

Pollen morphology of the Central European broomrapes (Orobanchaceae: *Orobanche*, *Phelipanche* and *Orobanchella*) and its taxonomical implications

Renata Piwowarczyk · Jacek Madeja ·
Marcin Nobis

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Abstract Pollen grains of 450 samples of 25 species of the genus *Orobanche* and *Phelipanche* occurring in Central Europe were investigated using light and scanning electron microscopy. Palynological data on 18 species are reported here for the first time. The usefulness of micromorphological studies on pollen of *Orobanche* and *Phelipanche* is demonstrated. Previous research showed the separation of *Phelipanche* from *Orobanche*, a finding which is also supported by pollen aperture type and exine ornamentation. The pollen of *Phelipanche* is tricolpate, while that of *Orobanche* is inaperturate. Our research showed that one of the species included so far within *Orobanche*, namely *O. coeruleascens*, has tricolpate pollen with microreticulate sculpture. As a consequence of the finding of tricolpate pollen and intermediate macromorphological characteristics of this species between *Orobanche* and *Phelipanche*, it is proposed to transfer it to a new genus, *Orobanchella*, described in the paper. A comparison of the main characteristics distinguishing *Orobanche*, *Phelipanche*, *Boulardia* and *Orobanchella* is also presented and discussed.

Keywords *Orobanche* · *Phelipanche* · *Orobanchella* · Pollen · Morphology · Systematic significance · SEM

Introduction

The family Orobanchaceae contains 15 obligate parasitic genera with ca 250 species (Pusch and Günther 2009). The family has a worldwide distribution, but the main centres of distribution are the Mediterranean, western and central Asia, northern Africa and North America (Kreutz 1995). *Orobanche* s.l. is the largest genus and comprises more than 200 species, which lack chlorophyll and are holoparasites of the roots of other vascular plants (Uhlich et al. 1995; Pusch and Günther 2009). In Europe, these species usually grow in the warmest regions, with the highest species diversity in the Mediterranean countries. In central and northern parts of Europe the genus comprises about 30 species, which are mostly rare, endangered or declining (e.g. Zázvorka 1997, 2000; Pusch and Günther 2009; Halamski and Piwowarczyk 2008; Piwowarczyk and Przemyski 2009, 2010; Piwowarczyk et al. 2009, 2010, 2011; Piwowarczyk 2011, 2012a, b, c, d, e, f, g, h, 2013; Nobis et al. 2014).

The genus *Orobanche* has traditionally been divided into four sections: *Trionychon*, *Orobanche*, *Gymnocaulis* and *Myzorrhiza* (Beck von Mannagetta 1930). In the most recent taxonomic treatments, these sections have been recognised as separate genera: *Aphyllon*, *Myzorrhiza*, *Phelipanche*, *Boulardia* and *Orobanche*. However, the classification of *Phelipanche* (syn. *O.* sect. *Trionychon*) and *Boulardia* (syn. *Orobanche latisquama*) as separate genera is still being discussed (Holub 1977, 1990; Ter-yokhin et al. 1993; Schneeweiss 2001; Manen et al. 2004; Schneeweiss et al. 2004a, b; Weiss-Schneeweiss et al.

R. Piwowarczyk (✉)

Department of Botany, Institute of Biology, Jan Kochanowski University, 15 Świętokrzyska St., 25-406 Kielce, Poland
e-mail: renka76@wp.pl; renata.piwowarczyk@ujk.edu.pl

J. Madeja

Department of Palaeobotany and Palaeoherbarium, Institute of Botany, Jagiellonian University, 46 Lubicz St., 31-512 Kraków, Poland
e-mail: jacek.madeja@uj.edu.pl

M. Nobis

Department of Plant Taxonomy, Phytogeography and Herbarium, Institute of Botany, Jagiellonian University, Kopernika 27 St., 31-501 Kraków, Poland
e-mail: m.nobis@uj.edu.pl

2006; Bennett and Mathews 2006; Schneeweiss 2007; Park et al. 2007a, b; Pujadas Salvà 2007; Carlón et al. 2008). Some authors still treat the genus *Orobanche* s.l. in a wide sense (Greuter and Raab-Straube 2009). The separation of *Phelipanche* and *Orobanche* is confirmed by recent molecular phylogenetic studies (e.g. Schneeweiss et al. 2004a, b; Manen et al. 2004; Park et al. 2007a, b); morphological and carpological differences (Holub 1977; Teryokhin 1997; Joel 2009a, b); chemotaxonomic study (Velasco et al. 2000) and micromorphology of seeds (e.g. Abu Sbaiah and Jury 1994; Plaza et al. 2004); and pollen grains (Zare et al. 2013). According to the authors of a recent study (Zare et al. 2013), verrucate exine ornamentation and inaperturate pollen type suggest a close relationship between *Orobanche*, *Boulardia* and *Diphelypaea*, whereas tricolpate pollen suggests a relationship between *Cistanche* and *Phelipanche*.

The flowers in *Orobanche* are always bisexual, with superior ovaries, more or less zygomorphic, with four didynamous stamens and two or three lobed stigma, characterised by cross-pollination or, rarely, by the absence of insects, i.e. self-pollination (Beck von Mannagetta 1930; Teryokhin 1997; Jones 1991). There are many adaptations of flowers for pollination: large or small flowers gathered in dense inflorescences; contrasting colouration and shine of the corolla and stigma; a wide range of often bright colours; the smell of the flowers, either pleasant (scent of cloves, e.g. *O. alba*, *O. caryophyllacea*, *O. crenata*, *O. gracilis*) or unpleasant (the smell of carrion, e.g. *O. foetida*, *O. rigens*, *O. rapum-genistae*, *O. variegata*) (the majority of species have a weak scent or none); the lower lip of the corolla serving as an alighting place for pollinators (two bright folds, coated with hairs); the production of nectar from coloured spots. Most species are pollinated by bumblebees and bees (Apidae), as well as, albeit less importantly, by species of the families Syrphidae, Vespidae, or Formicidae or carrion flies (Diptera) (Piwowarczyk, unpublished). Preliminary studies in relation to pollination and other shared interactions between parasitic plants and their hosts are presented by Ollerton et al. (2007).

Orobanchaceae is a eurypalynous family, with different types of pollen. Nevertheless, in many cases the biometric and morphological features of pollen grains possess a high diagnostic significance and are used in taxonomy. Pollen taxonomy can also explain the relationship and phylogenetic connections between particular genera of the family. Exine sculpture is one of the most important features and is a good tool in the recognition of genera or sections within a genus. Unfortunately, there are still not many papers dedicated to the pollen morphology of the family Orobanchaceae (e.g. Tiagi 1951; Rao 1963; Erdtman 1966; Polo 1987; Minkin and Eshbaugh 1989; Abu Sbaiah et al. 1994; Teryokhin 1997; Shahi Shavvon and Saeidi Mehrvarz

2010; Zare and Dönmez 2013; Zare et al. 2013; Piwowarczyk et al. 2014) and further studies within the whole family are required.

The aim of the study was to present (1) the macro- and micromorphological variability of pollen in the Central European *Orobanche* and *Phelipanche* taxa; (2) the taxonomic implications of pollen morphology in the examined taxa.

Materials and methods

Plant material

This study is based on plant material deposited mainly in the KTC herbarium. In total, 25 taxa of three genera, *Orobanche*, *Phelipanche* and *Orobanchella*, were analysed. A list of voucher specimens used in the study is given in Table 1. Each sample corresponds to a single plant, but pollen grains originating from different flowers have not been distinguished.

