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# Palynological study of Polish taxa of *Potentilla* subsect. Collinae (Rosaceae)

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Pollen grains of *Potentilla* subsect. Collinae Juz., i.e. *P. collina* Wibel, *P. leucopolitana* P.J. Müller, *P. thyrsiflora* Zimmeter, *P. silesiaca* Uechtr. and *P. wimannania* Günther et Schummel were studied with light and scanning electron microscope. Both viable and sterile pollen appeared in all taxa examined. The number of viable pollen ranged from 45.86% in *P. leucopolitana* to 59.95% in *P. wimannania.* The shape of pollen grains varies from prolate (dominating in all taxa studied except *P. collina)* to prolate-spheroidal. A size-diameter of the polar and equatorial axis of 18.3–22.9 × 11.9–17.5 μm was typical of *P. collina* and *P. leucopolitana*; and larger diameter of 23.1–30.6 × 15.8–22.4 μm of *P. thyrsiflora, P. silesiaca* and *P. wimannania*. The pollen is tricolporate with slightly striate ornamentation. Surface sculpture is not a good criterion to identify particular taxa from *Potentilla* subsect. Collinae. Except for *P. leucopolitana*, the pollen of which is characterized by much more marked striation, the pollen ornamentation was similar in all the specimens. Other characters such as the shape of style, the sepal to petal length ratio, the type of leaf division, the leaf pubescence of leaves as well as the sculpture of the fruitmay be valuable taxonomical criteria.

**Keywords**: pollen grains, SEM, LM, *Potentilla*

## Introduction

*Potentilla* subsect. Collinae Juz. includes 20 taxa, most of them (15 taxa) growing in Europe, i.e. *P. alpicola* De la Soie, *P. alsatica* T. Gregor, *P. argenteiformis* Kauffm., *P. aspegrenii* Kurtto, *P. collina* Wibel (= *P. collina* s.str.), *P. johanniniana* Goiran, *P. leucopolitana* P. J. Müller, *P. lindackeri* Tausch, *P. praec*ox F. W. Schultz, *P. rhenana* Zimmeter, *P. silesiaca* Uechtr., *P. thyrsiflora* Zimmeter, *P. wimannania* Günther et Schummel, *P. sterneri* T. Gregor et Karlsson and *P. wismariensis* T. Gregor et Henker. The remaining five taxa, i.e. *P. armeniaca* Siegfr., *P. lazica* Boiss. et Ball., *P. sommieri* Siegfr. et Kell., *P. svanetica* Siegfr. et Kell. and *P. radiata* Lehm. are found in Turkey.

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In Poland five taxa of *Potentilla* subsect. Collinae have been identified, i.e. *P. collina*, *P. thyrsiflora*, *P. silesiaca, P. wimannania* and *P. leucopolitana* (WOLF 1908, SZAFER and PAWŁOWSKI 1955, GERSTBERGER 2002, SZELĄG 2002, KURTTO et al. 2004). However, *P. silesiaca* has not been seen in Poland for more than 100 years. Probably it became extinct as early as the 19$^{th}$ century.

Some taxa in *P.* subsect. Collinae are apomicts, although sexual reproduction also occurs. In addition, they occasionally hybridize with each other and with their parents (MÜNTZING 1928a, ASKER and FRŐST 1970, ASKER 1977, KURTTO et al. 2004). Hybridisation and apomixis result in a diversity of forms, and, hence, *Potentilla* subsect. Collinae Juz., belongs among »critical« and systematically difficult taxa. According to some authors these taxa are intermediate between *P. argentea* L. and *P. tabernaemontani* Ascherson and *P. incana* P. Gaertner, B. Meyer et Scherb. (WOLF 1908, SZAFER and PAWŁOWSKI 1955, GERSTBERGER 2002, KURTTO et al. 2004).

Identification of taxa from *P.* subsect. Collinae is based on the length ratio of sepals to petals, the number of leaflets of basal leaves, the pattern of pubescence of the upper and lower surfaces of leaf blades (ASCHERSON and GRAEBNER 1904, WOLF 1908, JUZEPCZUK 1941, BALL et al. 1968, SOJÁK 1995), as well as on the anatomy of leaves (KOŁODZIEJEK and GABARA 2003) and achene morphology (KOŁODZIEJEK and GABARA 2007).

The pollen grains of the taxa from the genus *Potentilla* L. are tricolporate (REITSMA 1966, ERDTMAN 1969, EIDE 1979, 1981, FAEGRI and IVERSEN 1989, PUNT et al. 1994). In addition to the taxonomic value of the pore structure the variations in the pollen grain sculpture are useful for taxonomic purposes. (REITSMA 1966, FAEGRI and IVERSEN 1989). All these features were used by LEHT (1990) to describe pollen grains of a single taxon from *P.* subsect. *Collinae*, i.e. *P. leucopolitana.* According to the above mentioned study, pollen of this taxon is isopolar, tricolporate with the exine meridionally striped and ranging in size between 17.9–34.6 × 14.5–17.3 μm. These features, however, are not sufficient to distinguish among the taxa of *Potentilla* subsect. Collinae.

