 | i | 14–16 | 0.81 | 14.07 | ij | 12–16 | 0.64 | 13.8 | def | 12–16 | 1.10 |
| S. douglasii | 15.4 | fgh | 14–18 | 1.50 | 14.47 | ghij | 12–16 | 1.25 | 14.47 | cdef | 12–18 | 1.80 |
| S. elegans | 15.6 | efg | 14–16 | 0.81 | 15.73 | cdef | 14–16 | 0.69 | 15 | cd | 12–16 | 1.15 |
| S. henryi | 15.2 | ghi | 14–16 | 1.00 | 14.93 | efgij | 14–16 | 1.02 | 14.2 | cdef | 12–16 | 1.42 |
| S. hypericifolia | 18.27 | ab | 16–20 | 1.26 | 19.13 | a | 16–22 | 1.36 | 17.5 | ab | 16–20 | 1.55 |
| S. japonica | 15.6 | efg | 14–18 | 0.97 | 14.87 | fghij | 14–16 | 1.01 | 14.07 | cdef | 12–16 | 1.44 |
| S. media | 15.67 | defg | 14–18 | 1.18 | 15.8 | cdef | 14–18 | 1.10 | 14.67 | cde | 12–18 | 1.69 |
| S. nipponica | 16.6 | cd | 14–22 | 1.40 | 15.67 | cdef | 14–20 | 1.58 | 14.93 | cd | 12–22 | 1.80 |
| S. pubescens | 14.53 | hi | 12–16 | 1.04 | 14 | j | 12–16 | 1.05 | 14 | cdef | 12–16 | 1.39 |
| S. salicifolia | 19 | a | 18–20 | 1.02 | 19.33 | a | 16–20 | 1.09 | 18.27 | a | 16–20 | 1.26 |
| S. splendens | 16.47 | de | 14–20 | 1.25 | 15.2 | defgh | 14–18 | 1.13 | 15.2 | c | 14–18 | 1.13 |
| S. thanbergii | 15.4 | fgh | 14–18 | 1.07 | 15 | efgi | 12–18 | 1.26 | 13.6 | ef | 12–16 | 1.52 |
| S. tomentosa | 16.33 | defg | 14–18 | 1.67 | 16.2 | c | 14–18 | 1.32 | 13.2 | f | 10–18 | 1.71 |
| S. trichocarpa | 15.8 | defg | 14–18 | 0.96 | 15.4 | cdefg | 14–16 | 0.93 | 14.6 | cde | 12–16 | 1.30 |
| S. uratensis | 17.53 | bc | 16–20 | 1.01 | 15.4 | cdefg | 14–16 | 0.93 | 17 | ab | 14–20 | 1.37 |
| S. veitchi | 16.53 | de | 14–20 | 1.28 | 15.87 | cde | 14–20 | 1.38 | 14.67 | cde | 12–20 | 2.12 |
| S. wilsonii | 15.73 | defg | 14–18 | 1.02 | 14.93 | efgij | 12–16 | 1.14 | 14.13 | cdef | 12–16 | 1.38 |
| S. xbillardii | 18.13 | ab | 14–20 | 1.38 | 16.27 | c | 14–20 | 1.64 | 17.13 | ab | 14–20 | 1.87 |
| S. xcinerea | 15.67 | defg | 14–18 | 1.18 | 14.27 | hij | 12–16 | 1.02 | 14.67 | cde | 12–18 | 1.69 |
| LSD0.001 | 0.989 | | 0.992 | | 1.317 | | 26.73 | | 0.001 | | 0.001 |

2° Exine ornamentation striate with perforations.                       6

3 Exine ornamentation subtype IA (grooves distinct and of medium width, striae narrow; perforations few or absent, small). 4

3° Exine ornamentation type IIIA (grooves distinct and wide, striae narrow; perforations few or absent, small). 5

4. Striae wavy.  S. dasyantha

4° Striae straight.  S. hypericifolia

5. Striae run across the P axis.  S. cinerea ‘Grefsheim’

5° Striae extending along the P axis.  S. splendens

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6 Exine ornamentation type I ........................................................................................................ 7
6* Other type of exine ornamentation .......................................................................................... 8

7 Exine ornamentation subtype IA (grooves distinct and of medium width, striae narrow; perforations few or absent, small). .............................................................................................. S. media

7* Exine ornamentation subtype IB (grooves distinct and of medium width, striae wide; perforations few or absent, small). .............................................................................................. S. cana

