Palynomorphological peculiarities of representatives of tribes Lindenbergieae and Cymbarieae and pollen evolution in early-branching lineages of Orobanchaceae

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Abstract. Pollen morphology of four species belonging to four genera of the tribe Cymbarieae and two species of Lindenbergia of the tribe Lindenbergieae (Orobanchaceae) was studied using light and scanning electron microscopy. Pollen grains in Lindenbergieae are 3-colporate (rarely 2-colporate), prolate, spheroidal and oblate-spheroidal, small-sized, with reticulate exine sculpture. Pollen grains in Cymbarieae are 3-colpate, rarely 4-colpate, prolate, spheroidal and oblate-spheroidal, medium-sized. Exine sculpture in Cymbarieae is reticulate and rugulate-reticulate. Pollen characters of Cymbarieae and Lindenbergieae are compared with pollen patterns in Paulowniaceae and crown clades of Orobanchaceae. The earliest-branching clade of Orobanchaceae (Lindenbergieae) is palynomorphologically similar to Paulowniaceae (the clade sister to all Orobanchaceae) and to Wightia. These palynomorphological findings confirm the phylogenetic patterns recently revealed in basal Orobanchaceae and their closest relatives. It is concluded that the colporate (most probably 3-colporate) type of pollen grains could be ancestral in Orobanchaceae. Pollen diversity in crown clades of Orobanchaceae evolved on the base of a few pollen types and subtypes, which were peculiar to hypothetical ancestors of Orobanchaceae and are probably preserved in the extant taxa of Paulowniaceae and Lindenbergieae.

Keywords: Cymbarieae, Lindenbergieae, Orobanchaceae, pollen evolution, pollen morphology, phylogeny, taxonomy

Introduction

The tribe Cymbarieae D. Don (Orobanchaceae Vént.) in its current circumscription includes six or seven genera and ca. 14 species of root hemiparasites (Fischer, 2004; Bennett, Mathews, 2006; Reveale, 2012; McNeal et al., 2013; Olmstead, 2016). The genera currently included in the tribe are Bungea C.A. Mey. (2 species, southwestern and Central Asia and China), Cymbaria L. sensu lato (4 species, southeastern Europe, Central and eastern Asia; many authors also include here Cymbochasma (Endl.) Klokov & Zoz), Lesqueruexia Boiss. (1 species, L. syriaca Boiss. & Reut. = Siphonostegia syriaca (Boiss. & Reut.) Boiss., Eastern Mediterranean; most probably nested in Siphonostegia; see McNeal et al., 2013), Monochasma Maxim. ex Franch. & Sav. (4 species, East Asia), Schwalbea L. (1 species, S. americana L., eastern North America), and Siphonostegia Benth. (2 species: S. chinesis Benth. and S. laeta S. Moore, East Asia; or 3 species, if S. syriaca is included) (Fischer, 2004; Bennett, Mathews, 2006; Takhtajan, 2009; McNeal et al., 2013; Olmstead, 2016).

The genus Lindenbergia Lehman is represented by non-parasitic taxa and includes 12–15 species occurring mostly in tropical regions, from northeastern Africa through southern and southeastern Asia (including the Himalayas) to Philippines (Mabberley, 1997; Stevens, 2001–onwards; Fischer, 2004; Olmstead, 2016).

In earlier variants of the system of A.L. Takhtajan (1987, 1997) the mentioned genera Schwalbea, Bungea, Siphonostegia, Cymbaria, and Monochasma were included (with many other genera) in the tribe Rhinantheae Lam. & DC. of Scrophulariaceae subfam. Rhinantheoideae. In the latest version of his system, Takhtajan (2009) placed the genera Schwalbea, Cymbaria (including Cymbochasma), Siphonostegia, Lesqueruexia, Bungea, Monochasma in the tribe Cymbarieae (also with some other genera), and their familial and subfamilial placement remained the same. Fischer (2004) included in Cymbarieae six genera: Schwalbea, Cymbaria (including Cymbochasma), Siphonostegia, Lesqueruexia, Bungea, and Monochasma.

Takhtajan (1987, 1997) initially placed Lindenbergia in Scrophulariaceae subfam. Scrophularioideae trib. Gratioleae Benth. In 2009 he moved that genus to the...

