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УДК 581.331.2:582.973

DOI 10.37555/2707-3114.1.2021.247735

## Morphology of pollen grains of *Weigela floribunda* (Caprifoliaceae): application in taxonomy

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## Морфологія пилоквих зерен *Weigela floribunda* (Caprifoliaceae): використання у таксономії

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**Abstract.** The aim of this research was to provide detailed quantitative and qualitative characteristics on pollen grains of *Weigela floribunda* (Siebold & Zucc.) K. Koch (Caprifoliaceae) as applied for taxonomy. Pollen morphology of *W. floribunda* was studied using both light microscopy and scanning electron microscopy. Pollen grains are 3-porate, rarely 4-porate, suboblate to spheroidal ( $P/E=0.80-1.05$ ); in equatorial view elliptic or circular, in polar view circular or subcircular; medium- or large-sized ( $P=42.56-61.18 \mu\text{m}$ ,  $E=42.56-66.50 \mu\text{m}$ ). Pores are circular with distinct or indistinct margins and distinct annuli. Exine sculpture is echinate-microechinate-nanoechinate. Pollen grains of *W. floribunda* were analyzed for the first time in the present study.

**Key words:** palynomorphology, pollen type, exine sculpture, taxonomy.

**Анотація.** Метою дослідження було надати детальну кількісну та якісну характеристику пилоквих зерен *Weigela floribunda* (Siebold & Zucc.) K. Koch (Caprifoliaceae) для цілей систематики. Морфологію пилку *W. floribunda* вивчали за допомогою світлової та сканувальної електронної мікроскопії. Пилкові зерна 3-порові, зрідка 4-порові, сплющено-сфероїдальні або сфероїдальні ( $P/E=0.80-1.05$ ) за формою; в обрисі з екватора еліптичні або округлі, в обрисі з полюса округлі; середніх або великих розмірів ( $P=42.56-61.18 \mu\text{m}$ ,  $E=42.56-66.50 \mu\text{m}$ ). Пори округлі, з чіткими або нечіткими краями та чітким обідком. Скульптура екзини шипувато-шипикувато-наношипикувата. Пилкові зерна *W. floribunda* вперше проаналізовані у цьому дослідженні.

**Ключові слова:** паліноморфологія, тип пилку, скульптура екзини, таксономія

**Introduction.** Recent molecular studies clarified considerably the phylogenetic relationships within the order Dipsacales (Bell et al., 2001; Donoghue et al., 2001; Zhang et al., 2003; Theis et al., 2008; Winkworth et al., 2008; Jacobs et al., 2011; Wang et al., 2015; Xiang et al., 2020). The family *Caprifoliaceae* s.l., as currently circumscribed in a wide sense, contains representatives of several other previously recognized families of *Dipsacales*, such as *Valerianaceae*, *Dipsacaceae*, *Morinaceae*, *Diervillaceae*, etc. (APG III, 2009; APG IV, 2016). According to new molecular phylogenetic data, the genus *Weigela* Thunb., together with the genus *Diervilla* Tourn. ex Mill., forms the clade of *Diervilloideae*, which is sister to the clade containing representatives of *Caprifolioideae* (Wang et al., 2021).

*Weigela* includes ca. 10 species native in North-Central, South-Central and Southeast China, with Inner Mongolia and Manchuria; Japan; Korea; Khabarovsk Region, Kuril Islands, Primorye, and Sakhalin of Russia (Barbarych, 1961; IPNI, 2021); the species *W. floribunda* (Siebold & Zucc.) K. Koch grows in Japan. In Ukraine it is cultivated in gardens and parks as a popular ornamental shrub (Barbarych, 1961; Mosyakin, Fedoronchuk, 1999).