8 Exine ornamentation type II ...................................................................................................... 9
8* Other types of exine ornamentation ........................................................................................... 10

9 Exine ornamentation subtype IIA (grooves very distinct and of medium width, striae narrow; perforations numerous, with different diameters (mostly small). ......................................... 11

Table 4. Minimal, maximal and mean values as well as coefficients of variation (CV) for exine thickness along the polar axis (Exp), exine thickness along the equatorial diameter (Exe) and P/E of analyzed species.

| Trait | Exp | Exe | P/E |
|-------|-----|-----|-----|
| Species | Mean | Min-Max | s.d. | Mean | Min-Max | s.d. | Mean | Min-Max | s.d. |
| S. alba | 0.98 | defg | 0.2–1.6 | 0.28 | 0.98 | cde | 0.2–1.6 | 0.28 | 1.019 | defghi | 0.88–1.14 | 0.066 |
| S. alba var. latifolia | 0.787 | gh | 0.2–1.6 | 0.34 | 0.793 | ef | 0.2–1.6 | 0.38 | 1.024 | defghi | 0.90–1.13 | 0.062 |
| S. betulifolia | 0.653 | h | 0.2–1 | 0.23 | 0.653 | f | 0.2–1 | 0.23 | 0.972 | hij | 0.80–1.25 | 0.086 |
| S. cana | 1.253 | bcd | 0.6–1.6 | 0.34 | 1.253 | abc | 0.6–1.6 | 0.34 | 1.089 | abcd | 0.88–1.33 | 0.134 |
| S. chamaedryfolia | 0.913 | efg | 0.2–1.6 | 0.43 | 0.913 | def | 0.2–1.6 | 0.43 | 0.943 | j | 0.80–1.00 | 0.057 |
| S. chinensis | 0.68 | h | 0.2–1 | 0.19 | 0.68 | f | 0.2–1 | 0.19 | 1.014 | efgij | 0.88–1.14 | 0.061 |
| S. dasyantha | 0.793 | fgh | 0.6–1.6 | 0.25 | 0.793 | ef | 0.6–1.6 | 0.25 | 1.026 | defghi | 0.88–1.17 | 0.075 |
| S. douglasii | 0.853 | efg | 0.6–1.6 | 0.28 | 0.853 | def | 0.6–1.6 | 0.28 | 1.067 | bcdef | 0.88–1.29 | 0.089 |
| S. elegans | 1.053 | bcdefg | 0.6–1.6 | 0.31 | 1.053 | abcde | 0.6–1.6 | 0.31 | 0.993 | ghij | 0.88–1.14 | 0.059 |
| S. henryi | 0.973 | defg | 0.6–2 | 0.4 | 0.973 | cde | 0.6–2 | 0.4 | 1.02 | defghi | 0.88–1.14 | 0.07 |
| S. hypericifolia | 0.873 | efg | 0.2–1.6 | 0.36 | 0.873 | def | 0.2–1.6 | 0.36 | 0.958 | ij | 0.80–1.13 | 0.076 |
| S. japonica | 1.013 | cddefg | 0.6–1.6 | 0.28 | 1.013 | bcde | 0.6–1.6 | 0.28 | 1.052 | bcdefg | 0.88–1.14 | 0.077 |
| S. media | 0.987 | cdefg | 0.6–1.6 | 0.3 | 0.987 | bcde | 0.6–1.6 | 0.3 | 0.996 | fghij | 0.78–1.29 | 0.096 |
| S. nipponica | 1.313 | b | 0.6–2 | 0.42 | 1.313 | a | 0.6–2 | 0.42 | 1.066 | bcdef | 0.89–1.12 | 0.098 |
| S. pubescens | 0.847 | efg | 0.2–1.6 | 0.29 | 0.847 | def | 0.2–1.6 | 0.29 | 1.041 | cdefg | 1.00–1.33 | 0.082 |
| S. salicifolia | 0.673 | h | 0.2–1.6 | 0.28 | 0.673 | f | 0.2–1.6 | 0.28 | 0.986 | ghij | 0.90–1.13 | 0.074 |
| S. splendidens | 1.053 | bcdefg | 0.6–2 | 0.36 | 1.053 | abcde | 0.6–2 | 0.36 | 1.086 | abcde | 0.88–1.14 | 0.075 |
| S. tananbergii | 1.087 | bcde | 0.6–1.6 | 0.38 | 1.087 | abcd | 0.6–1.6 | 0.38 | 1.03 | cdefg | 0.88–1.17 | 0.076 |
| S. tomentosa | 1.667 | a | 02-sty | 0.48 | 1.273 | ab | 0.6–2 | 0.49 | 1.016 | efgi | 0.78–1.29 | 0.138 |
| S. trichocarpa | 1.08 | bcdefg | 0.6–1.6 | 0.29 | 1.08 | abcde | 0.6–1.6 | 0.29 | 1.028 | cdefg | 0.88–1.14 | 0.066 |
| S. uratensis | 1.22 | bc | 0.6–1.6 | 0.36 | 1.22 | abc | 0.6–1.6 | 0.36 | 1.142 | a | 1.00–1.43 | 0.085 |
| S. vetchii | 1.02 | cdefg | 0.6–1.6 | 0.37 | 1.02 | bcde | 0.6–1.6 | 0.37 | 1.046 | cdefg | 0.88–1.14 | 0.086 |
| S. wilsonii | 0.907 | efg | 0.6–1.6 | 0.34 | 0.893 | def | 0.6–1.6 | 0.34 | 1.057 | bcdefg | 0.88–1.17 | 0.079 |
| S. x billardii | 0.84 | efg | 0.6–1.6 | 0.28 | 0.84 | def | 0.6–1.6 | 0.28 | 1.121 | ab | 1.00–1.29 | 0.095 |
| S. xcinerea | 1.273 | bc | 0.6–2 | 0.42 | 1.273 | ab | 0.6–2 | 0.42 | 1.1 | abc | 1.00–1.29 | 0.077 |
| LSD0.001 | 0.288 | 0.29 | 0.072 | 10.13 | F value | <0.001 | <0.001 | <0.001 | 0.004 |