Many authors studied and discussed pollen morphology of representatives of *Scrophulariaceae* and *Orobanchaceae* in general (Minkin, Eshbaugh, 1989), or specifically *Rhinantheae* (Inceoğlu, 1982; Lu et al., 2007; Tsymbalyuk, Mosyakin, 2017), and its taxonomic and evolutionary significance (see also Tsymbalyuk, Mosyakin, 2013a, b; Mosyakin, Tsymbalyuk, 2015a, b, 2017 and references therein). However, there are only a few publications reporting data on pollen of taxa now placed in *Cymbariaeae*; these publications provide some data based on light and scanning electron microscopy (Inceoğlu, 1982; Minkin, Eshbaugh, 1989; Lu et al., 2007). Pollen grains of two species (*Bungea trifida* and *Lesquereuxia syriaca*) currently placed in *Cymbariaeae* were also studied using transmission electron microscopy (Inceoğlu, 1982). However, no comprehensive analysis of palynomorphological peculiarities of *Cymbariaeae* in its updated phylogeny-based circumscription has been done until now.

Prijanto (1969) provided the following information about pollen grains of *Lindenber gia*: "Pollen grains small, 3-colporate, rarely 2-colporate, oblate spheroidal to prolate spheroidal (polar axis 12–19 µm long, equatorial diameter 13–18 µm; apocolpium diameter 3–5 µm; exine about 0.8–1.5 µm thick, sexine as thick as nexine, reticulate". However, no images of pollen grains were published by Prijanto (1969). Hjertson (1995) updated the information of Prijanto (1969) and provided SEM images of pollen grains of *L. muraria* (Roxb. ex D. Don) Brüthl and *L. grandiflora* (Buch.-Ham. ex D. Don) Benth. He concluded that "pollen grains in *Lindenber gia* are tricolporate, oblate to prolate in shape, with a 12–19 µm long polar axis and an equatorial diameter of 13–18 µm. The exine as well as the sexine is about 1 µm thick, and the surface is reticulate".

Molecular phylogenetic evidence indicate that *Cymbariaeae* is one of the early-branching (basal) lineages of *Orobanchaceae*, most probably its second-branching clade, following the basalmost clade of *Lindenber gia* (*Orobanchaceae* trib. *Lindenber giaeae* (= *Lindenber giaeae*)) (McNeal et al., 2013; Cusimano, Wicke, 2016; Schneider et al., 2016; Schneider, Moore, 2017, etc.). According to molecular phylogenetic studies (Bennett, Mathews, 2006; McNeal et al., 2013), the clade of *Cymbariaeae* is subdivided into two subclades: one of these clades includes *Bungea, Cymbaria* (including *Cymbochasma*), and *Monochasma*; the other houses *Siphonostegia* (including *Lesquereuxia*) and *Schwalbea*.

Our earlier studies and analysis of pollen morphology in basal clades of *Scrophulariaceae* sensu stricto allowed us to outline the main trends of morphological pollen evolution in the family in its new circumscription and to hypothesize on possible ancestral pollen types in the group (Mosyakin, Tsymbalyuk, 2015, 2017). Because of that, we may expect that a comprehensive analysis of pollen morphology in early-branching clades of *Orobanchaceae* could bring comparable results and shed light on main trends of pollen evolution in that group as well.

The purpose of the present research was to study and analyze the morphological features of pollen grains of representatives of *Cymbariaeae* and *Lindenber giaeae* in the updated taxonomic circumscriptions of these tribes, and to compare the pollen patterns with existing systems and molecular phylogenetic data.

**Materials and methods**

Pollen of four species belonging to four genera of *Cymbariaeae* (*Bungea, Cymbaria, Cymbochasma*, and *Siphonostegia*) was sampled in the National Herbarium of Ukraine (KW – herbarium of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv, Ukraine). Pollen grains of two species of *Lindenber gia* were sampled in the herbarium of the Missouri Botanical Garden (MO; St. Louis, Missouri, USA). Data of the studied specimens are cited exactly according to the label information, in English translation and in original languages.

In general, the methods used in the present study are essentially the same as those we used earlier (see Mosyakin, Tsymbalyuk, 2015a, b, 2017). Pollen morphology was studied using light microscopy and scanning electron microscopy. For light microscopy studies (LM, Biolar, × 700), the pollen was acetolyzed following Erdtman (1952). For size determinations, 20 measurements were taken along the polar (P) and equatorial (E) axes for each species. For scanning electron microscopy (SEM, JSM-6060LA), pollen grains were treated with 96%-ethanol, then these samples were sputter-coated with gold and investigated at the Center of Electron Microscopy of the...
General description of pollen grains

**Lindenbergeae**

Pollen grains in monads, radially symmetrical, isopolar, 3-colporate (rarely 2-colporate, according to Prijanto, 1969), prolate, spheroidal or oblate-spheroidal; mainly small-sized: \( P = 13.3–18.6 \) \( \mu m \) (12–19 \( \mu m \), according to Prijanto, 1969), \( E = 13.3–18.6 \) \( \mu m \). Outline in polar view 3-lobate, in equatorial view elliptical or circular. Colpi long, narrow, 1.3–2.7 \( \mu m \) wide, with mainly indistinct (sometimes distinct) more or less strict margins, with acute ends. Endoapertures mainly indistinct, circular, 2.4–2.7 \( \mu m \) long and wide. Exine 0.7–1.6 \( \mu m \). Tectum nearly equal to infratectum. Columellae indistinct or distinct, thin, or exine layers invisible. Exine sculpture reticulate. Colpus membrane smooth.

