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Research paper

Pollen morphology of European bladderworts (*Utricularia* L., Lentibulariaceae)

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ABSTRACT

The pollen morphology of the seven known European bladderworts: *Utricularia australis*, *Utricularia bremii*, *Utricularia intermedia*, *Utricularia minor*, *Utricularia ochroleuca*, *Utricularia stygia* and *Utricularia vulgaris* was studied.

Their pollen grains, coming from different populations, were investigated using both light microscopy and scanning electron microscopy to give data on size, shape (P/E ratio), number of colpori and exine ornamentation: important diagnostic characteristics for *Utricularia* pollen.

Within the investigated species, the pollen grains were usually medium sized (~30 μm), sub-isopolar, radially symmetric and zonocolporate. For the non-fruiting species *Utricularia bremii*, *Utricularia stygia* and *Utricularia ochroleuca*, the grains were often malformed, asymmetric or in the form of gigapollen or micropollen. A significant number of gigapollen grains were observed in *Utricularia stygia* while micropollen was observed in *Utricularia ochroleuca*. The shape of the normal grains was variable from suboblate to prolate spheroidal and they were (10)-11-18-(19)-zonocolporate. The prevalent ornamentations were psilate (on mesocolpi) and fossulate (on apocolpium) except for *Utricularia bremii*, which had a somewhat perforate ornamentation. The pollen of *Utricularia stygia* is described here for the first time. A pollen key, based on these micromorphological data, is presented for European *Utricularia* species.

1. Introduction

Utricularia L. is the largest genus of the family Lentibulariaceae. Taylor (1989), with a monograph on this genus, reported 214 species but Fleischmann (2012) has more recently increased this number up to at least 228 currently accepted species. These rootless carnivorous plants, commonly known as bladderworts, have been reported on all continents except Antarctica and they occupy aquatic, terrestrial and epiphytic habitats (Taylor, 1989; Król et al., 2012). Taylor (1989) recognized only six species for Europe: *Utricularia australis* R. Br., *Utricularia bremii* Heer, *Utricularia intermedia* Hayne, *Utricularia minor* L., *Utricularia ochroleuca* R. W. Hartm. and *Utricularia vulgaris* L., as he tentatively treated under *Utricularia ochroleuca* the seventh species, *Utricularia stygia* G. Thor, which had been described contemporarily (Thor, 1988). All European bladderworts are aquatic perennials, having filiform, branched foliar organs bearing small traps designed mainly to attract, capture and digest live microzooplankton. The flowers are zygomorphic and yellow; the fruits are capsules. Bladderworts usually grow in nutrient-poor habitats with shallow standing waters such as small lakes, ponds, oligotrophic marshes and *Sphagnum* bogs and their distribution is highly fragmented. *U. australis*, *U. bremii*, *U. ochroleuca* and *U. stygia* are considered non-fruiting as they do not produce seed (Thor, 1988; Taylor, 1989).

The published data on the pollen morphology of bladderworts are based mainly on light microscopy observations (e.g., Huynh, 1968; Casper and Manitz, 1975). *Utricularia* pollen grains are released as free monads, permanent tetrads have been observed only in *Utricularia punctata* (Thanikaimoni, 1966) and *Utricularia quelchii* (Huynh, 1968; Taylor, 1989). *Utricularia* pollen is described as fairly homogeneous for the ornamentation's pattern with the exception of two South American species, *Utricularia oliveriana* and *Utricularia neottioides*, which present a microechinate pattern across their whole surface (Lobreau-Callen et al., 1999). The pollen grains of the European species are suboblate-prolate, polycolpor(oid)ate with 13 colpi in *Utricularia minor*, 16 in *Utricularia intermedia* and 18 in *Utricularia vulgaris* (Erdtman, 1952). Scandinavian populations of *Utricularia australis* (under the name of *Utricularia neglecta*) were described by Erdtman et al. (1961). The grains of *Utricularia bremii* were reported as heteropolar and spiraperturate but also malformed as frequent and deep disturbations occur during microsporogenesis (Huynh, 1968; Casper and Manitz, 1975; Käsermann and Moser, 1999). According to Casper and Manitz (1975), gigapollen, with a strikingly large diameter

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of 35–75 μm, have been observed mainly in *Utricularia ochroleuca* and *U. australis* but more rarely in *U. vulgaris* and *U. intermedia*. SEM observations were provided by Lobreau-Callen et al. (1999) and Danylyk et al. (2007) for *U. australis* and by Tsymbalyuk et al. (2008) who described all Ukrainian species (*U. australis*, *U. minor*, *U. intermedia* and *U. vulgaris*).

There is scant data on fossil pollen from the pre-Quaternary period comparable with those of modern *Utricularia*, while there are many reports from Pleistocene and Holocene deposits (Halden, 1917; Jonas, 1952; Iversen, 1954; Godwin, 1956; Nakamura and Tsukada, 1960; Zagwijn, 1960; Thanikaimoni, 1966; Bartlett and Barghoorn, 1973; Sohma, 1975a,b).

The aims of this study were: a) to increase the knowledge of the pollen biology and morphology of European bladderworts in association with plant reproduction and b) to compile a pollen key based on micromorphological and comparative data resulting from the study of different populations of bladderworts as a valuable tool for constructing pollen diagrams.