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Table 5. Minimal, maximal and mean values as well as coefficients of variation (CV) for Le/P, Exp/P and Exe/E of analyzed species.

| Trait | Le/P | Exp/P | Exe/E |
|-------|------|-------|-------|
|       | Min-Max | s.d. | Min-Max | s.d. | Min-Max | s.d. |
| Species | Mean | | | Mean | | | Mean | | |
| S. alba | 0.935 | abcdef | 0.75–1 | 0.083 | 0.06 | def | 0.013–0.100 | 0.018 | 0.061 | cdefg | 0.013–0.100 | 0.018 |
| S. alba var. latifolia | 0.961 | abc | 0.70–1 | 0.07 | 0.043 | fg | 0.011–0.089 | 0.019 | 0.045 | fgh | 0.011–0.089 | 0.022 |
| S. betulifolia | 0.894 | ef | 0.78–1 | 0.067 | 0.035 | g | 0.010–0.063 | 0.013 | 0.034 | h | 0.010–0.063 | 0.012 |
| S. cana | 0.908 | abcd | 0.75–1 | 0.066 | 0.079 | bc | 0.033–0.114 | 0.022 | 0.085 | ab | 0.038–0.133 | 0.025 |
| S. chamaedryfolia | 0.95 | abcd | 0.80–1 | 0.06 | 0.049 | efg | 0.011–0.089 | 0.023 | 0.047 | efg | 0.010–0.089 | 0.022 |
| S. chinensis | 0.945 | abcd | 0.75–1 | 0.079 | 0.043 | fg | 0.013–0.063 | 0.012 | 0.044 | gh | 0.013–0.063 | 0.012 |
| S. dasyantha | 0.959 | abcd | 0.86–1 | 0.064 | 0.055 | def | 0.038–0.100 | 0.016 | 0.056 | defg | 0.043–0.114 | 0.017 |
| S. douglasii | 0.939 | ab | 0.86–1 | 0.067 | 0.056 | def | 0.033–0.114 | 0.019 | 0.059 | defg | 0.038–0.114 | 0.019 |
| S. elegans | 0.962 | ab | 0.86–1 | 0.059 | 0.068 | bcd | 0.038–0.114 | 0.02 | 0.067 | bcd | 0.038–0.100 | 0.02 |
| S. henryi | 0.935 | abcd | 0.75–1 | 0.081 | 0.064 | bcde | 0.038–0.125 | 0.025 | 0.066 | bcde | 0.038–0.143 | 0.029 |
| S. hypericifolia | 0.959 | abcd | 0.80–1 | 0.064 | 0.048 | efg | 0.010–0.089 | 0.019 | 0.046 | fgh | 0.010–0.089 | 0.019 |
| S. japonica | 0.903 | bcdef | 0.75–1 | 0.091 | 0.065 | bcde | 0.033–0.114 | 0.019 | 0.068 | bcd | 0.038–0.114 | 0.019 |
| S. media | 0.935 | abcd | 0.86–1 | 0.067 | 0.063 | cde | 0.033–0.100 | 0.018 | 0.063 | cdefg | 0.033–0.114 | 0.019 |
| S. nipponica | 0.899 | def | 0.78–1 | 0.062 | 0.079 | bc | 0.043–0.125 | 0.024 | 0.084 | ab | 0.043–0.125 | 0.026 |
| S. pubescens | 0.963 | ab | 0.86–1 | 0.062 | 0.058 | def | 0.013–0.100 | 0.019 | 0.061 | cdefg | 0.013–0.114 | 0.021 |
| S. salicifolia | 0.963 | ab | 0.80–1 | 0.062 | 0.036 | g | 0.010–0.089 | 0.015 | 0.034 | h | 0.010–0.089 | 0.015 |
| S. splendidens | 0.926 | ab | 0.78–1 | 0.076 | 0.064 | bcde | 0.033–0.114 | 0.022 | 0.07 | abcd | 0.038–0.125 | 0.024 |
| S. thunbergii | 0.884 | f | 0.75–1 | 0.082 | 0.07 | bcd | 0.038–0.114 | 0.024 | 0.072 | abcd | 0.038–0.114 | 0.023 |
| S. tomentosa | 0.81 | g | 0.63–0.9 | 0.085 | 0.103 | a | 0.056–0.143 | 0.032 | 0.079 | abc | 0.038–0.143 | 0.03 |