**Cymbarieae**

Pollen grains in monads, radially symmetrical, isopolar, mainly 3-colporate, rarely 4-colporate, prolate, spheroidal, or oblate-spheroidal. The outlines in equatorial view elliptical or circular, in polar view sub-circular, circular, and sub-triangular; medium- or large-sized: \( P = 22.6–47.9 \) \( \mu m \) (up to 50 \( \mu m \), according to Lu et al., 2007), \( E = 25.3–43.9 \) \( \mu m \). Colpi long or medium-length, narrow, medium-width to wide, 1.3–6.6 \( \mu m \) wide, with indistinct, uneven margins, and blunt, acute, or indistinct ends. Exine 0.7–3.3 \( \mu m \). Tectum nearly equal to infratectum, columellae mainly indistinct or thin and arranged more or less regularly. Exine sculpture reticulate, rarely rugulate-reticulate. Capita (pila heads) arranged in circle in most or all regions of pollen surface. Caput of pila 0.16–0.67 \( \mu m \) (up to 0.90 \( \mu m \), according to Lu et al., 2007). Colpus membrane granulate.

Pollen types and subtypes

Based on their aperture types, pollen grains of the studied species belong to the same basic pollen types. The second type (3-colpate) contains four subtypes segregated according to their exine sculpture, pollen size, details of colpi, and thickness of the exine.

**Type I — 3-colporate**

Sculpture reticulate.
\( P = 13.3–18.6 \) \( \mu m \), \( E = 13.3–18.6 \) \( \mu m \).
Colpi long, narrow, 1.3–2.7 \( \mu m \) wide, with acute ends.
Endoapertures mainly indistinct, circular, 2.4–2.7 \( \mu m \) long and wide.
Exine 0.7–1.6 \( \mu m \). *Lindenbergia philippensis*, L. sinaica.

**Type II — 3-colpate**

**Subtype 1.** Sculpture retipilate. Caput of pila 0.17–0.33 \( \mu m \).
\( P = 38.6–47.9 \) \( \mu m \), \( E = 34.6–43.9 \) \( \mu m \).
Colpi long, medium-width, 2.4–3.3 \( \mu m \) wide, ends acute.
Exine 1.3–3.3 \( \mu m \). *Bungea trifida*.

**Subtype 2.** Sculpture retipilate and rugulate-retipilate. Caput of pila 0.41–0.67 \( \mu m \).
\( P = 37.2–47.9 \) \( \mu m \), \( E = 33.2–42.6 \) \( \mu m \).
Colpi medium-length, wide, 4.0–6.6 \( \mu m \) wide, ends blunt, sometimes acute.
Exine 1.6–2.7 \( \mu m \). *Cymbaria dahurica*.

**Subtype 3.** Sculpture retipilate. Caput of pila 0.17–0.25 \( \mu m \).
\( P = 29.3–33.2 \) \( \mu m \), \( E = 30.6–35.9 \) \( \mu m \).
Colpi medium-length, medium-width, 2.7–5.3 \( \mu m \) wide, ends blunt, indistinct.
Exine 0.7–1.3 \( \mu m \). *Cymbochasma boryschenica*.

**Subtype 4.** Sculpture retipilate. Caput of pila 0.16–0.25 \( \mu m \).
\( P = 22.6–29.3 \) \( \mu m \), \( E = 25.3–29.3 \) \( \mu m \).
Colpi long, narrow, 1.3–2.4 \( \mu m \) wide, ends blunt.
Exine 1.3–2.4 \( \mu m \). *Siphonostegia chinensis*.