The purpose of the present paper was to make a more extensive analysis of vitality, shape and dimensions, as well as surface sculpture of pollen grains of five taxa of *P.* subsect. Collinae in order to find possible taxon-specific features.

## Materials and methods

Pollen grains of 5 taxa, i.e. *Potentilla collina*, *P. leucopolitana*, *P. thyrsiflora*, *P. silesiaca*. and *P. wimannania* were obtained from herbarium sheets as well as from living plant specimens (except *P. silesiaca*) growing in the experimental garden in Łódź. A list of voucher specimens is provided at the end of this paper.

The numbers of fertile and sterile pollen in three newly opened flowers of each *Potentilla* taxon were determined after staining with acetocarmine.

For the scanning electron microscope analysis of shape and dimensions as well as of exine sculpture, pollen grains were mounted on metal stubs, sputtered with technical gold (Pelco S.C 6 coating system), examined and photographed in a Tesla BS 340 microscope. The length of the polar axis (P) and equatorial axis (E) were measured in 30 pollen grains from each taxon (enlarged × 10,000, equatorial view) and the P/E ratio was calculated. Analysis of the pollen grain sculpture was done on the polar area (× 30,000). The data obtained were statistically analyzed with Student's test. A difference was considered statistically significant when $P< 0.01$.

### Specimens investigated

Abbreviations for herbaria after HOLMGREN et al. (1990).

1. *Potentilla collina* Wibel GERMANY: Wertheim, 1799, A. Wibel (LE 2174, type).
2. *P. leucopolitana* P.J. Müller FRANCE: Wissenbourg, 27.5.1857, F. Schultz herbarium normale Cent. 3 no. 256 (BP 165401, type).
3. *P. thyrsiflora* Zimmeter UKRAINE: Kleparów bei Lemberg, 1885, B. BŁocki (BP 167319).
4. *P. silesiaca* Uechtr. POLAND: Flora von Schlesien, Breslau: Hügel Nimkau und Nippern, 28.6.1863, R. Uechtritz (PRA).
5. *P. wimannania* Gűnther et Schummel POLAND: Dolny Śląsk, Scheitnich, Pepelwitz, Friedewalde, 1813, Wimann (LE, type).

## Results

In all taxa of *Potentilla* subsect. *Collinae*, fertile and sterile pollen grains are present. The highest proportion of sterile pollen is observed in *P. leucopolitana* (54.14%) while the lowest is observed in *P. thyrsiflora* (40.05%) (Tab. 1).

**Tab. 1.** Percentage of prolate and prolate-spheroidal pollen and proportion of viable pollen grains in four taxa of *Potentilla* subsect. *Collinae*.

| Taxa | Viable | | |
|---|---|---|---|
| | Prolate | Prolate-spheroidal | Proportion viable |
| *P. collina* | 46.57 | 53.43 | 53.59 |
| *P. leucopolitana* | 61.77 | 38.23 | 45.86 |
| *P. thyrsiflora* | 81.52 | 18.48 | 59.95 |
| *P. wimanniana* | 81.75 | 18.25 | 54.55 |

According to the classification of FAEGRI and IVERSEN (1989) based on the length of the polar (P) and equatorial axes (E) two types of pollen grains can be distinguished in taxa *P.* subsect. C*ollinae*, the first: small, 18.3–22.9 × 11.9–17.5 µm typical for *P. collina* and *P. leucopolitana* and larger, 23.1–30.6 × 15.8–22.4 µm observed in *P. thyrsiflora, P. silesiaca* and *P. wimannania* (Tab. 2). Larger pollen is characteristic of flowers having a diameter of > 1.2 mm, as in *P. thyrsiflora*, while smaller pollen is produced in flowers with diameter < 1.2 mm as observed in *P. collina*.