| S. trichocarpa | 0.924 | ab | 0.86–1 | 0.063 | 0.069 | bcd | 0.033–0.100 | 0.018 | 0.07 | abcd | 0.038–0.114 | 0.019 |
| S. uratensis | 0.969 | a | 0.88–1 | 0.052 | 0.07 | bcd | 0.033–0.100 | 0.021 | 0.079 | abc | 0.038–0.114 | 0.022 |
| S. veitchii | 0.886 | f | 0.75–1 | 0.095 | 0.062 | cde | 0.033–0.114 | 0.022 | 0.064 | cdefg | 0.038–0.114 | 0.022 |
| S. wilsonii | 0.9 | cdef | 0.75–1 | 0.083 | 0.058 | def | 0.033–0.114 | 0.023 | 0.061 | cdefg | 0.038–0.114 | 0.025 |
| S. xbilliardii | 0.945 | ab | 0.78–1 | 0.07 | 0.047 | efg | 0.030–0.089 | 0.017 | 0.053 | defgh | 0.030–0.114 | 0.022 |
| S. xscirpifolia | 0.935 | abcd | 0.86–1 | 0.066 | 0.082 | b | 0.038–0.125 | 0.028 | 0.089 | a | 0.043–0.143 | 0.028 |

LSD0.001 0.061 0.018 0.019
ANOVA F 7.52 16.31 14.44
F value <0.001 <0.001 <0.001

https://doi.org/10.1371/journal.pone.0273743.1005
13 Exine ornamentation type IV (grooves distinct and of medium width, flat, striae very wide; perforations very numerous and most extremely large). ...................................... S. chinensis, S. japonica, S. uratensis

13° Exine ornamentation type V (grooves indistinct, flat and blurred, striae wide; perforations numerous and usually large). ...................................... S. nipponica, S. salicifolia

**Intrageneric and interspecific variability of pollen grains**

The results of the MANOVA performed indicated that all the samples were significantly different with regard to all of the nine quantitative traits (Wilk’s $\lambda = 0.05895$; $F_{24,725} = 11.20$; $P<0.0001$). The results of ANOVA indicated that the main effects of the species were significant for all the nine observed traits (Tables 3–5 and S1 Table). The mean values and standard deviations for the observed traits indicated high variability among the tested samples, for which significant differences were found in terms of all the analyzed morphological traits (Tables 3–5 and S1 Table) (Figs 6–9).

The interspecific variability of the *Spiraea* pollen grains was examined based on nine selected quantitative features. Statistical analysis of the studied features indicated high variability among the tested species. The most variable biometric traits were E and P (Table 3), while lower variability was observed in the Le/P ratio and Exe (Tables 4 and 5). A ranking of variability of the observed traits is as follows: $E > P > Le > Exp/P > Exe/E > Exp > P/E > Exe > Le/P$. The most variable species for all the nine traits jointly were *S. ×billardii*, *S. veitchii*, *S. nipponica* and *S. cana*, while those with lower variability were *S. dasyantha*, *S. elegans*, *S. trichocarpa* and *S. chamaedryfolia*.