Descriptions of pollen grains

**Genus Lindenberge Lehm.**

*Lindenbergia philippensis* (Cham.) Benth. (Fig. 1, a–c; Fig. 4, a–d)

LM. Pollen grains 3-colporate, oblate-spheroidal and spheroidal, occasionally prolate, in polar view 3-lobate, in equatorial view elliptical and circular. \( P = 13.3–17.3 \) \( \mu m \), \( E = 14.6–18.6 \) \( \mu m \). Colpi long, narrow, 1.3–2.7 \( \mu m \) wide, with indistinct (some distinct) more or less strict margins, tapering to acute ends; colpus membrane smooth. Endoapertures indistinct, circular, 2.4–2.7 \( \mu m \) wide, 2.4–2.7 \( \mu m \) long. Mesocolpium = 9.3–10.6 \( \mu m \), apocolpium = 2.7–4.0 (5.3) \( \mu m \). Exine 1.1–1.6 \( \mu m \) thick. Tectum nearly equal to infratectum. Columellae indistinct or distinct, thin. Exine sculpture indistinct or distinct, microreticulate.
SEM. Sculpture reticulate. Colpus membrane smooth.

**Specimen investigated:** Philippines; Mountain Province; Bontoc Municipality; Bontoc territory; Caluttit; 17°05' N, 120°58' E; 1000 m elev. 17 November 1982. C.C. Bodner. No 131 (MO).

**Lindenbergia sinaica** Benth. (Fig. 1, d–f; Fig. 4, e–h)

LM. Pollen grains 3-colporate, prolate and spheroidal, occasionally oblate-spheroidal, in polar view 3-lobate, in equatorial view elliptical and circular. P = 14.6–18.6 µm, E = 13.3–17.3 µm. Colpi long, narrow, 2.0–2.4 µm wide, with indistinct (occasionally some distinct) more or less strict margins, tapering to acute ends; colpus membrane smooth. Endoapertures indistinct, more or less circular, 2.7 µm wide, 2.4–2.7 µm long. Mesocolpium = 7.9–10.6 µm, apocolpium = 4.0 µm. Exine 0.7–1.1 µm thick, exine layers invisible. Exine sculpture indistinct, foveolate.

SEM. Sculpture reticulate. Colpus membrane smooth.

**Specimen investigated:** [Ethiopia]. About 150 km. SW of Assab [Eritrea], along road to Kombolcha [Ethiopia]. Alt.: 400 m. Dry, rocky slope. 18.1 1966. W. de Wilde. No 9771 (MO).

**Genus Buncea C.A. Mey.**

**Buncea trifida** (Vahl) C.A. Mey. (Fig. 2, a–c; Fig. 5, a–d)

LM. Pollen grains 3-colpate, prolate and occasionally spheroidal, in polar view sub-triangular and/or circular, in equatorial view elliptical and circular. P = 38.6–47.9 µm, E = 34.6–43.9 µm. Colpi long, medium-width, 2.4–3.3 µm wide, with indistinct, uneven margins, tapering to more or less acute or indistinct ends; colpus membrane granulate. Mesocolpium = 26.6–34.6 µm, apocolpium = 6.6–13.3 µm. Exine 1.3–3.3 µm thick. Tectum nearly equal to infratectum. Columellae invisible. Exine sculpture indistinct, microreticulate.

SEM. Sculpture reticulate. Caput of pila 0.17–0.33 µm. Colpus membrane granulate.


**Genus Cymbaria L.**

**Cymbaria dahurica** L. (Fig. 2, d–f; Fig. 5, e–h)

LM. Pollen grains 3-colpate, prolate and occasionally oblate-spheroidal, in polar view sub-triangular, in equatorial view elliptical. P = 37.2–47.9 µm, E = 33.2–42.6 µm. Colpi medium-length, 4.0–6.6 µm wide, with indistinct, uneven margins, tapering to blunt, sometimes acute ends, colpus membrane smooth and granulate. Mesocolpium = 26.6–33.2 µm, apocolpium = 10.6–19.9 µm. Exine 1.6–2.7 µm thick. Tectum nearly equal to infratectum. Columellae indistinct. Exine sculpture indistinct, sometimes distinct, microreticulate.

SEM. Sculpture reticulate and rugulate-retipilate. Caput of pila 0.41–0.67 µm. Colpus membrane granulate.


**Genus Cymboschasma (Endl.) Klokov & Zoz**

**Cymboschasma borythesthica** (Pall. ex Schlecht.) Klokov & Zoz (Cymbaria borythesthica Pall. ex Schlecht.) (Fig. 3, a–c; Fig. 5, i–l)

LM. Pollen grains 3-colpate, occasionally 4-colpate, oblate-spheroidal, in polar view sub-circular, in equatorial view elliptical. P = 29.3–33.2 µm, E = 30.6–35.9 µm. Colpi medium-length, medium-width, 2.7–5.3 µm wide, with indistinct, uneven margins, tapering to blunt, indistinct ends, colpus membrane smooth and granulate. Mesocolpium = 22.6–26.6 µm, apocolpium = 6.6–9.3 µm. Exine 0.7–1.3 µm thick. Tectum nearly equal to infratectum. Columellae indistinct. Exine sculpture indistinct, sometimes distinct, microreticulate.