It is well known that morphological characters of pollen grains as additional diagnostic features are often efficiently used for taxonomy (Jacobs et al., 2011; Mosyakin, Tsymbalyuk, 2015, 2017; Tsymbalyuk et al., 2018, 2019a, b, 2020, 2021; Dirmenci et al., 2019, etc.). However, the available information about pollen grains of representatives of the genus *Weigela* is limited. Donoghue (1985) investigated pollen grains of *W. florida* (Bunge) A. DC. using both light microscopy and scanning electron microscopy. Jacobs et al. (2011) investigated pollen grains of three species (*W. coraeensis* Thunb., *W. decora* (Nakai) Nakai, *W. Maximowiczii* (S. Moore) Rehder) of the genus *Weigela* using scanning electron microscopy; however, the photomicrographs are absent in that publication. The PalDat online database contains some information on pollen grains and photomicrographs of *W. floribunda* using light microscopy (Auer, 2021).

The aim of the present research was to provide detailed quantitative and qualitative characteristics of the pollen grains of *W. floribunda* and to evaluate the importance of these data for taxonomy.

**Materials and methods.** Pollen grains of *W. floribunda* were sampled at the National Herbarium of Ukraine (KW – Herbarium of the M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv, Ukraine). Herbarium acronyms are given following *Index Herbariorum* (Thiers, 2008–onward, continuously updated). Abbreviations of names of taxon authors mainly follow Brummitt, Powell (1992), with additions and updates available from IPNI (2021).

In general, the methods used in the present study were the same as those we used earlier (Tsymbalyuk et al., 2020, 2021). Pollen grains were studied using both light microscopy (LM) and scanning electron microscopy (SEM). For LM studies (Biolar,  $\times 700$ ), the pollen was acetolyzed following the method by Erdtman (1952), mounted on slides with glycerinated gelatin, and analyzed and photomicrographed using a light microscope. For size determinations, 30 measurements were taken along the polar axis (P) and equatorial diameter (E); 20 measurements of the exine and annulus thickness, width and length of pores were performed. The P/E ratio was calculated in order to determine the pollen shape. For all quantitative characters, descriptive statistics was applied and the range (minimum and maximum values), arithmetic mean and standard deviation were calculated. The number of echini/microechini/nanoechini per unit area ( $100 \mu\text{m}^2$ ) was determined. The slides are deposited in the Palynotheca (reference pollen collection) at the National Herbarium of Ukraine (Bezusko, Tsymbalyuk, 2011).

For SEM (JEOL JSM-6060LA), dry pollen grains were treated with 96%-ethanol, then samples were sputter-coated with gold and investigated at the Center of Electron Microscopy of the M. G. Kholodny Institute of Botany. The measurements of the echini, microechini and nanoechini were taken on five pollen grains from SEM micrographs and made using the program AxioVision Rel. 4.8.2. Terminology used in descriptions of pollen grains follows the glossaries by Punt et al. (2007) and Halbritter et al. (2018).

**Results and discussion.** The original data on quantitative pollen characters are summarized in Table. LM and SEM photomicrographs of pollen grains are shown in Figures 1 and 2. Description of pollen grains of *W. floribunda*.

**LM.** Pollen grains 3-porate, rarely 4-porate, suboblate to spheroidal, in equatorial view elliptic or circular, in polar view circular or subcircular. Pores circular, rarely elliptic, with distinct or indistinct margins, and distinct annuli, pore membrane psilate. Exine very thin, undifferentiated into layers. Columellae absent. Exine sculpture echinate-microechinate-nanoechinate.