The correlation analysis performed indicated statistically significant correlation coefficients for 25 out of 36 coefficients (Table 7, Fig 10). Twelve out of 25 significantly correlated pairs of traits were characterized by positive correlation coefficients. Negative correlation coefficients were observed between $P$ and $Exp/P$ (-0.486), $P$ and $Exe/P$ (-0.530), $E$ and $Exe$ (-0.376), $E$ and $P/E$ (-0.629), $E$ and $Exp/P$ (-0.524), $E$ and $Exe/E$ (-0.666), $Le$ and $Exp$ (-0.432), $Le$ and $Exe$ (-0.396), $Le$ and $Exp/P$ (-0.645), $Le$ and $Exe/E$ (-0.600), $Exp$ and $Le/P$ (-0.592), $Exe$ and $Le/P$ (-0.430), and $Le/P$ and $Exp/P$ (-0.586). In the case of eleven pairs of traits, no significant correlation was established (Table 7, Fig 10).

In the presented clustering according to the neighbor method of Euclidean distances, all the examined *Spiraea* species were divided into three groups (Fig 11). The first group included eight species: *S. alba var. latifolia*, *S. ×billardii*, *S. chamaedryfolia*, *S. hypericifolia*, *S. betulifolia*,...
The second one consisted of five species: *S. henri*, *S. wilsonii*, *S. douglasii*, *S. dasyantha* and *S. pubescens*. The third one comprised all the other species and was divided into two subgroups: A—*S. alba*, *S. media*, *S. elegans*, and B—all other species (Fig 11). The phylogenetic tree of distribution of pollen morphological characters was presented on the Fig 12. The obtained divisions were very similar to those presented in the dendrogram (Fig 11).

Fig 13 shows variability of the quantitative traits in the 25 *Spiraea* taxa under investigation in terms of the first two canonical variables. In the graph coordinates of the point for particular species are values for the first and second canonical variables, respectively. The first two canonical variables accounted for 80.08% of the total multivariate variability between individual species. Significant positive linear relationships with the first canonical variable were found for Exe, P/E, Exp/P and Exe/E (Table 8), while the first canonical variable correlated negatively

**Fig 6.** The density plot of P by *Spiraea* taxa.

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with P, E and Le (Table 8). The second canonical variable had a significant positive correlation with Exp, Exe, Exp/P and Exe/E, but a significant negative correlation with Le/P (Table 8).

The greatest variation in terms of all the traits, based on the Mahalanobis distances measured, was found for the species *S. salicifolia* and the invasive *S. dasyantha* (the Mahalanobis distance between them amounted to 6.138). The greatest similarity was found for the species *S. japonica* and *S. wilsonii* (0.432). The values of the Mahalanobis distances for all the pairs of treatments are presented in Table 9. Pollen of the invasive *S. tomentosa* was distinctive due to it exhibiting the greatest variation in terms of all the traits together, based on the Mahalanobis distances measured. Pollen of these species was characterized by a relatively narrow range of E values (14–18 μm), the largest range of the P/E ratio (0.78–1.29) and the highest number of pollen grains (20) with the thickest exine (2 μm) (Tables 3–5).
Discussion

Palynological studies on the species from the genus *Spiraea* L. are not numerous. The least amount of research has been carried out in Europe [32], perhaps due to the small number of native species (7), and also in North America [23]. Most research papers come from Asia, which is the world center for the distribution of this genus [38–40, 63]. According to previous studies, the diagnostic features of the *Spiraea* pollen grains were exine ornamentation (striae and groove length, width and direction, and perforation number and diameter) and P and the P/E ratio [23, 32, 38–40], as well as endoaperture diameter [38]. In the opinion of Liu et al. [39] as well as the authors of this study, P or the P/E ratio were of a lesser diagnostic value, because the values of both these traits were often quite similar in all the studied taxa. Neither this study nor studies carried out by other researchers confirmed the observation of Polyakova...
and Gataulina [38] that endoapertures of some Spiraea species can be wide (S. hypericifolia, S. salicifolia), while others narrow (S. alpine, S. crenata, S. schlothaurae).