Two types of viable pollen grains in *P.* subsect. *Collinae* are present: one prolate in shape with P/E ratio from 1.53 in *P. leucopolitana* to 1.92 in *P. collina* and a second prolate-spheroidal with P/E ratio from 1.01 in *P. thyrsiflora* to 1.10 in *P. leucopolitana* (Tab. 2). Prolate pollen generally dominated in *P. wimannania* (81.75%), *P. thyrsiflora* (81.52%), and *P. leucopolitana* (61.77%) and is least abundant in *P. collina* (46.57%). Prolate-spheroidal pollen accounted for about 18% in *P. wimannania* and *P. thyrsiflora* up to 53.43% in *P. collina.*

**Tab. 2.** Measurements of *Potebntilla* subsect. *Collinae* pollen: average ± confidence limit of length of polar (P) and equatorial axes (E) (in µm) and P/E ratio.

| Taxa | Prolate | | | Prolate spheroidal | | |
|---|---|---|---|---|---|---|
| | P | E | P/E | P | E | P/E |
| *P. collina* | 22.9±1.8 | 11.9±0.9 | 1.92±0,15 | 18.3±0.7 | 17.5±1.6 | 1.05±0,12 |
| *P. leucopolitana* | 22.3±1.8 | 14.6±0.8 | 1.53±0,14 | 19.2±1.4 | 17.3±1.2 | 1.10±0,16 |
| *P. thyrsiflora* | 26.9±0.8 | 16.9±0.6 | 1.59±0,10 | 24.4±0.8 | 24.1±1.0 | 1.01±0,11 |
| *P. silesiaca* | 26.5±0.8 | 15.8±0.9 | 1.67±0,16 | 23.1±1.2 | 22.4±2.4 | 1.03±0,15 |
| *P. wimannania* | 30.6±1.3 | 16.8±0.3 | 1.82±0,18 | 24.3±1.6 | 23.9±1.1 | 1.02±0,18 |

No significant differences in the surface of pollen grains of the examined taxa are seen (Figs. A–O). The exine sculpture in all the taxa studied is delicately striate on both the operculum and tectum. The striate ornamentation consisting of elongated muri is separated by grooves. The muri run in different directions in the mesocolpium and in the apocolpium area. In the mesocolpium area the muri are arranged parallel to the major (P) axis, whereas in the apocolpium, they meander, resembling a ball of wool. The colpi in all pollen are covered with thin apertural membranes, the widest occurring in the mesocolpium and gradually narrowing towards the poles.

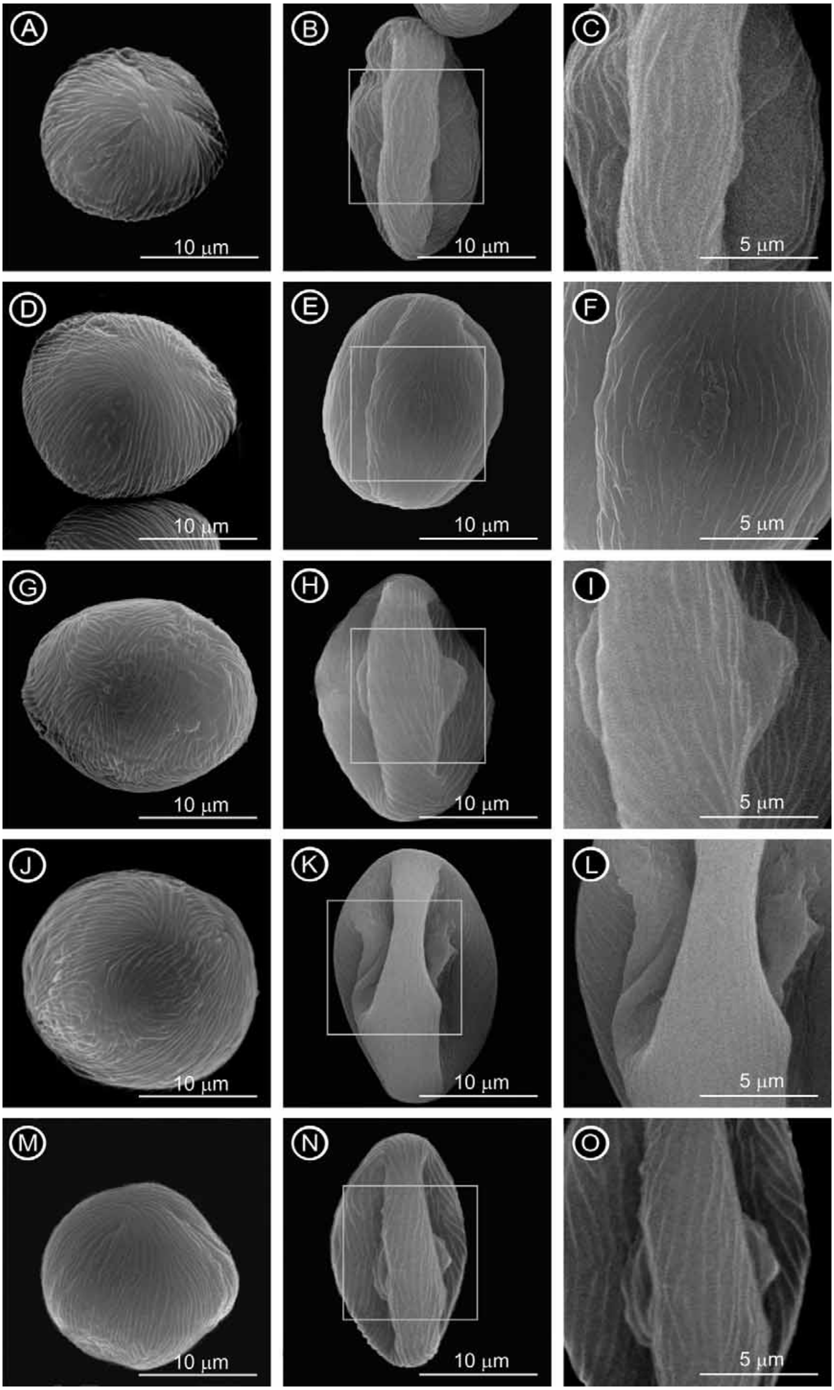
The only differences (Figs. D–F) refer to the depth of the striation. This feature distinguishes *P. leucopolitana* from the other taxa studied here.