**SEM.** Exine sculpture echinate-microechinate-nanoechinate. Echini cylindrical, 1.32–2.64  $\mu\text{m}$  high, 0.96–1.62  $\mu\text{m}$  wide at base, with straight sides and acute apices, echini higher than basal width, (2–5/100  $\mu\text{m}^2$ ). Echini with convex sides, acute or obtuse apices, 1.02–1.61  $\mu\text{m}$  high, 0.95–1.48  $\mu\text{m}$  wide at base, echini higher than basal width, (2–3/100  $\mu\text{m}^2$ ); microechini with convex sides and obtuse apices, 0.76–0.82  $\mu\text{m}$  high, 0.74–0.98  $\mu\text{m}$  wide at base, about as broad as height (3–5/100  $\mu\text{m}^2$ ); nanoechini with convex sides and obtuse apices, 0.38–0.59  $\mu\text{m}$  high, 0.67–0.81  $\mu\text{m}$  wide at base, broader than high (1–5/100  $\mu\text{m}^2$ ); tectum psilate-granulate in area between echini. Pore membranes granulate.

**Specimen investigated:** Hort. Bot. Jurjevensis [now Tartu, Estonia]. Japonia (Culta) fl. 10.IV 1886. Teste: Prof. N. Kusnezow et F.F. Wagner (KW).

**Table. Pollen morphometric characters: mean  $\pm$  standard deviation; range (min-max) (all measurements given as  $\mu\text{m}$ ).**

Characters	Measurements
Polar axis	49.61 $\pm$ 4.79 (42.56–61.18)
Equatorial diameter	52.48 $\pm$ 6.94 (42.56–66.50)
P/E ratio	0.95 $\pm$ 0.06 (0.80–1.05)
Pore length	6.56 $\pm$ 1.19 (3.99–9.31)
Pore width	5.90 $\pm$ 0.93 (3.99–6.65)
Annulus thickness	2.33 $\pm$ 0.50 (1.33–3.32)
Exine thickness	1.22 $\pm$ 0.32 (0.66–1.99)

The obtained data demonstrated that pollen grains of *W. floribunda* have a porate type of apertures. A characteristic feature of pollen grains of this species is also a very thin exine undifferentiated into layers, as well as the absence of columellae. The exine sculpture is represented by echini of two types: (1) echini with a cylindrical, parallel-sided basal part with a conical acute apex; the basal part is normally longer than the apex, height of echini exceeding their basal width; and (2) echini with a more or less circular basal part, with an obtuse or rarely acute apex; height of echini exceeding their basal width. Microechini about as broad as high, nanoechini broader than high.

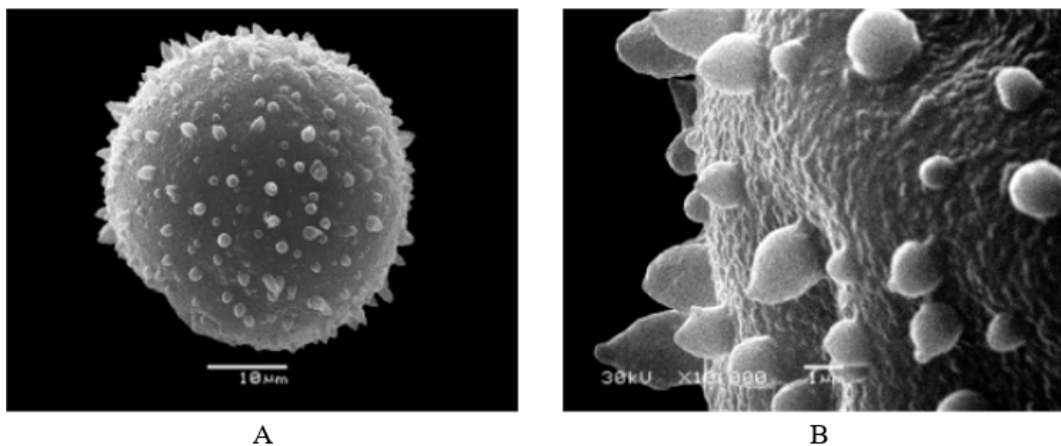


Fig. 1. Pollen grains of *W. floribunda* (SEM): A – equatorial view, B – exine sculpture.