For the genus Spiraea other palynologists distinguished a striate or rarely striate-granulate exine ornamentation [23, 38, 39, 63]. Only the striate exine ornamentation was reported by Wrońska-Pilarek et al. [32] in S. tomentosa, while in the study presented here striate were observed in only one species, where it was the striate-reticulate ornamentation. All authors agree that the most important features of Spiraea pollen were the striae and the groove course and dimensions, as well as the presence or absence of perforations. Polyakova and Gataulina [38] reported that in the meadowsweet species the striae were variable in length and width. They crossed and branched, for the most part meridionally and in different directions. In the opinion of the above-mentioned authors, S. salicifolia was characterized by the ‘four-grooved’ pollen grains. However, such a feature was not found in the study presented here. Liu et al.
Table 7. Correlation coefficients between observed traits.

| Trait | P | E | Le | Exp | Exe | P/E | Le/P | Exp/P | Exe/E |
|-------|---|---|----|-----|-----|-----|------|-------|-------|
|       |   |   |    |     |     |     |      |       |       |
| P     | 1 |   |    |     |     |     |      |       |       |
| E     | 0.898*** | 1 |     |     |     |     |      |       |       |
| Le    | 0.914*** | 0.815*** | 1 |     |     |     |      |       |       |
| Exp   | -0.245 | -0.347 | -0.444' | 1 |     |     |      |       |       |
| Exe   | -0.285 | -0.426' | -0.409' | 0.946*** | 1 |     |      |       |       |
| P/E   | -0.241 | -0.641*** | -0.212 | 0.365 | 0.471' | 1 |      |       |       |
| Le/P  | 0.132 | 0.109 | 0.522** | -0.587** | -0.422' | -0.025 | 1 |      |       |
| Exp/P | -0.481' | -0.542** | -0.644*** | 0.966*** | 0.922*** | 0.382 | -0.579* | 1 |       |
| Exe/E | -0.527** | -0.683*** | -0.596** | 0.870*** | 0.948*** | 0.614** | -0.367 | 0.918*** | 1 |       |

* P<0.05  
** P<0.01  
*** P<0.001

https://doi.org/10.1371/journal.pone.0273743.t007

Fig 10. Heatmap for Pearson’s correlation coefficients between observed traits for Spiraea taxa ($r_{0.05} = 0.38; r_{0.01} = 0.49; r_{0.001} = 0.60$).

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Song et al. [40] recognized that all Spiraeeae taxa have a striate exine ornamentation. Indeed, in their opinion four types of striate exine ornamentation could be recognized. The first three types could each be divided into two subtypes based on the diameter of the perforations, which may be small or large. The significant importance of the diameters and number of perforations in the diagnosis of *Spiraea* species was also emphasized by Wrońska-Pilarek et al. [32].

The pollen grains of *Spiraea* species were described most often as small-sized [32, 38–40, 43, 44, 64] and rarely as medium-sized [38, 40]. In the research presented here, all the studied pollen grains were small. Additionally, Polyakova and Gataulina [38] reported that the
significant variability of pollen size was typical of *S. media* and *S. alpine*. However, these results (P of 17.8–21.5 μm) for *S. media* were not confirmed in this study—the P and E values were average, ranging between 14 and 18 μm (Table 3). The differences may be due to the fact that the cited authors examined more samples (5) of *S. media* from populations scattered from Siberia to the Far East. Palynologists have mentioned different pollen shape types in the meadowsweet species. These pollen grains ranged from prolate and subspheroidal, spherical,
Fig 13. Distribution of 28 *Spiraea* taxa in space of two first canonical variables.

https://doi.org/10.1371/journal.pone.0273743.g013

Table 8. Correlation coefficients between the first two canonical variables and original traits.

| Trait          | First canonical variable | Second canonical variable |
|----------------|--------------------------|---------------------------|
| P              | -0.9376***               | 0.067                     |
| E              | -0.986***                | 0.054                     |
| Le             | -0.869***                | -0.271                    |
| Exp            | 0.381                    | 0.817***                  |
| Exe            | 0.441*                   | 0.612***                  |
| P/E            | 0.537**                  | 0.022                     |
| Le/P           | -0.160                   | -0.811***                 |
| Exp/P          | 0.581**                  | 0.730***                  |
| Exe/E          | 0.680**                  | 0.459*                    |
| Percentage of explained multivariate variability | 66.06                         | 15.75                     |

* P < 0.05  
** P < 0.01  
*** P < 0.001

https://doi.org/10.1371/journal.pone.0273743.t008
Table 9. Mahalanobis distances between analyzed *Spiraea* species were calculated based on nine quantitative traits.