## Discussion

According to many authors (MÜNTZING 1928a, KUPRIYANOV and ZHOLOBOVA 1975, OCKENDON and GATES 1976, ASKER and JERLING 1992, CZAPIK 1994) a high number of irregularly shaped and sterile pollen grains is a reliable apomictic indicator. If this is true, pollen sterility from 40.05% in *P. thyrsiflora* to 54.14% in *P. leucopolitana* as observed in the present paper, suggests that apomixis occurs in taxa of *P.* subsect. *Collinae.*

Moreover, environmental factors such as temperature and day length affect pollen viability. For example, JONES and CLARKE (1943) reported a reduction in the number of fertile pollen in plants growing at high glasshouse temperature. On the other hand a temperature of about 25.3 °C enhanced pollen fertility in male sterile and male fertile lines (BARHAM and MUNGER 1950). Furthermore, this effect was strongest 12 days before anthesis, coinciding with the time of break-up of the pollen tetrads. Since the plants used in the present studies bloomed during the second week of July 2005 when the mean day temperature was 25.5–28.5 °C, the abundance of sterile pollen, which was observed in taxa of *P.* subsect. *Collinae,* is consistent with the observation of JONES and CLARKE (1943).

There are only few data concerning the size of pollen grains in the taxa of *P.* subsect. *Collinae*. According to LEHT (1990), pollen grain dimensions of *P. leucopolitana* were in the range of 17.9–34.6 × 14.5–17.3 µm, while those described by us were larger, i.e., 19.2–22,3 × 14.6–22.3 µm. The analysis of the pollen size and P/E ratio (Tab. 2) revealed the lack of clear differences among taxa of the *P.* subsect. *Collinae*. although it allowed us to differentiate two subgroups: the first including *P. collina* and *P. leucopolitana*, with pollen size between 18.3–22.9 × 11.9–17.5 µm and the second including *P. thyrsiflora*, *P. silesiaca* and *P. wimannania* having a pollen size of 23.1–30.6 × 15.8–22.4 µm.




**Figs.** **A–O.** Surface sculpture of the pollen grains of *Potentilla* subsect. Collinae Juz. **A–C.** – *Potentilla collina* Wibel.; **D–F** – *P. leucopolitana* P. J. Müller; **G–I** – *P. thyrsiflora* Zimmeter; **J–L** – *P. silesiaca* Uechtr.; **M–O** – *P. wimannania* Gunther et Schummel.

It is well known that pollen grain dimensions in many genera are correlated with chromosome number (MÜNTZING 1928b, KAPADIA and GOULD 1964, KATSIOTIS and FORSBERG 1995, ANCHEV and DENEVA 1997). However, SÁNCHEZ AGUDO et al. (1998) showed in P. *neumanniana* Rchb., *P. pyreneica* Ramond ex DC. and *P. argentea* that the size of pollen grains was not strictly correlated with ploidy level. Similarly, no correlation was observed between pollen size and chromosome number in the taxa of *P.* subsect. *Collinae*. *Potentilla thyrsiflora* and *P. wimannania* with ploidy level 2n = 21–35 produced pollen 24.4–26.9 µm and 24.3–30.6 µm in diameter, respectively, while *P. collina* with 2n = 28, 35 produced smaller pollen, 15.1–17.0 µm in diameter (ILNICKI and KOŁODZIEJEK 2008).

The results of the present paper demonstrated that surface sculpture is not a good criterion for the identification of particular taxa from *Potentilla* subsect. *Collinae.* Except for *P. leucopolitana*, the pollen of which is characterized by much more marked striation (Figs. D–F), pollen ornamentation was similar in all the specimens. Other characters such as the shape of style, the sepal to petal length ratio, the type of leaf division, the leaf pubescence of leaves as well as the sculpture of the fruit, may be valuable taxonomical criteria. on

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