Light microscopy observations

The pollen grains were prepared with the standard method, Erdtman's acetolysis (Erdtman 1969; Faegri and Iversen 1989). Following acetolysis, the pollen grains were mounted in glycerine jelly and prepared for LM observation. Some characteristics, such as polar and equatorial axis and exine thickness, were examined by LM (Axio Imager 2, Zeiss) for 30 pollen grains under 1,000× magnifications with the help of the AxioVision computer program (version 4.8.1.0, Carl Zeiss Imaging Solutions). Measurements were taken under the light microscope only in accordance with changes in pollen shape and dimensions occurring during its preparation for SEM.

Scanning electron microscopy (SEM) observation

Using SEM observation, we analysed 450 samples of 25 taxa. For SEM observation, samples were coated with gold using a JFC-1100E Ion Sputter manufactured by JEOL. Micromorphological structures of pollen grains were observed and photographs taken by means of Hitachi S-4700 and Philips XL 20 scanning electron microscopes at various magnifications. The terminology was adopted from Punt et al. (2007) and Hesse et al. (2009).

Morphometric analysis

The statistical analyses are based on 1,170 fully-developed pollen grains from 25 taxa. Characteristics were measured in 30 grains of each specimen studied (Table 1). Finally, a

Table 1 Taxa used in this study and voucher information

Number	Taxon	Locality	Voucher	Host
<i>Orobanchae</i>				
1	<i>O. alba</i> subsp. <i>alba</i>	Poland	Bieszczady Mts, 14 Aug 2009, R. Piwowarczyk (KTC)	<i>Thymus pulegioides</i>
2a	<i>O. alba</i> subsp. <i>major</i>	Poland	Lasocin, 27 Jul 2006, R. Piwowarczyk (KTC)	<i>Salvia verticillata</i>
2b	<i>O. alba</i> subsp. <i>major</i>		Kąty II, 15 Jul 2006, R. Piwowarczyk (KTC)	<i>Salvia verticillata</i>
3	<i>O. alsatica</i>	Poland	Broczówka, 8 Jun 2007, R. Piwowarczyk (KTC)	<i>Peucedanum cervaria</i>
4	<i>O. bartlingii</i>	Poland	Podzamcze, 4 Jun 2009, R. Piwowarczyk (KTC)	<i>Libanotis pyrenaica</i>
5a	<i>O. caryophyllacea</i>	Poland	Teresin, 8 Jun 2007, R. Piwowarczyk (KTC)	<i>Galium mollugo</i>
5b	<i>O. caryophyllacea</i>		Bogucice, 6 Jun 2007, R. Piwowarczyk (KTC)	<i>Galium odoratum</i>
5c	<i>O. caryophyllacea</i>		Pieniny Mts, Kozia Mt, 23 Jul 2010, R. Piwowarczyk (KTC)	<i>Galium mollugo</i>
6	<i>O. crenata</i>	Spain	Cordoba, 2 May 2012, R. Piwowarczyk (KTC)	<i>Vicia</i> sp.
7a	<i>O. elatior</i>	Poland	Baldran, 07 Jul 2010, R. Piwowarczyk (KTC)	<i>Centaurea scabiosa</i>
7b	<i>O. elatior</i>		Wólka Leszczańska, 08 Jun 2008, R. Piwowarczyk (KTC)	<i>Centaurea scabiosa</i>
8a	<i>O. flava</i>	Poland	Tatra Mts, Mała Łąka Valley, 22 Jun 2009, R. Piwowarczyk (KTC)	<i>Petasites kablikianus</i>
8b	<i>O. flava</i>	Slovakia	Nizkie Tatra Mts, 7 Aug 2011, R. Piwowarczyk (KTC)	<i>Petasites kablikianus</i>
9	<i>O. gracilis</i>	Austria	Hundsheim, 14 Jun 2012, R. Piwowarczyk (KTC)	<i>Anthyllis vulneraria</i>
10	<i>O. hederæ</i>	Spain	Elx, palm garden, 28 Apr 2009, R. Piwowarczyk (KTC)	<i>Hedera helix</i>
11a	<i>O. kochii</i>	Poland	Pęczelice, 13 Jun 2010, R. Piwowarczyk (KTC)	<i>Centaurea scabiosa</i>
11b	<i>O. kochii</i>		Toporowice, 5 Jun 2008, R. Piwowarczyk (KTC)	<i>Centaurea scabiosa</i>
12	<i>O. lucorum</i>	Poland	Warsaw, botanical garden, 10 July 2009, R. Piwowarczyk (KTC)	<i>Berberis vulgaris</i>
13a	<i>O. lutea</i>	Poland	Wesołówka, 17 Jun 2006, R. Piwowarczyk (KTC)	<i>Medicago falcata</i>
13b	<i>O. lutea</i>		Chomentówek, 6 Jun 2007, R. Piwowarczyk (KTC)	<i>Medicago sativa</i>
13c	<i>O. lutea</i>		Młodzawy Duże, 2 Jun 2007, R. Piwowarczyk (KTC)	<i>Medicago falcata</i>
14	<i>O. mayeri</i>	Poland	Pieniny Mts, Trzy Korony, 30 Jun 2009, R. Piwowarczyk (KTC)	<i>Laserpitium latifolium</i>
15	<i>O. minor</i>	Poland	Żywiec, 19 Jun 2009, R. Piwowarczyk (KTC)	<i>Trifolium repens</i>
16a	<i>O. pallidiflora</i>	Poland	Bieszczady Mts, 14 Aug 2009, R. Piwowarczyk (KTC)	<i>Carduus personata</i>
16b	<i>O. pallidiflora</i>		Lubiatowo, 24 Jun 2009, R. Piwowarczyk (KTC)	<i>Cirsium oleraceum</i>
16c	<i>O. pallidiflora</i>		Cieple, 5 Jul 2010, R. Piwowarczyk (KTC)	<i>Cirsium arvense</i>
16d	<i>O. pallidiflora</i>		Potok Mały, 29 Jun 2009, R. Piwowarczyk (KTC)	<i>Cirsium arvense</i>
17a	<i>O. picridis</i>	Poland	Pasturka, 25 Jun 2007, R. Piwowarczyk (KTC)	<i>Picris hieracioides</i>
17b	<i>O. picridis</i>		Machnów Stary, 9 Jun 2007, R. Piwowarczyk (KTC)	<i>Picris hieracioides</i>
18	<i>O. reticulata</i>	Slovakia	Nizkie Tatra Mts, Puste, 5 Aug 2011, R. Piwowarczyk (KTC)	<i>Carduus glaucus</i>
19	<i>O. teucrii</i>	Austria	Hundsheim, 14 Jun 2012, R. Piwowarczyk (KTC)	<i>Teucrium montanum</i>
<i>Phelipanche</i>				
20a	<i>P. arenaria</i>	Poland	Zawiercie-Bzów, 11 Jun 2010, R. Piwowarczyk (KTC)	<i>Artemisia campestris</i>
20b	<i>P. arenaria</i>		Pasturka, 25 Jun 2007, R. Piwowarczyk (KTC)	<i>Artemisia campestris</i>
21	<i>P. bohemica</i>	Poland	Zawiercie-Bzów, 11 Jun 2010, R. Piwowarczyk (KTC)	<i>Artemisia campestris</i>
22	<i>P. caesia</i>	Ukraine	Askania Nova, 16 Jun 2011, R. Piwowarczyk (KTC)	<i>Artemisia austriaca</i>
23	<i>P. purpurea</i>	Poland	Chrzanów, 18 Jun 2009, R. Piwowarczyk (KTC)	<i>Achillea millefolium</i> s.l.
24	<i>P. ramosa</i>	Poland	Zysławice, 7 Sep 2008, R. Piwowarczyk (KTC)	<i>Nicotiana tabacum</i>
<i>Orobanchella</i>				
25a	<i>O. coerulescens</i>	Poland	Dobrowoda, 19 Jun 2010, R. Piwowarczyk (KTC)	<i>Artemisia campestris</i>
25b	<i>O. coerulescens</i>		Pasturka, 25 Jun 2007, R. Piwowarczyk (KTC)	<i>Artemisia campestris</i>

total of the seven most informative quantitative and qualitative morphological characteristics was chosen for the analyses: width of exine (mean μm); polar axis (mean μm); equatorial diameter (mean μm); polar/equatorial ratio; number of furrows on grain; sculpture of pollen grains