| Species | S. alba | S. alba var. latifolia | S. betulifolia | S. cana | S. chamaedryfolia | S. chinensis | S. dasyantha | S. douglasi | S. elegans | S. henryi | S. hypericifolia | S. japonica | S. media | S. nepetosum | S. pauciflora | S. salicifolia | S. spicata | S. thunbergii | S. tomentosa | S. trilobata | S. uratensis | S. wilsonii | S. × billardii | S. × cinerea | S. × thunbergii | S. × wilsonii | S. × strophante | S. × sieboldii | S. × veitchii |
|---------|---------|-----------------------|---------------|--------|------------------|-------------|-------------|-------------|-----------|---------|----------------|-------------|---------|----------------|-------------|----------------|----------------|-------------|-------------|----------------|-------------|-------------|----------------|-------------|-------------|----------------|-------------|-------------|
| 1       |        | 2.35                  |               | 4.19   | 2.59             |             | 1.82        | 3.44        | 4.80      | 4.12    | 1.87            | 4.70         | 2.35   | 3.44           | 4.80         | 1.87           | 3.44           | 4.80        | 2.35        | 3.44           | 4.80         | 2.35        | 3.44           | 4.80         | 2.35        |
| 2       | 2.35   |                      |               |        |                  |             | 2.36        | 4.38        | 5.02      | 2.77    | 6.10            | 1.68         |        |                |              |                |                |              |            |                |              |                |                |              |
| 3       | 4.19   | 2.59                  | 4.12          |        |                  |             | 4.12        | 1.87        | 4.70      | 2.35    | 3.44            | 4.80         | 2.35   | 3.44           | 4.80         | 1.87           | 3.44           | 4.80        | 2.35        | 3.44           | 4.80         | 2.35        | 3.44           | 4.80         | 2.35        |
| 4       | 1.82   | 3.44                  | 4.80          | 4.12   | 1.87             | 4.70        | 2.35        | 3.44        | 4.80      | 2.35    | 3.44            | 4.80         |        |                |              |                |                |              |            |                |              |                |                |              |
| 5       | 3.44   | 4.80                  | 1.87          | 4.70   | 2.35             | 3.44        | 4.80        | 2.35        | 3.44      | 4.80    | 2.35            | 3.44         | 4.80   |                |              |                |                |              |            |                |              |                |                |              |
| 6       | 4.12   | 1.87                  | 4.70          | 2.35   | 3.44             | 4.80        | 2.35        | 3.44        | 4.80      | 2.35    | 3.44            | 4.80         |        |                |              |                |                |              |            |                |              |                |                |              |
| 7       | 1.87   | 4.70                  | 2.35          | 3.44   | 4.80             | 2.35        | 3.44        | 4.80        | 2.35      | 3.44    | 4.80            | 2.35         | 4.80   |                |              |                |                |              |            |                |              |                |                |              |
| 8       | 4.70   | 2.35                  | 3.44          | 4.80   | 2.35             | 3.44        | 4.80        | 2.35        | 3.44      | 4.80    | 2.35            | 3.44         |        |                |              |                |                |              |            |                |              |                |                |              |
| 9       | 2.35   | 3.44                  | 4.80          | 2.35   | 3.44             | 4.80        | 2.35        | 3.44        | 4.80      | 2.35    | 3.44            | 4.80         | 4.80   |                |              |                |                |              |            |                |              |                |                |              |
| Dα = 3.73 |        |                       |               |        |                  |             | 2.35        | 3.44        | 4.80      | 2.35    | 3.44            | 4.80         | 4.80   |                |              |                |                |              |            |                |              |                |                |              |

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trilobate, dolicho-round, oblong and fusiform [39], through elongated spheroidal [38], and from oblate to prolate [40]. The shape of the pollen in S. tomentosa was most frequently spheroidal, rarely oblate-spheroidal or prolate-spheroidal and very rarely subprolate or prolate [32]. The results in this study indicate that pollen shape in the 25 studied taxa was most frequently spheroidal and prolate-spheroidal, much less often oblate-spheroidal, suboblate or subprolate, and very rarely prolate. The reason for these differences may be connected with the detailed description of pollen shape classes in the last two studies.

The authors, as well as the other cited researchers, did not find Ubisch bodies (orbicules) in the studied taxa. These results were confirmed by Song et al. [40], who noted that orbicule distribution patterns indicated that the absence of orbicules was a synapomorphic condition of the more derived clade, comprising the genera Pentactina, Petrophytum, Kelseya and Spiraea.