The pore type of apertures and echinate sculpture are characteristic of pollen grains of the genera *Knautia* L., *Dipsacus* L., *Cephalaria* Schrad. (Tsybalyuk et al., 2018, 2019 a, b, 2020). However, pollen grains of *W. floribunda* are morphologically different from pollen grains of other studied species. The pollen characteristics described here may be used in future studies aiming at completing the knowledge on all *Weigela* species and at understanding the evolution of pollen morphology in *Caprifoliaceae* in general.

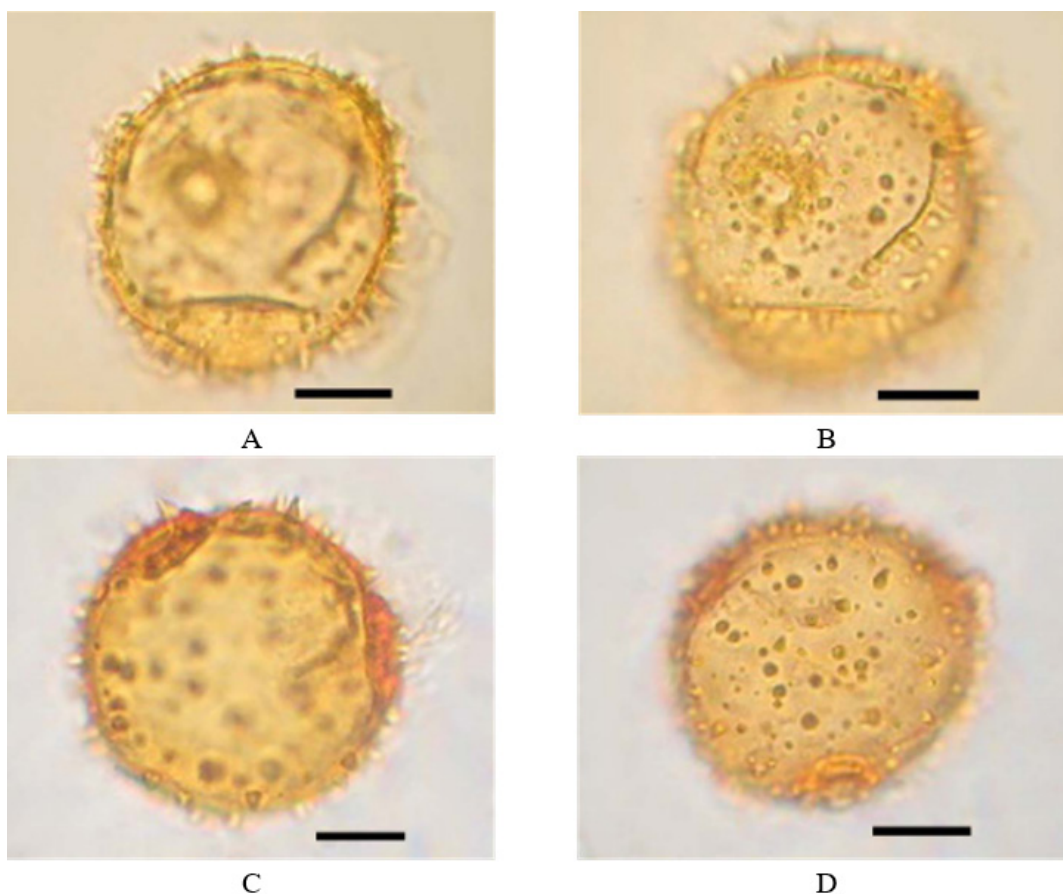


Fig. 2. Pollen grains of *W. floribunda* (LM): A, B — equatorial view, C, D — polar view. Scale bars = 10  $\mu$ m.

### **Acknowledgements**

The authors express their gratitude to Natalia M. Shyian, Head Curator of the National Herbarium of Ukraine (KW; herbarium of the M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine). Kind help and cooperation of Dmytro O. Klymchuk, Head of the Centre of Electron Microscopy (M. G. Kholodny Institute of Botany), is greatly appreciated.

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