(1—granules look like a ball of wool, 2—granules consisted of a few smaller round elements knitted together into a smooth surface, 3—microreticulate); arrangement of granules on the pollen surface: 1—no areas without granules are present, 2—areas without granules are present,

Table 2 Characters used in cluster analysis (comp. “Materials and methods”)

Species	Character						
	Polar axis (mean μm)	Equatorial diameter (mean μm)	Width of exine (mean μm)	Polar/equatorial ratio	Furrows number on grain	Sculpture of pollen grains	Arrangement of granules
<i>Orobanchella coeruleascens</i>	20.4	21.9	1.4	0.934	3	3	3
<i>Phelipanche arenaria</i>	22.1	24.5	1.4	0.903	3	3	3
<i>P. bohemica</i>	22.9	24.2	1.7	0.944	3	3	3
<i>P. caesia</i>	25.0	23.8	1.3	1.053	3	3	3
<i>P. purpurea</i>	21.6	22.1	1.5	0.977	3	3	3
<i>P. ramosa</i>	19.3	21.3	1.4	0.905	3	3	3
<i>Orobanche alba</i> subsp. <i>alba</i>	22.6	19.8	0.9	1.142	0	1	2
<i>O. alba</i> subsp. <i>major</i>	22.5	18.9	0.9	1.191	0	1	2
<i>O. alsatica</i>	27.5	22.6	0.9	1.219	0	2	2
<i>O. bartlingii</i>	26.3	22.4	0.8	1.174	0	2	2
<i>O. caryophyllacea</i>	25.0	21.7	1.1	1.152	0	2	2
<i>O. crenata</i>	20.2	18.2	0.9	1.110	0	2	2
<i>O. elatior</i>	26.2	22.9	1.1	1.144	0	2	2
<i>O. flava</i>	27.5	23.7	1.1	1.163	0	2	1
<i>O. gracilis</i>	23.6	20.3	0.9	1.165	0	2	2
<i>O. hederiae</i>	24.8	21.3	1.1	1.162	0	1	2
<i>O. kochii</i>	25.1	22.2	1.1	1.130	0	2	2
<i>O. lucorum</i>	27.4	23.7	1.0	1.157	0	2	1
<i>O. lutea</i>	23.8	20.8	0.8	1.144	0	2	2
<i>O. mayeri</i>	28.8	24.2	1.0	1.189	0	2	2
<i>O. minor</i>	23.3	19.6	0.8	1.188	0	2	1
<i>O. pallidiflora</i>	23.9	20.8	0.8	1.151	0	2	2
<i>O. picridis</i>	23.9	20.0	0.9	1.195	0	2	2
<i>O. reticulata</i>	27.0	23.2	0.9	1.165	0	2	2
<i>O. teucrii</i>	26.3	22.6	0.8	1.162	0	2	2

3—no granules are present (different type of pollen sculpture). The characteristics used are listed in Table 2. The mean value of the characteristics measured or scored from all samples of the taxon was analysed using cluster analysis to illustrate the general relationships and similarities between them. The analysis was performed in order to obtain results providing a general overview and to determine whether different morphological groups exist and are separated into distinct clusters. Similarity between samples was calculated on the basis of Gower’s general similarity coefficient. The dendrogram was prepared using the UP-GMA method. Subsequently, principal coordinate analysis (PCoA) was performed on the basis of all quantitative and qualitative features. The goal of PCoA was the positioning of objects (individuals) in a space of reduced dimensionality

while preserving their distance relationships. Data analyses were performed using MVSP 3.1 (Kovach 1999).

Results

LM and SEM study

Both LM and SEM observations enabled the team to distinguish, among *Orobanche* and *Phelipanche* pollen, two main groups with distinctly different features regarding pollen shape and exine micromorphology.

Group 1 contains pollen grains in monads, suboblate (*P. arenaria*), oblate spheroidal (*P. purpurea*, *P. bohemica*, *P. ramosa* and *Orobanche coeruleascens*) (according to P/E

Table 3 Characters of tricolpate *Phelipanche*-like type pollen morphology

Taxon	Number	Polar axis (µm)			Equatorial diameter (µm)			P/E ratio	Number of colpi	Exine (mean, µm)	Pollen shape (acc. to Erdtman 1966)	Exine sculpture
		Min	Max	Mean ±SD	Min	Max	Mean ±SD					
<i>Phelipanche arenaria</i>	20a	19.85	24.95	22.24 ±1.37	21.46	28.22	25.11 ±1.66	0.89	3 (2)	1.32	Oblate spheroidal	Microreticulate
<i>Phelipanche arenaria</i>	20b	18.84	25.61	21.53 ±1.42	19.91	29.16	25.73 ±1.92	0.84	3	1.40	Suboblate	Microreticulate
<i>Phelipanche bohémica</i>	21	20.82	28.46	22.89 ±1.56	23.46	27.43	25.09 ±0.87	0.91	3 (2.4)	1.67	Oblate spheroidal	Microreticulate
<i>Phelipanche caesia</i>	22	21.01	28.89	25.01 ±1.34	22.00	26.24	23.75 ±1.21	1.05	3	1.28	Prolate spheroidal	Microreticulate
<i>Phelipanche purpurea</i>	23	19.35	24.49	21.60 ±1.20	20.31	25.77	23.15 ±1.31	0.93	3	1.46	Oblate spheroidal	Microreticulate
<i>Phelipanche ramosa</i>	24	17.10	22.68	19.29 ±1.14	19.22	23.87	21.87 ±1.33	0.89	3	1.38	Oblate spheroidal	Microreticulate
<i>Orobanchella coeruleascens</i>	25a	17.57	22.95	21.00 ±1.43	20.81	26.25	23.30 ±1.49	0.90	3 (2)	1.32	Oblate spheroidal	Microreticulate
<i>Orobanchella coeruleascens</i>	25b	16.49	24.82	20.22 ±2.03	17.66	24.52	22.19 ±1.73	0.91	3 (4)	1.38	Oblate spheroidal	Microreticulate

ratio) and prolate spheroidal (*P. caesia*), and tricolpate (but some single grains with 2 or 4 colpi) forms. Some differences in colpi shape and distribution are visible (colpi were sometimes fused, wide at the equator and narrow near the poles) but these features appear in all members of group 1 (Tables 2, 3; Figs. 3, 5).

Group 2 contains pollen grains in monads, prolate spheroidal (*O. kochii* and *O. crenata*) to subprolate (other) and inaperturate forms, or with irregular colpi. The basic exine ornamentation type is granulate. Visible differences in the size of granules and their distribution are present between some species. Three types of granule surface were distinguished: (1) granules with almost smooth surfaces (*O. caryophyllacea*, *O. flava*, *O. elatior*, *O. lucorum*); (2) granules consisting of a few smaller round elements knitted together (*O. caryophyllacea*, *O. picridis*, *O. minor*, *O. alba* subsp. *major*, *O. kochii*, *O. lutea*, *O. reticulata*, *O. teucrii*, *O. gracilis*, *O. crenata*); and (3) granules consisting of elongated elements visible on granule surfaces (like balls of wool) (*O. alba* subsp. *alba*, *O. hederæ*, *O. kochii*, *O. flava*) (Tables 2, 4; Figs. 1, 2, 4, 5).