SEM. Sculpture retipilate. Caput of pila 0.17–0.25 µm. Colpus membrane granulate.
Fig. 1. Pollen grains of *Lindenber gia* (scanning electron microscopy): *a*–*c* – *L. philippensis*; *d*–*f* – *L. sinaica*; *a* – polar and equatorial view; *e* – equatorial view; *b*–*d*, *f* – reticulate exine sculpture.
Fig. 2. Pollen grains of *Bunaea* and *Cymbaria* (scanning electron microscopy): *a–c* — *B. trifida*; *d–f* — *C. dahurica*; *a, e* — equatorial view; *b, c* — retipilate exine sculpture; *d, f* — rugulate-retipilate exine sculpture
Fig. 3. Pollen grains of *Cymbochasma* and *Siphonostegia* (scanning electron microscopy): *a–c* – *C. borystenica*; *d–f* – *S. chinensis*; *a, e* – equatorial view; *b–d, f* – retipilate exine sculpture
**Specimens investigated:**


**Genus Siphonostegia Benth.**

*Siphonostegia chinensis* Benth. (Fig. 3, d–f; Fig. 5, m–p)

LM. Pollen grains 3-colpate, oblate-spheroidal, spheroidal, occasionally prolate, in polar view circular, in equatorial view circular. \( P = 22.6–29.3 \mu m \), \( E = 25.3–29.3 \mu m \). Colpi long, narrow, 1.3–2.4 \( \mu m \) wide, with indistinct, uneven margins, tapering to blunt ends, colpus membrane granulate. Mesocolpium = 18.6–22.6 \( \mu m \), apocolpium = 5.3–9.3 \( \mu m \). Exine 1.3–2.4 \( \mu m \) thick. Tectum nearly equal to infratectum. Columellae distinct, thin, arranged more or less regularly. Exine sculpture distinct, microreticulate.

SEM. Sculpture retipilate. Caput of pila 0.16–0.25 \( \mu m \). Colpus membrane granulate.


Original and published data on quantitative and qualitative pollen characters of representative of *Cymbarieae* and *Lindenbergieae* are summarized in Tables 1 and 2.

**Comparative pollen morphology of genera of *Cymbarieae* and *Lindenbergieae***

In general, our data are in good agreement with the results of previous studies (İnceoğlu, 1982; Minkin, Eshbaugh, 1989; Lu et al., 2007). Analysis of our original palynomorphological data and literature records demonstrated that pollen grains of representatives of all genera of *Cymbarieae* are characterized by the 3-colpate type of apertures, mainly with retipilate sculpture (Table 2).
Fig. 5. Pollen grains of Bungea, Cymbaria, Cymbochasma, and Siphonostegia (light microscopy): a–d — B. trifida; e–h — C. dahurica; i–l — C. borysstenica; m–p — S. chinensis. a, b, e, f, i, j, m, n — equatorial view; c, d, g, h, k, l, o, p — polar view. Scale bars: 10 µm.
Table 1. Summary of pollen morphometric features

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Polar axis (µm)</th>
<th>Equatorial axis (µm)</th>
<th>Colpi width (µm)</th>
<th>Mesocolpium (µm)</th>
<th>Apocolpium (µm)</th>
<th>Exine (µm)</th>
<th>Reference</th>
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<tr>
<td>Lindenberga philippensis</td>
<td>13.3–17.3</td>
<td>14.6–18.6</td>
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<td>9.3–10.6</td>
<td>2.7–4.0 (5.3)</td>
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<td>7.9–10.6</td>
<td>3.3–4.0</td>
<td>0.7–1.1</td>
<td>original data</td>
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<td>34.6–43.9</td>
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<td>26.6–34.6</td>
<td>6.6–13.3</td>
<td>1.3–3.3</td>
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<td>39</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td>1.1</td>
<td>Inceoğlu, 1982</td>
</tr>
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<td>–</td>
<td>–</td>
<td>Lu et al., 2007</td>
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<td>–</td>
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<td>–</td>
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<td>Inceoğlu, 1982</td>
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<td>–</td>
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<td>–</td>
<td>Minkin, Eshbaugh, 1989</td>
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Original data are those reported here and in Table 2; “–” means no data reported.