Of the three invasive species studied (S. douglasii, S. japonica and S. tomentosa), the first two were not distinguished by specific pollen features, while S. tomentosa was distinctive as showing the greatest variation in terms of all the traits jointly, based on the Mahalanobis distances measured. It was found that the pollen features of this species differed from the other studied taxa in terms of the relatively narrow range of E, the largest range of the P/E ratio and the highest number of pollen grains with the thickest exine. In earlier studies by Wróńska-Pilarek et al. [32] carried out on a sample of 900 pollen grains of S. tomentosa, similar data was obtained. Thus, it could be concluded that the invasive S. tomentosa described shows a large variability of pollen features and a thick exine, which may indicate a greater ability to adapt its pollen to changing environmental conditions, guaranteeing it greater efficiency in taking over new areas. However, in the authors’ opinion to confirm this thesis the pollen of other invasive plant species should be compared, not only pollen from the genus Spiraea.

For the first time the intrageneric and interspecific variability of Spiraea pollen grains were studied based on nine quantitative traits. Statistical analysis of the investigated features indicated high variability among the species. The most variable species for all the nine traits jointly were S. ×billardii, S. veitchii, S. nipponica and S. cana, while those with lower variability were S. dasyantha, S. elegans, S. trichocarpa and S. chamaedryfolia. The most variable biometric traits were the E and P, while lower variability was recorded in the Le/P ratio and Exe. The ranking of variability in the observed traits is as follows: E > P > Le > Exp/P > Exe/E > Exp > P/E > Exe > Le/P.

It needs to be stressed here that the above conclusions were drawn based on studies conducted applying the method of collecting flowers from single shrubs of individual taxa, an approach commonly used in palynology. Thus the recorded results may not illustrate the entire pollen variability in the investigated species. We are aware of this limitation in the sampling procedure; nevertheless, we are of an opinion that due to the definitely conservative character of pollen grains the obtained results are representative. This thesis is confirmed by the results of our numerous palynological studies, such as Wróńska-Pilarek et al. [31, 34, 65, 66] concerning pollen morphology and variability in dozens of species from many genera (e.g. Crataegus L., Rosa L., Rubus L., Salix L., Spiraea L.). They were performed using the same method and they were typically comparable to data cited in the above-mentioned papers given by other palynologists. A similar situation is found in the genus Spiraea.

Conclusions

- The most useful pollen morphological features included exine ornamentation type or subtype determined based on traits of grooves and striae (length, width and course) and perforations (presence or absence, number, diameter). The other biometric features (pollen size and shape, exine thickness) turned out to be useless, as they were similar in all the studied taxa.
The presented results showed that the morphological traits of pollen grains from 25 *Spiraea* taxa made it possible to isolate then individual species, while the other species formed groups of typically 2–3, up to 8 species. The same groups included species both from different and the same sections and series, including related and unrelated ones. Therefore, the pollen features do not fully confirm the classical taxonomic division of the studied genus. Thus, the morphological features of the pollen can be used in the taxonomy of the genus *Spiraea* as auxiliary traits to describe a particular species and taxonomic relations within the examined genus.

The statistical analysis of the studied pollen traits indicated high variability among the tested species. The most variable biometric traits were E and P, while lower variability was observed for the Le/P ratio and Exe.

Pollen of the invasive *S. tomentosa* differed from the other studied taxa in terms of the narrow range of the E values, the largest range of the P/E ratio and the highest number of pollen grains with the thickest exine. Pollen of the other invasive species (*S. douglasi* and *S. japonica*) did not differ significantly from the other studied species.

Supporting information

S1 Table. Complete morphological observations of quantitative features. (PDF)

S1 Fig. LM micrographs of equatorial view of pollen showing colpori and exine ornamentation features (exine thickness). A, *S. alba*; B, *S. betulifolia*; C, *S. cana*; D, *S. chamaedryfolia*; E, *S. chinensis*; F, *S. dasyantha*; G, *S. douglasii*; H, *S. elegans*; I, *S. henryi*; J, *S. hypericifolia*; K, *S. alba* var. *latifolia*; L, *S. media*; M, *S. media*; N, *S. nipponica*; O, *S. pubescens*; P, *S. salicifolia*; Q, *S. splendens*; R, *S. thunbergii*; S, *S. trichocarpa*; T, *S. uratensis*; U, *S. veitchii*; V, *S. wilsonii*; W, *S. ×billardii*; X, *S. ×cinerea*; Y, *S. tomentosa*, A-Y. (PDF)

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