According to differences in the size and distribution of granules, two main patterns of pollen surface ornamentation can be found: 1—granules distributed over the whole pollen grain, but small areas without granules are present; the granules are of different sizes but always smaller than 1 µm; most granules are joined to others, but isolated granules are also present; 2—the surfaces are similar to those described above, but there are no areas without granules (Figs. 1, 2).

Statistical analysis

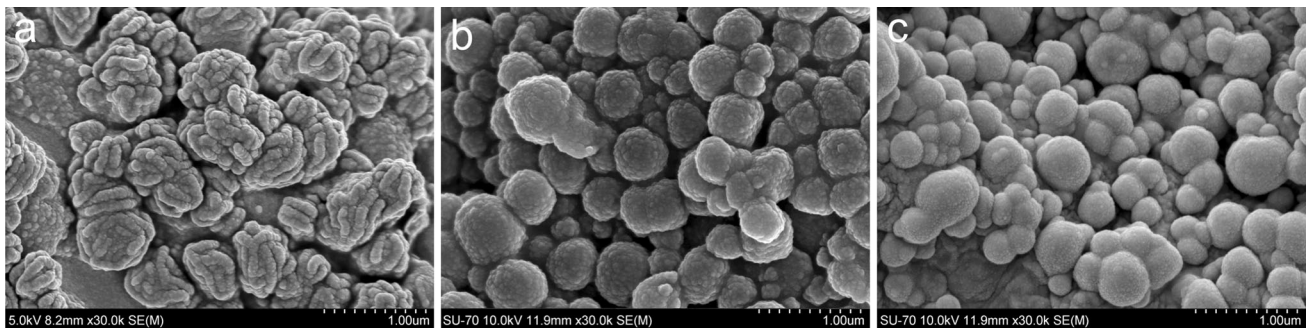
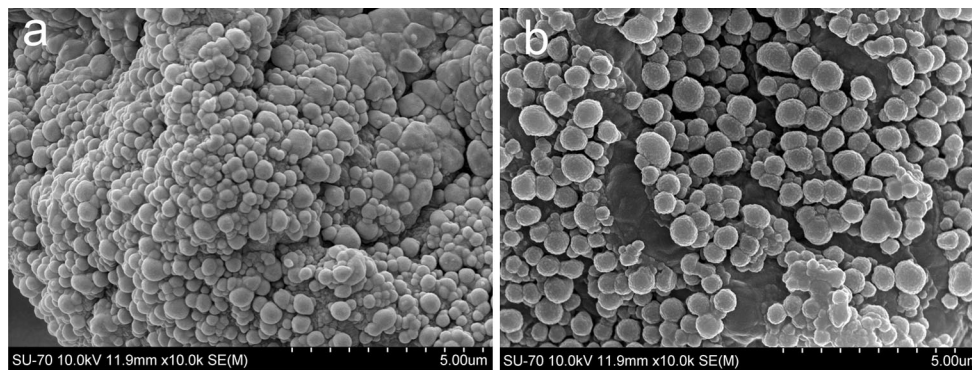
Cluster analysis (UPGMA) performed on the complete set of data (Table 2) resulted in the delimitation of two main subclusters. The first comprises a group of taxa with distinctly tricolpate pollen grains with reticulate surfaces, whereas the second comprises uncolpate grains with granulate surfaces (Figs. 3, 4, 5, 6). A similar pattern of results is shown by the ordination diagrams from the principal coordinate analysis (PCoA, not shown), also based on the complete quantitative and qualitative set of data. The first subcluster in UPGMA effectively separates the species *P. ramosa*, *P. bohémica*, *P. arenaria*, *P. purpurea*, *P. caesia* and *Orobanchella coeruleascens* from all the rest of the specimens belonging to *Orobanchella* placed in the second cluster. In the *Phelipanche* subcluster, all taxa are very similar in general habit, with tricolpate pollen grains. In cluster analysis, *P. caesia* differs slightly from the other members, with a higher polar/equatorial ratio and a thinner exine. However, pollen characteristics in this group of species are characterised by generally higher variance (Fig. 7). Although all the species located in the

Table 4 Characters of inaperturate *Orobanche*-like type pollen morphology

Taxon	Number	Polar axis (μm)				Equatorial diameter (μm)				P/E ratio	Exine (mean, μm)	Pollen shape (acc. to Erdtman 1966)	Exine sculpture
		Min	Max	Mean	$\pm\text{SD}$	Min	Max	Mean	$\pm\text{SD}$				
<i>Orobanche alba</i> subsp. <i>alba</i>	1	17.44	26.77	22.62	2.20	15.88	22.21	19.80	1.75	1.15	0.86	Subprolate	Granulate
<i>Orobanche alba</i> subsp. <i>major</i>	2a	18.72	29.79	23.10	2.86	16.24	23.86	19.11	1.92	1.21	0.89	Subprolate	Granulate
<i>Orobanche alba</i> subsp. <i>major</i>	2b	17.94	25.52	21.84	1.77	14.11	21.33	18.63	1.92	1.18	0.84	Subprolate	Granulate
<i>Orobanche alsatica</i>	3	21.25	33.45	27.50	3.12	15.46	29.08	22.57	2.86	1.23	0.87	Subprolate	Granulate
<i>Orobanche bartlingii</i>	4	21.32	30.84	26.33	2.51	16.72	28.30	22.43	2.71	1.18	0.77	Subprolate	Granulate
<i>Orobanche caryophyllacea</i>	5a	21.20	28.06	24.52	1.44	18.36	23.65	21.29	1.30	1.15	1.20	Subprolate	Granulate
<i>Orobanche caryophyllacea</i>	5b	19.68	30.21	25.44	2.55	17.24	27.79	22.10	2.65	1.16	1.04	Subprolate	Granulate
<i>Orobanche caryophyllacea</i>	5c	19.83	28.00	23.17	1.99	15.22	24.67	19.50	2.03	1.19	1.09	Subprolate	Granulate
<i>Orobanche crenata</i>	6	16.37	23.28	20.19	1.94	13.79	20.99	18.18	1.93	1.11	0.85	Prolate spheroidal	Granulate
<i>Orobanche elatior</i>	7a	21.52	30.83	25.91	2.44	17.71	27.52	22.67	2.48	1.15	1.12	Subprolate	Granulate
<i>Orobanche elatior</i>	7b	20.30	34.47	26.44	3.75	17.52	32.19	23.07	3.78	1.15	1.11	Subprolate	Granulate
<i>Orobanche flava</i>	8a	18.90	30.42	24.88	2.59	15.78	25.06	21.40	2.33	1.17	1.09	Subprolate	Granulate
<i>Orobanche flava</i>	8b	24.09	35.11	30.11	2.88	22.17	31.97	26.01	2.74	1.16	1.16	Subprolate	Granulate
<i>Orobanche gracilis</i>	9	17.38	27.39	23.59	2.14	13.82	24.16	20.25	2.09	1.17	0.86	Subprolate	Granulate
<i>Orobanche hederæ</i>	10	21.59	30.67	24.77	2.40	17.21	25.30	21.31	2.02	1.17	1.11	Subprolate	Granulate
<i>Orobanche kochii</i>	11a	19.91	27.50	23.84	1.95	17.31	24.83	21.21	2.04	1.14	1.13	Subprolate	Granulate
<i>Orobanche kochii</i>	11b	22.17	32.22	26.26	2.81	18.56	29.72	23.15	2.46	1.14	1.11	Subprolate	Granulate
<i>Orobanche lucorum</i>	12	21.11	35.52	27.39	3.32	19.00	29.95	23.67	3.16	1.16	1.05	Subprolate	Granulate
<i>Orobanche lutea</i>	13a	18.36	26.78	22.26	2.36	15.54	22.33	19.31	1.71	1.15	0.75	Subprolate	Granulate
<i>Orobanche lutea</i>	13b	20.71	29.98	25.43	2.25	15.67	25.70	22.48	2.29	1.15	0.77	Subprolate	Granulate
<i>Orobanche lutea</i>	13c	22.19	39.21	29.16	3.39	16.97	31.17	24.86	3.12	1.18	1.02	Subprolate	Granulate
<i>Orobanche mayeri</i>	14	25.05	34.69	28.77	2.66	19.55	29.50	24.19	2.46	1.19	1.04	Subprolate	Granulate
<i>Orobanche minor</i>	15	18.08	27.80	23.28	1.93	15.60	24.30	19.59	2.37	1.20	0.78	Subprolate	Granulate
<i>Orobanche pallidiflora</i>	16a	18.75	27.16	22.23	2.15	16.37	23.90	19.43	2.03	1.15	0.70	Subprolate	Granulate
<i>Orobanche pallidiflora</i>	16b	19.95	30.96	26.11	2.50	17.61	27.19	23.08	2.14	1.14	0.84	Subprolate	Granulate
<i>Orobanche pallidiflora</i>	16c	19.52	31.36	24.08	2.39	16.85	25.60	21.07	2.15	1.16	0.84	Subprolate	Granulate
<i>Orobanche pallidiflora</i>	16d	18.03	28.42	23.02	3.10	14.71	24.19	19.55	2.49	1.18	0.70	Subprolate	Granulate
<i>Orobanche picridis</i>	17a	18.59	33.38	22.80	3.06	15.12	23.49	19.50	2.27	1.17	0.87	Subprolate	Granulate