Table 2. Summary of pollen morphological characteristics

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<th>Taxon</th>
<th>Apertures</th>
<th>Shape</th>
<th>Polar view</th>
<th>Colpi</th>
<th>Colpus membrane</th>
<th>Exine sculpture / caput of pila</th>
<th>Reference</th>
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<td>Lindenberga philippensis</td>
<td>3-colporate</td>
<td>oblate-spheroidal, spheroidal (some prolate)</td>
<td>trilobate</td>
<td>long, narrow, acute ends</td>
<td>smooth</td>
<td>reticulate</td>
<td>original data</td>
</tr>
<tr>
<td>Lindenberga sinaica</td>
<td>3-colporate</td>
<td>prolate, spheroidal (some oblate-spheroidal)</td>
<td>trilobate</td>
<td>long, narrow, acute ends</td>
<td>smooth</td>
<td>reticulate</td>
<td>original data</td>
</tr>
<tr>
<td>Bungea trifida</td>
<td>3-colpate</td>
<td>prolate (some spheroidal)</td>
<td>sub-triangular, circular</td>
<td>long, medium-width, acute ends</td>
<td>reticulate, 0.17–0.33</td>
<td>–</td>
<td>original data</td>
</tr>
<tr>
<td>Bungea trifida</td>
<td>3-colpate</td>
<td>oblate-spheroidal</td>
<td>semi-angular</td>
<td>long, acute ends</td>
<td>coarsely granulate</td>
<td>retipilate</td>
<td>Inceoğlu, 1982</td>
</tr>
<tr>
<td>Cymbaria dahurica</td>
<td>3-colpate</td>
<td>prolate (some oblate-spheroidal)</td>
<td>sub-triangular</td>
<td>medium-length, wide, blunt or acute ends</td>
<td>granulate</td>
<td>–</td>
<td>original data</td>
</tr>
<tr>
<td>Cymbaria dahurica</td>
<td>3-colpate</td>
<td>subprolate</td>
<td>sub-triangular</td>
<td>with granules</td>
<td>retipilate, 0.45–0.90</td>
<td>–</td>
<td>Lu et al., 2007</td>
</tr>
<tr>
<td>Cymbaria mongolica</td>
<td>3-colpate</td>
<td>subprolate</td>
<td>sub-triangular</td>
<td>with granules</td>
<td>retipilate, 0.16–0.40</td>
<td>–</td>
<td>Lu et al., 2007</td>
</tr>
<tr>
<td>Cymbochasma borysthenica</td>
<td>3-colpate (some 4-colpate)</td>
<td>oblate-spheroidal</td>
<td>sub-circular</td>
<td>medium-length, medium-width, blunt, indistinct ends</td>
<td>granulate</td>
<td>–</td>
<td>original data</td>
</tr>
<tr>
<td>Monochastra savatteri</td>
<td>3-colpate</td>
<td>prolate, spheroidal (some oblate-spheroidal)</td>
<td>circular</td>
<td>wide at equator and narrow near poles</td>
<td>with fine granules</td>
<td>retipilate, 0.19–0.39</td>
<td>Lu et al., 2007</td>
</tr>
<tr>
<td>Siphonostegia chinensis</td>
<td>3-colpate</td>
<td>spheroidal, oblate-spheroidal (some prolate)</td>
<td>circular</td>
<td>long, narrow, blunt ends</td>
<td>granulate</td>
<td>–</td>
<td>original data</td>
</tr>
<tr>
<td>Siphonostegia chinensis</td>
<td>3-colpate</td>
<td>spheroidal</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>retipilate</td>
<td>Minkin, Eshbaugh, 1989</td>
</tr>
<tr>
<td>Siphonostegia chinensis</td>
<td>3-colpate</td>
<td>spheroidal</td>
<td>circular</td>
<td>wide at equator, but not narrow near poles</td>
<td>with coarse granules</td>
<td>retipilate, 0.18–0.31</td>
<td>Lu et al., 2007</td>
</tr>
<tr>
<td>Siphonostegia syriaca</td>
<td>3-colpate</td>
<td>prolate, spheroidal</td>
<td>circular</td>
<td>long, acute ends</td>
<td>coarsely granulate</td>
<td>retipilate</td>
<td>Inceoğlu, 1982</td>
</tr>
<tr>
<td>Schwalbea americana</td>
<td>3-colpate</td>
<td>spheroidal</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>retipilate</td>
<td>Minkin, Eshbaugh, 1989</td>
</tr>
</tbody>
</table>
Pollen grains in Cymbochasma borysthenica are 3-colpate and occasionally 4-colpate (Tsymbalyuk, 2011). They have the thinnest exine (0.7–1.3 μm) among pollen grains of the studied species of the tribe. Pollen grains of Cymbochasma borysthenica are smaller as compared to pollen of Cymbalaria dahurica and C. mongolica, and also differ from species of Cymbalaria sensu stricto in the pollen shape and outline (see Table 2). Thus, pollen characters may provide additional evidence in favor of recognition of Cymbochasma as a separate genus.