Table 4 continued

Taxon	Number	Polar axis (μm)				Equatorial diameter (μm)				P/E ratio	Exine (mean, μm)	Pollen shape (acc. to Erdtman 1966)	Exine sculpture
		Min	Max	Mean	$\pm\text{SD}$	Min	Max	Mean	$\pm\text{SD}$				
<i>Orobanche picridis</i>	17b	18.92	33.61	25.01	3.49	13.34	25.45	20.52	2.70	1.23	0.88	Subprolate	Granulate
<i>Orobanche reticulata</i>	18	19.56	31.04	27.00	2.63	19.35	27.83	23.17	1.84	1.19	0.85	Subprolate	Granulate
<i>Orobanche teucris</i>	19	22.67	31.29	26.31	1.86	16.09	25.75	22.63	2.12	1.17	0.83	Subprolate	Granulate

**Fig. 1** Micromorphology of granules in selected species of *Orobanche*: **a** *Orobanche hederae* (granules like balls of wool), **b** *O. reticulata* (granules consisted of a few smaller round elements knitted together), **c** *O. flava* (granules glabrous or almost so)**Fig. 2** Arrangement of granules on the pollen surface: **a** areas without granules not present (*Orobanche flava*), **b** bald areas without granules present (*O. reticulata*)

Orobanche subclade are very similar according to the general habit of pollen grains, two smaller subgroups of taxa can be distinguished. They differ mainly in the size of grains and width of exines (cf Fig. 8). In the first group, which comprises *Orobanche alba* s. lato, *O. crenata*, *O. gracilis*, *O. hederae*, *O. lutea*, *O. minor*, *O. pallidiflora* and *O. picridis*, the mean polar axis of the pollen grains is <23.9 , the equatorial mean is <20.8 and the exine is generally narrower ($0.8\text{--}0.9\ \mu\text{m}$). In all the remaining taxa from the subclade, the mean polar axis of pollen grains

varies from 23.3 to 28.8, the equatorial mean is 21.3–24.2 and the exine width is $(0.8\text{--})\ 0.9\text{--}1.1\ \mu\text{m}$ (Table 2; Fig. 8).

In the *Phelipanche* group, a thinner exine has been noted in *P. caesia*, but at the same time, it has the highest value for equatorial and polar axis. The largest pollen grains in the *Orobanche* group were noted e.g. in *O. elatior*, *O. flava* or *O. lucorum*, the smallest in e.g. *O. crenata* and *O. picridis*. The thickest exines were recorded in *O. caryophyllacea*, *O. elatior* and *O. flava*, and the thinnest in *O. lutea* and *O. bartlingii* (Tables 2, 3, 4).

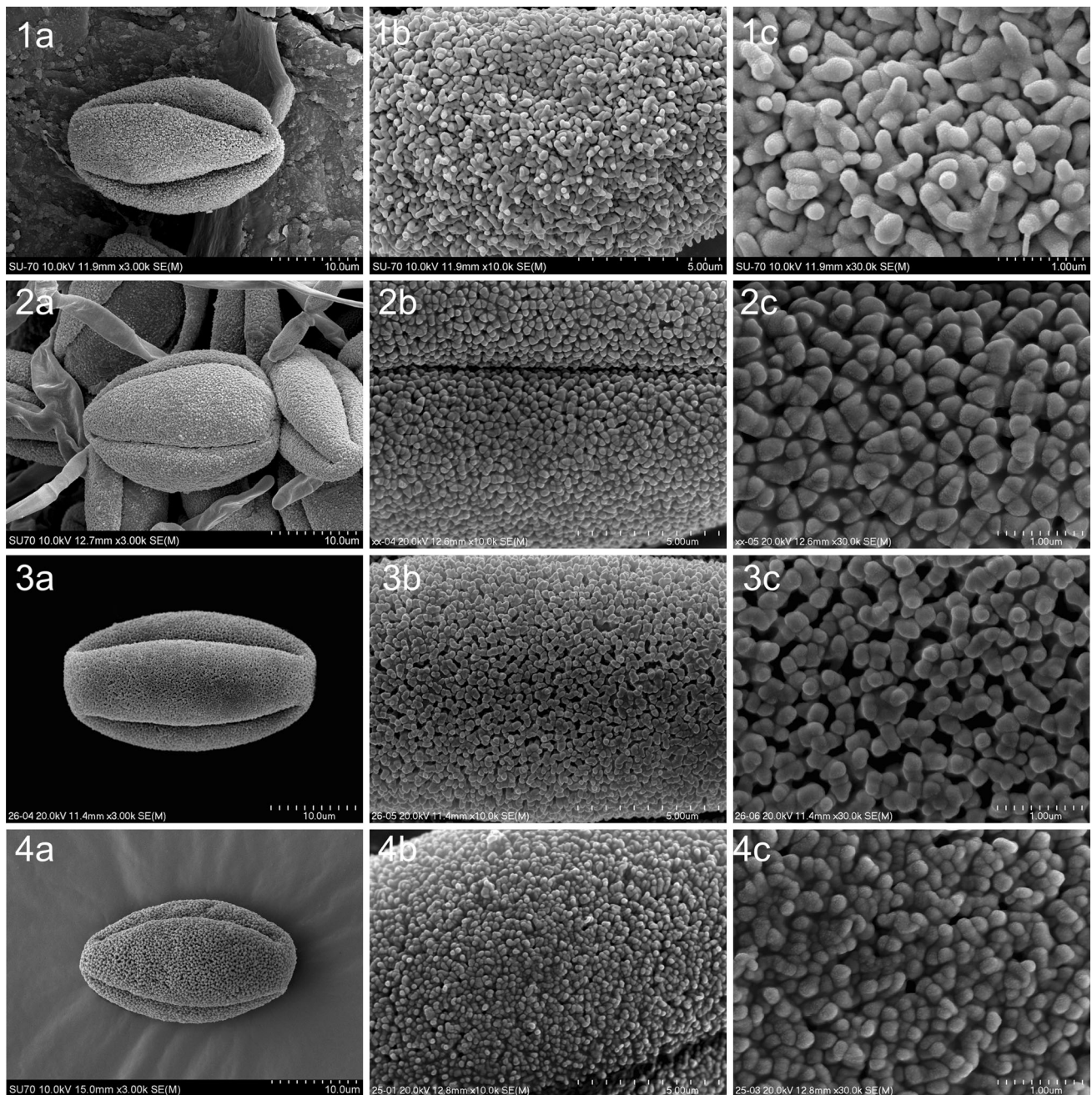


Fig. 3 Pollen grains and its micromorphology in: *Orobanchella coerulea* (1), *Phelipanche caesia* (2), *P. arenaria* (3), *P. ramosa* (4); **a** general habit of pollen grain (magnification $\times 3,000$), **b, c** microstructure of pollen grain (magnification $\times 10,000$ and $\times 30,000$)

Discussion

Analysis of the pollen grains of Central European representatives of *Orobanche* s.l. showed that they are characterised by high variability, both inter- and intra-specific, as well as in relation to oligo- and polyphagous species parasitic on various hosts. In addition, some taxa may produce heteromorphic pollen grains (Zare et al. 2013), but this issue requires further study. Surely pollen characteristics, i.e. the number of apertures and the ornamentation of

exines, cannot be overlooked, because they bear valuable information to help analyse evolutionary relationships and classifications within the Orobanchaceae family.

The characteristics of pollen are important criteria for distinguishing genera or sections, but are less important in distinguishing species. However, it is worth mentioning the subtle yet noticeable differences in the size and sculpture of pollen of species treated in different taxonomic units, i.e. species of the problematic complex *O. alsatica* s.l., namely *O. alsatica*, *O. bartlingii* and *O. mayeri*, all three of which

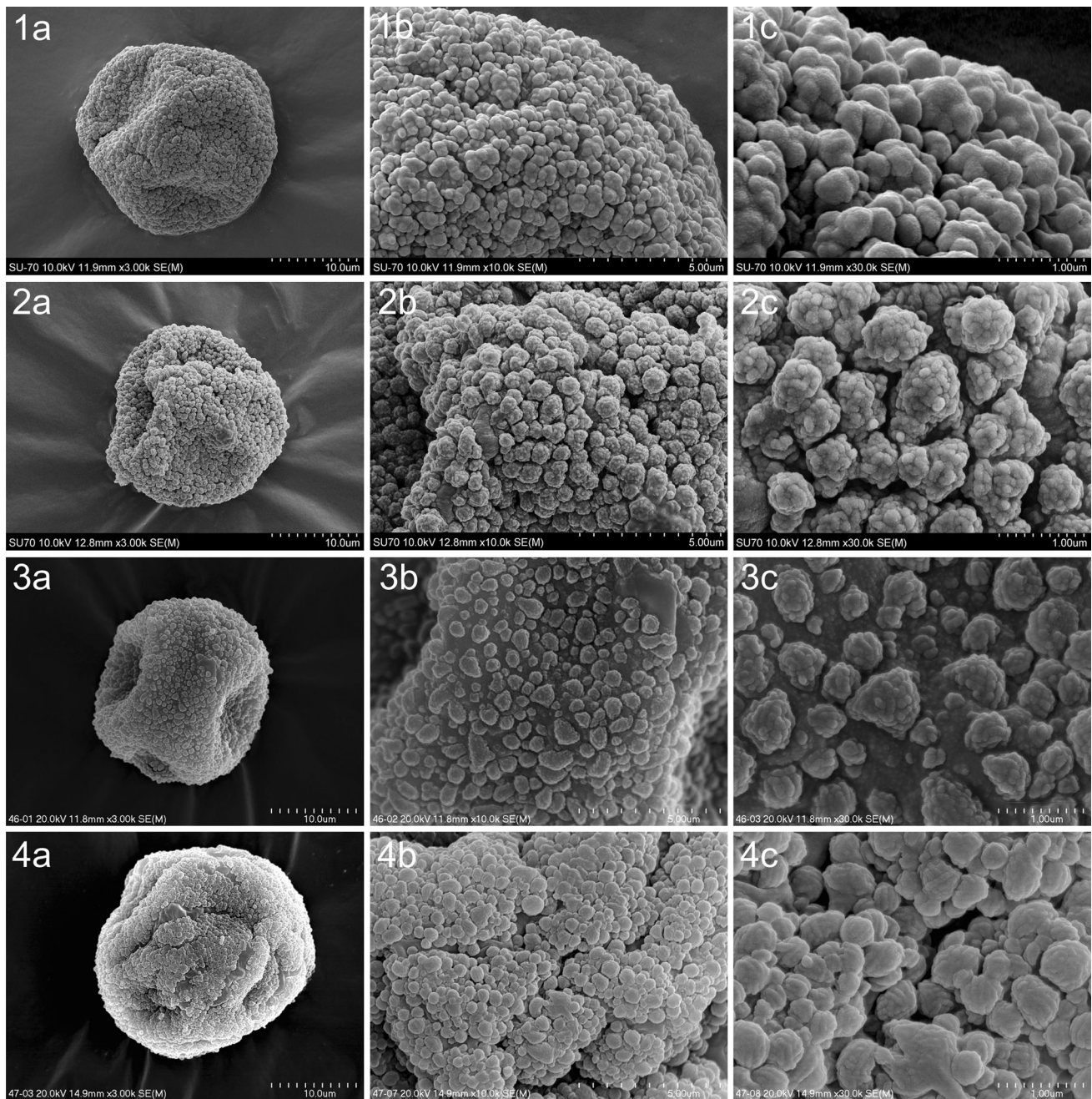


Fig. 4 Pollen grains and its micromorphology in: *Orobanche caryophyllacea* (1), *O. alba* subsp. *alba* (2), *O. lutea* (3), *O. flava* (4); **a** general habit of pollen grain (magnification $\times 3,000$), **b, c** microstructure of pollen grain (magnification $\times 10,000$ and $\times 30,000$)

are parasites of Apiaceae (Piwowarczyk 2011, 2012c; Piwowarczyk et al. 2014). In another example, *O. pallidiflora*, the species is treated differently in various studies: as a separate taxon, or in the rank of subspecies or variety within *Orobanche reticulata* (Beck von Mannagetta 1890, 1930; Zázvorka 2000; Pusch and Günther 2009). *O. reticulata* and *O. pallidiflora* differ in morphology, habitat and altitudinal ranges as well as host preferences (e.g. Kreutz 1995; Piwowarczyk et al. 2010). Our study showed that

O. pallidiflora has thinner exines and smaller equatorial and polar axes than *O. reticulata*, along with differences in sculpture. *Orobanche bohémica* as a separate species was first described by Čelakovský (1879). It has been assigned to different taxonomic ranks, e.g., that of a variety, by Beck von Mannagetta (1930) and Hayek (1914); as a subspecies of *Phelipanche purpurea*, by e.g. Zázvorka (2000) and Carlón et al. (2008); or as a separate species, by Holub and Zázvorka (1999), Pusch (2006), Pusch and Günther (2009), and