The two studied species of Cymbalaria sensu stricto are similar in their pollen size, shape, and outline; however, they differ in their colpi structure and exine sculpture. In pollen grains of Cymbalaria dahurica, colpi are medium-length, wider than in C. mongolica, and exine sculpture is retipilate (caput of pila 0.41–0.90 μm) or rugulate-retipilate, while C. mongolica has narrower and longer colpi (as compared to those in C. dahurica) and retipilate exine sculpture (caput of pila 0.16–0.40 μm) (Lu et al., 2007, and original data).

In general, pollen grains of Bungea trifida and Cymbalaria mongolica (Lu et al., 2007) are similar in their shape, outline, size, and exine sculpture; however, Bungea trifida has colpi wider than those in C. mongolica, and the thickest exine (1.3–3.3 μm) among all studied species.

The smallest sizes are characteristic of pollen grains of Siphonostegia chinensis (Table 1). This species also has the narrowest colpi among all studied species. Columellae in all species studied here are mainly indistinct, while in Siphonostegia chinensis those are distinct, thin, and arranged more or less regularly.

Pollen grains of Siphonostegia syriaca (Lesqueruexia syriaca) (Inceoğlu, 1982) and S. chinensis are similar in their shape, outline, size, exine sculpture, and length of colpi; however, in S. syriaca the colpi ends are acute, while in S. chinensis they are obtuse (with blunt ends).

Pollen grains of Schwalbea (Minkin, Eshbaugh, 1989) are similar to those of Siphonostegia in their outline, size, and exine sculpture. Unfortunately, the characters reported by Minkin and Eshbaugh (1989) are insufficient for a more detailed comparative analysis.

The two studied species of Lindenbergia are similar to each other in having the 3-colporate aperture type, reticulate exine sculpture, and long and narrow colpi. They, however, differ by the exine thickness: in L. philippensis the exine is 1.1–1.6 μm thick, the tectum is nearly equal to the infratectum, and columellae are indistinct or distinct, while in L. sinaica the exine is thinner (0.7–1.1 μm thick), and exine layers are invisible (indistinct).

**Comparison of palynomorphological and molecular phylogenetic evidence**

According to molecular phylogenetic studies (Bennett, Mathews, 2006; McNeal et al., 2013), the clade of Cymbalariae is subdivided into two subclades. The subclade that includes Bungea, Cymbalaria, Cymbochasma, and Monochasma is characterized by larger pollen grains and wider colpi, as compared to the subclade of Siphonostegia (including Lesqueruexua) and Schwalbea.

The earliest-branching clade of Lindenbergia is sister to the clade containing all other members of Orobanchaceae. Small-sized 3-colporate pollen grains with reticulate exine revealed in all studied taxa of Lindenbergia are similar to pollen of some representatives of Plantaginaceae, in particular, those of Gratioleae (Tsymbalyuk, Mosyakin, 2013a, 2014; Tsymbalyuk, 2016). In our opinion, that superficial similarity does not reflect direct phylogenetic relationships of these taxa, but rather some recurrent patterns (plesiomorphic characters) appearing is several clades of Lamiales.

The genus Paulownia Siebold & Zucc. is currently placed phylogenetically as a group sister to Orobanchaceae (Oldestad et al., 2001; Oxelman et al., 2005; Bennet, Mathews, 2006; Schäferhoff et al., 2010; McNeal et al., 2013). Pollen grains of Paulownia are 3-colporate, with reticulate exine (Erdtmann, 1952; Chen, 1983; Tsymbalyuk, 2014). In these characters, Paulownia is rather similar to Lindenbergia. However, Paulownia differ from Lindenbergia in having small- and medium-sized pollen grains (small in Lindenbergia), trilobate and sub-triangular in outline (only trilobate in Lindenbergia), with distinct orae (indistinct in Lindenbergia) and the colpus membrane smooth and granulate (only smooth in Lindenbergia). Some similarity with pollen of Paulownia and Lindenbergia is also observed in pollen grains of the phylogenetically still problematic genus Wightia Wall. (Zhou et al. 2014), which also has 3-colporate pollen with reticulate exine sculpture (Wei, 1989; Tsymbalyuk, 2014, 2016). However, in Paulownia and Lindenbergia orae are circular and colpi are tapered to acute ends, while Wightia has elliptical orae and colpi expanded to rounded ends.