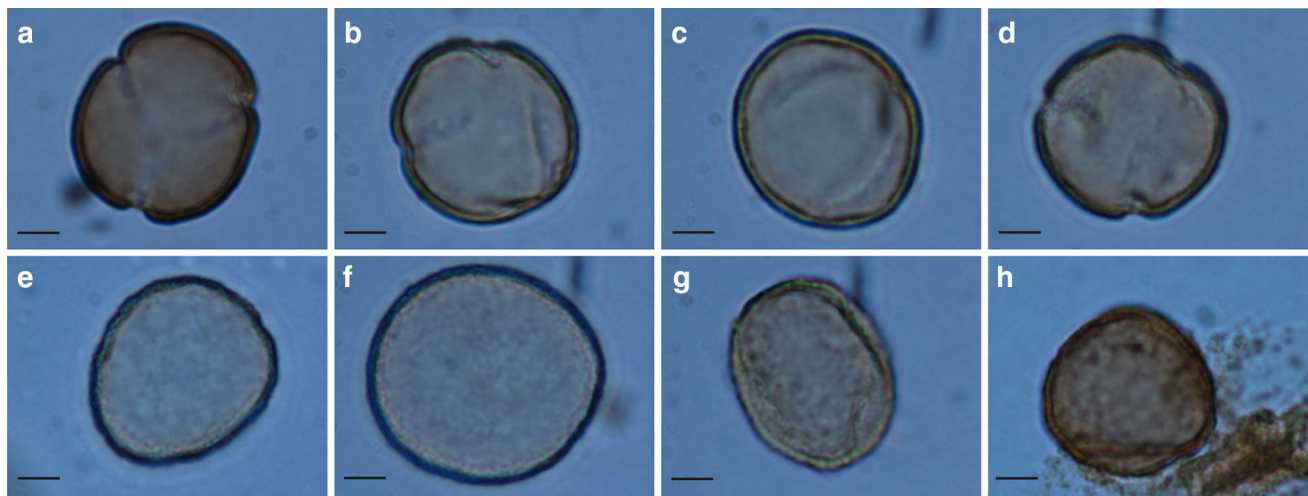


Fig. 5 Pollen grains of *Orobanchella coerulescens* (a), *Phelipanche caesia* (b), *P. arenaria* (c), *P. ramosa* (d), *Orobanchella caryophyllacea* (e), *O. alba* subsp. *alba* (f), *O. lutea* (g) and *O. flava* (h). Scale bar 5 μ m

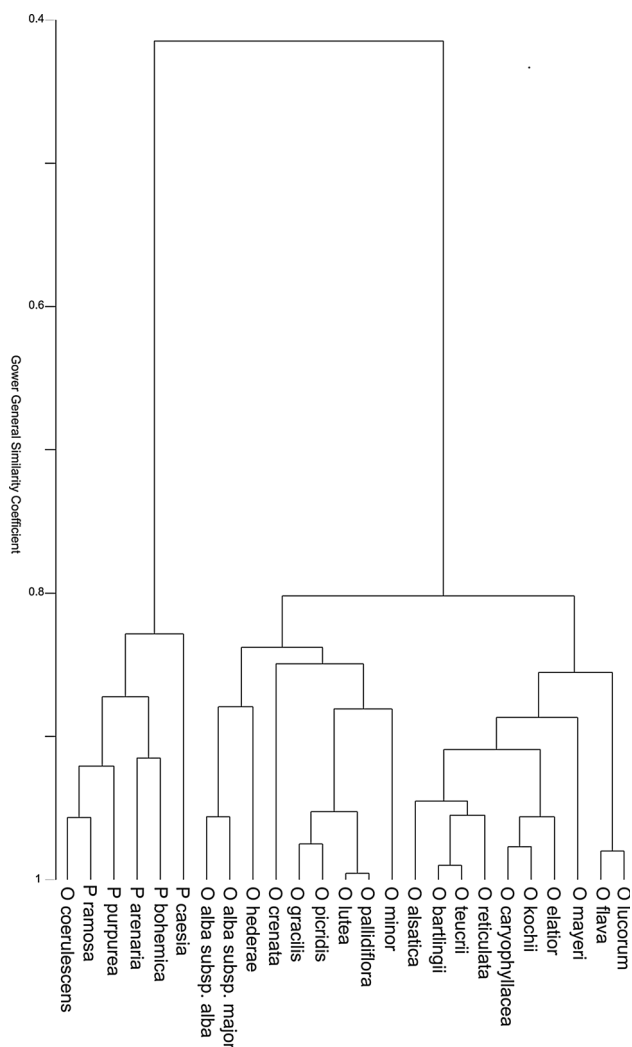


Fig. 6 Cluster analysis (UPGMA method of classification and Gower's general similarity coefficient) performed on seven quantitative and qualitative characters of pollen grains

Piwowarczyk (2012f, g). Palynological analysis also revealed auxiliary micromorphological differences: *P. bohemica* has a thicker exine and larger equatorial and polar axes than *P. purpurea*, along with differences in sculpture. A Central European species, parasitic mainly on *Centaurea*, considered until recently as *O. elatior* s.l. and now recognised as two distinct species, *O. elatior* and *O. kochii* (Zázvorka 2010), also demonstrates subtle differences in the size and level of sculpture of pollen grains (Tables 2, 3, 4).

Our study of pollen morphology showed various characteristics of *Orobanchella coerulescens*. *O. coerulescens* has a Eurasian distribution. It occurs in the Far East, Japan, China, the Caucasus, Latvia, Ukraine and Romania. Very rare in Central Europe, it is known from Slovakia, the Czech Republic, Austria, Germany and Poland. As a typical steppe plant, it has relict features of post-glacial *Artemisia* steppes in the western part of its range. The species is recognised as extinct in the majority of its localities at the western limit of its distribution and its populations are very scarce in Central Europe. It is mainly parasitic on *Artemisia campestris* (Zázvorka 2000; Pusch and Günther 2009; Piwowarczyk 2012d). Traditionally, the species was included in the section *Orobanchella*, grex *Coerulescentes* Beck (Beck von Mannagetta 1890), or, according to the new classification, in the section *Inflatae* (Beck) Tzvel., subsection *Coerulescentes* (Beck) Teryokhin (Teryokhin et al. 1993). This subsection contains about 10 species, poorly known and often lacking a very clear taxonomic position, of mainly steppe parasites involving the genus *Artemisia*. They are mostly endemic, occurring mainly in eastern Asia and Russia (Teryokhin et al. 1993; Piwowarczyk 2012d). We have shown that the pattern of pollen grains of *O. coerulescens* clearly belongs to a *Phelipanche* tricolpate type of pollen with microreticulate

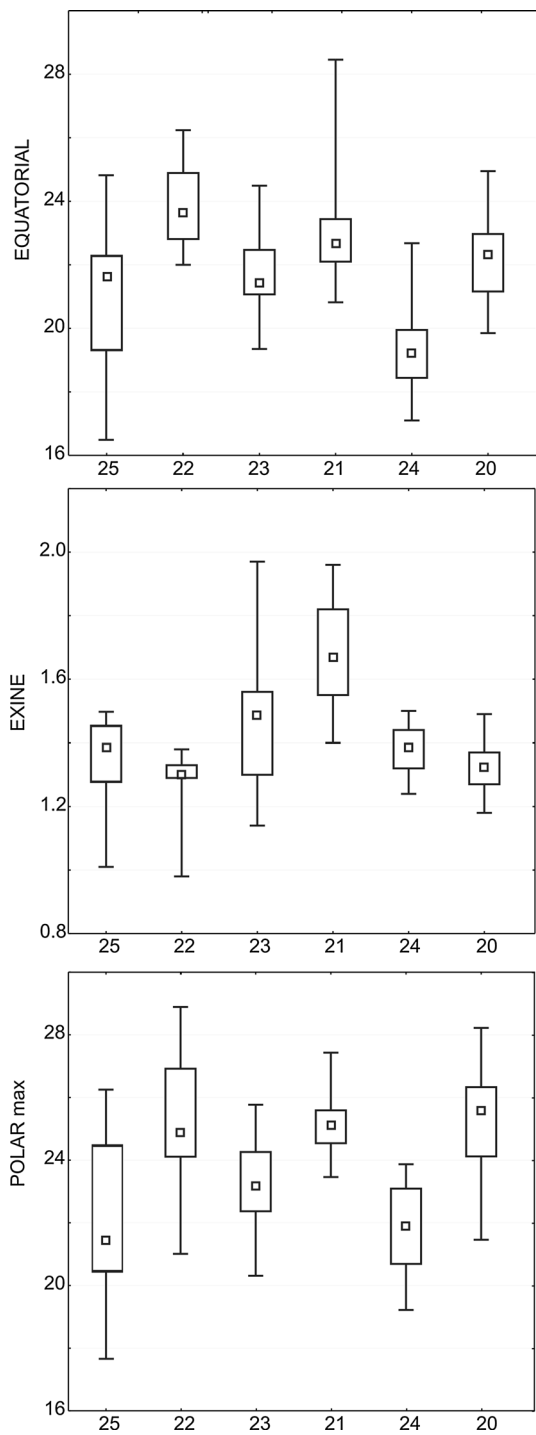


Fig. 7 Box and whisker plots of selected macromorphological characters of tricolpate *Phelipanche*-like pollen grains. Points indicate the mean value (open square), boxes represent 25 and 75 % percentile, and range (whiskers) represents 1 and 99 % percentile. Numbers indicated examined species (comp. Table 1)

sculpture (Figs. 3, 5). Previously, microreticulate sculpture of the exine was known only in *Cistanche* and *Phelipanche* (Zare et al. 2013). At the same time this contradicts the classification of the pollen of *O. coerulescens* by Zare et al.

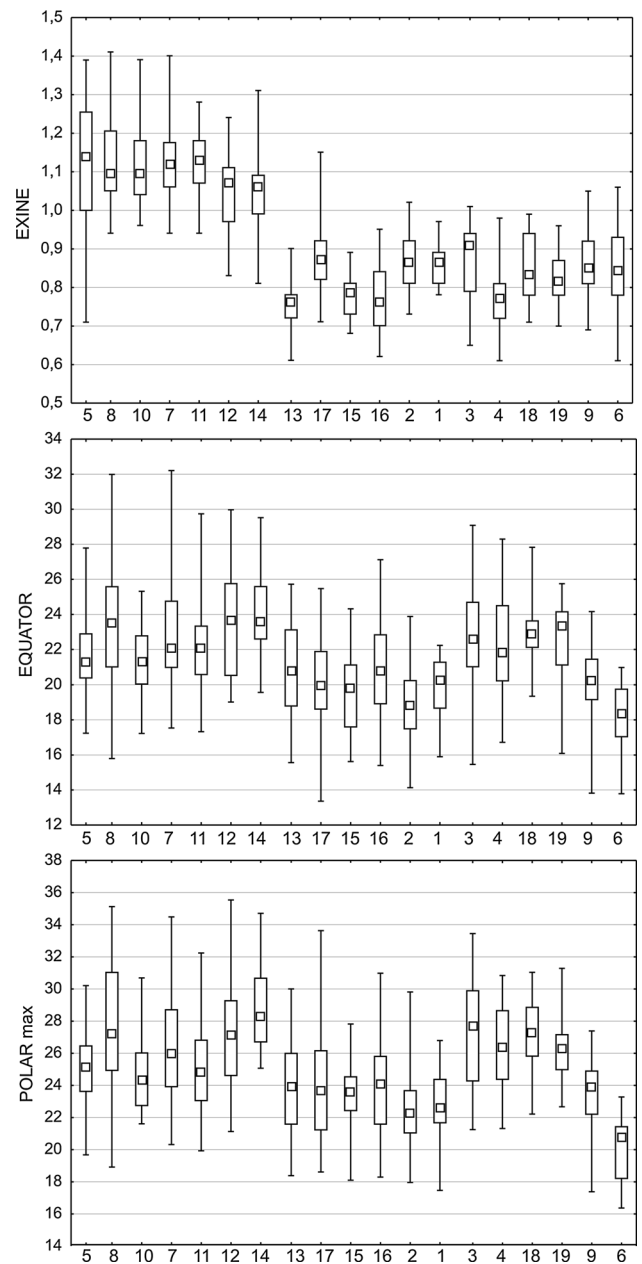


Fig. 8 Box and whisker plots of selected macromorphological characters of inaperturate *Orobanche*-like pollen grains. Points indicate the mean value (open square), boxes represent 25 and 75 % percentile, and range (whiskers) represents 1 and 99 % percentile. Numbers indicated examined species (comp. Table 1)

(2013) into the inaperturate type. Macromorphological characteristics indicate its intermediate position between *Orobanche* and *Phelipanche*. *O. coerulescens* is quite easy to distinguish from related species because of its characteristic thick stem, especially near the inflorescence, and numerous tiny, geniculately bent, pale blue flowers. The entire plant, the stem in particular, is very hairy and, when dry, looks as if covered with mould (Piwowarczyk and Przemyski 2009). Features that place it within *Orobanche*

Table 5 A comparison of the main characters distinguishing *Orobanche*, *Phelipanche*, *Boulardia* and *Orobanchella*

Character	Genus			
	<i>Orobanche</i>	<i>Phelipanche</i>	<i>Boulardia</i>	<i>Orobanchella</i>
Bracteoles	Absent	2 bracteoles under or attached the calyx	Absent	Absent
Stem	Simple	Branched or simple	Simple	Simple
Calyx	Divided into 2 lateral segments	Cylindrical or campanulate with 4–5 teeth	Divided into 2 lateral segments	Divided into 2 lateral segments
Fruit	Style persists, lateral opening	Style falls off, top opening	Style persists, lateral opening	Style persists, lateral opening
Colour of flowers	Yellow, red, brown	Usually blue, violet	Violet, purple, rarely white–pink, yellow	Light-blue, blue–violet
Colour of stigma and anthers	Yellow, orange, purple, brown	Usually white, rare yellow–white	Usually white, rare yellow–white	Usually white, rare yellow–white
Type of pollen				
Aperture	Inaperturate	Tricolpate	Inaperturate	Tricolpate
Sculpture of the exine	Granulate	Microreticulate	Granulate	Microreticulate
Type of seeds	Outer tangential wall pitted or rarely smooth	Wall with fibrillar ornamentation	Wall with fibrillar ornamentation	Periclinal wall granulate rugged or smooth, very rare weakly pitted
Chromosomes	$n = 19$	$n = 12$	$n = 38$	$n = 19$

are: absence of bracteoles, calyx divided into 2 lateral segments, style persist, lateral opening, number of chromosomes $n = 19$; however, it is close to *Phelipanche* by virtue of such features as: blue or violet flowers, white stigma and anthers, and the presence of tricolpate pollen with microreticulate sculpture (Table 5). In the work of Manen et al. (2004), Schneeweiss et al. (2004b), and Weiss-Schneeweiss et al. (2006), it is shown for the first time to be a member of the Eurasian lineage *O. coerulescens*. Phylogenetic relationships also show a degree of separation of *O. coerulescens* from the rest of the species of the section *Orobanche* and place it in an intermediate position between *Orobanche*, *Boulardia* and *Phelipanche* (Schneeweiss et al. 2004a; Piednoël et al. 2012).

Summarising the above, we proposed to transferred *Orobanche coerulescens* to a new genus *Orobanchella*. A comparison of the main characters distinguishing *Orobanche*, *Phelipanche*, *Boulardia* and *Orobanchella* is presented in Table 5.

New genus and combination

Orobanchella R. Piwowarczyk, M. Nobis & J. Madeja **gen. nov.**

Type: *Orobanchella caerulescens* (Stephan in Willdenow) R. Piwowarczyk, M. Nobis & J. Madeja

Orobanchella coerulescens (Stephan in Willdenow) R. Piwowarczyk, M. Nobis & J. Madeja **comb. nov.**

Basionym: *Orobanche coerulescens* Stephan in Willdenow, Sp. Pl. 3(1): 349, 1800.

Type: “Habitat in Sibiria versus Mare Caspium”. Willdenow, 1800 (p. 349). According to Novopokrovskij and Tzvelev, 1958 (p. 72), described from area in vicinity of lake Inder “iz raiona Inderskogo ozera” (W Kazakhstan) (type in LE).

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