Thus, the earliest-branching clade of Orobanchaceae (Lindenbergieae) is palynomorphologically similar to the clade sister to all Orobanchaceae (Paulowniaeae).
and to the currently phylogenetically unplaced (?) genus *Wightia*. These palynomorphological findings confirm the phylogenetic patterns currently revealed in basal *Orobanchaceae* and their closest relatives.

**Main trends of pollen evolution in early-branching *Orobanchaceae***

We may conclude that the colporate (most probably 3-colporate) type of pollen grains could be ancestral in *Orobanchaceae*. This type is peculiar to members of Clade I (*sensu* McNeal et al., 2013) that includes *Lindenbergia* (3-colporate pollen with reticulate exine sculpture and indistinct orae). Then, the colpate type probably evolved by reduction of orae; this type, with retipilate exine sculpture, is characteristic of representatives of Clade II, including *Siphonostegia, Schwalbea, Monochasma, Cymbaria, Cymbodisma*, and *Bungea*. A sculpture type transitional between reticulate and retipilate exine could be the rugulate-retipilate type observed in *Cymbaria dahurica*. In the large and diverse Clade III, which includes holoparasitic members of *Boschniakia* C.A. Mey. ex Bong., *Xylanche* Beck, *Kopsiopsis* (Beck) Beck, *Epifagus* Nutt., *Conopholis* Wallr., *Mannagetta* Harry Sm., *Cistanche* Hoffmanns. & Link, *Dipheylpaea* Nicolson, *Orobanche* L., *Phelipanche* Pomel, and the recently resurrected genus *Aphyllost Monch.* (Schneider, 2016; Schneider, Moore, 2017), a greater diversity of aperture and exine sculpture types and patterns is observed (Tsymbalyuk, Mosyakin, 2013b, c; Zare et al., 2014; Piwowarczyk et al., 2015; Tsymbalyuk, 2016).

Thus, available palynomorphological data are well consistent with the phylogenetic patterns in early-branching *Orobanchaceae* (*Lindenbergieae* and *Cymbarieae*) and their relatives, which are currently revealed by molecular phylogenetic evidence (Bennett, Mathews, 2006; McNeal et al., 2013). The considerable pollen diversity in crown clades and subclades of *Orobanchaceae* evolved on the base of a few pollen types and subtypes, which were peculiar to hypothetical ancestors of *Orobanchaceae* and are probably preserved until now in the extant taxa of *Paulowniaceae* and *Orobanchaceae* trib. *Lindenbergieae*.

Data on pollen morphology also confirm the updated phylogeny-based taxonomic circumscription of *Cymbarieae* as outlined by Fischer (2004) and Olmstead (2016).

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**REFERENCES**


Цимбалюк З.М., Мосякін С.Л. Палиноморфологічні особливості представників триб *Lindenbergieae* і *Cymbarieae* та еволюція пильку в базальних кладах *Orobanchaceae*.  
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Сполучення светлової та сканируючої електронної микроскопії із використанням електронної мікроскопії досліджено пилькові зерна чотирьох видів з чотирьох родів триб *Cymbarieae* та *Lindenbergieae* у базальних кладах *Orobanchaceae*. Установлено, що пилькові зерна триб *Lindenbergieae* та *Cymbarieae* з різними типами пилькових зерен середніх розмірів, з різною структурою екзини. Особливості пилькових зерен представників триб *Lindenbergieae* та *Cymbarieae* порівнюються з такими в *Paulowniaceae* і верхівкових кладах *Orobanchaceae*. Базальна клада *Orobanchaceae* (Lindenbergieae) за палиноморфологічними особливостями подібна до *Paulowniaceae* (сестринська клада до всіх *Orobanchaceae*) і *Wightia*. Палиноморфологічні висновки підтверджують філогенетичні закономірності, які виявлені у базальних *Orobanchaceae* та їхніх найближчих родичів. Зроблено висновок, що бороздно-оровий (іноді 3-бороздно-оровий) тип пилькових зерен може бути предковим у родині *Orobanchaceae*. Різноманітність пилькових зерен у верхівкових кладах *Orobanchaceae* еволюціонувала на основі дієльних та паліитипів пильку, властивих гіпотетичним предкам *Orobanchaceae*; такі типи, ймовірно, збереглися в сучасних представниках *Paulowniaceae* та *Lindenbergieae*.  

Ключові слова: *Cymbarieae*, *Lindenbergieae*, *Orobanchaceae*, еволюція пильку, морфологія пильку, систематика, філогенія