2. Materials and methods

Flowers of the seven European species of *Utricularia*, originating from different populations, were collected (and then dried) from the field or ex situ cultures (Table 1) from the Spring and Summer seasons between 2003 and 2011. The plants were identified according to Casper (1974), Thor (1979, 1988), Pignatti (1982), Taylor (1989), Moeslund et al. (1990) and Aeschimann et al. (2004). Only flowers at their anthesis were picked, with ~10 flowers taken from each population.

The *exsiccata* are housed in the Herbarium Universitatis Mediolanensis (MI), Department of Biosciences, University of Milano, Italy. The *exsiccata* of *Utricularia bremii*, originated from Mercurago (Italy), are kept in the Herbarium of Museo Regionale di Scienze Naturali (MRSN), Torino, Italy. Those of *Utricularia vulgaris*, originated from Doubrava (Czech Republic), are housed in the Herbarium of the Department of Botany and Zoology of the Masaryk University (BRNU), Brno, Czech Republic. The palynological terminology used is according to Punt et al. (2007) and Hesse et al. (2009).

2.1. Light microscopy (LM)

Pollen grains were acetolyzed according to Erdtman (1960) and the measures of P (= polar axis) and E (= equatorial diameter) of the pollen grains, mounted in glycerine jelly, were taken at a 100× immersion objective lens under a Leitz Wetzlar (Germany) microscope, equipped with a Leica camera. The mean of P and E and the ratio P/E, used to determine the shape, were obtained from the measurements of 50 pollen grains in the equatorial view for each population. In case of species (and populations) with prevalent malformed grains, the number of measurable grains was lower. This was true for all populations of *Utricularia bremii* and *Utricularia stygia* and the Beuren population of *Utricularia ochroleuca* (see Table 2).

2.2. Scanning electron microscopy (SEM)

Acetolyzed pollen, air dried, was sputtered with 25 nm layer of gold in argon plasma (AGAR Automatic Sputter Coater B7341 equipped with a quartz crystal thickness monitor) and then was studied using a Leo 1430 and a Cambridge Stereoscan 360 scanning electron microscope.

3. Results

3.1. LM survey (Plate 1; Table 2)

The mature pollen grains of the European species of *Utricularia* are released as free monads of medium size (~30 μm). The color of fresh pollen is whitish.

The grains are usually isopolar or sub-isopolar (Plate 1, figs. 1, 2, 6–15, 17, 18) and zonocolporate. Pollen from *Utricularia bremii* (Plate 1, figs. 3–5), *Utricularia stygia*, here described for the first time (Plate 1, fig. 16), and more rarely *Utricularia ochroleuca*, can be strongly

Table 1
Sampling and ecology of the examined taxa.

Species	Site	Elevation (m a.s.l.)	Habitat
U. australis R. Br.	Lago di Annone (Italy)	224	Meso-eutrophic lake
	Lago di Caldaro (Italy)	216	Mesotrophic lake, sedge stand
	Pian di Gembro (Italy)	1350	*Sphagnum* bog
	Suchdol nad Lužnicí, S Bohemia (Czech Republic)	454	Shallow sand-pit
	Lagoni di Mercurago, Arona (Italy)	290	Swamp
	Lago di Candia, Candia Canavese (Italy)	226	Marshes
	Parco “Le folaghe”, Casei Gerola (Italy)	70	Pond
U. bremii Heer	Lago di Caldaro (Italy)	216	Mesotrophic lake, sedge stand
	Suchdol nad Lužnicí, S Bohemia (Czech Republic)	454	Shallow sand-pit
	Lagoni di Mercurago, Arona (Italy),	290	Cultivated ex situ at Rea Botanic Garden, Trana (Italy), 450 m a.s.l.
	Pian del Tivano, Nesso (Italy)	946	Peat bog
U. intermedia Hayne	Nový Vdovec fishpond, Třeboň, S Bohemia (Czech Republic)	430	Peat bog
	W Ohlstadt, Upper Bavaria (Germany)	650	Ditches in wet meadow
	Výtopa fishpond, S Bohemia (Czech Republic)	445	Peat bog
U. minor L.	Pian di Gembro (Italy)	1350	*Sphagnum* bog
	Vizír fishpond, S Bohemia (Czech Republic)	440	Peat bog
	Fetzachmoos, N Beuren, Isny im Allgäu, Baden-Württemberg (Germany)	700	*Sphagnum* bog
	Hliníř fishpond, S Bohemia (Czech Republic)	420	Peat bog
U. ochroleuca Hartman	W Ohlstadt, Upper Bavaria (Germany)	650	Ditches in wet meadow
	Fetzachmoos, N Beuren, Isny im Allgäu, Baden-Württemberg (Germany)	700	*Sphagnum* bog
	Nadějský fishpond, Klec, S Bohemia (Czech Republic)	425	Peat bog
U. stygia Thor	Vizír fishpond, S Bohemia (Czech Republic)	440	Peat bog
	Fetzachmoos, N Beuren, Isny im Allgäu, Baden-Württemberg (Germany)	700	*Sphagnum* bog
	Karštejn fen, S Bohemia (Czech Republic)	430	Fen pools
	Hliníř fishpond, S Bohemia (Czech Republic)	420	Peat bog
	Rod fishpond, Frahelž (Czech Republic)	430	Peat bog
U. vulgaris L.	Oasi “Le Foppe”, Trezzo sull'Adda (Italy)	210	Eutrophic pools in old shallow clay-pit
	La Polada, Lonato del Garda (Italy)	160	Peat bog
	Doubrava, Hodonin, S Moravia (Czech Republic)	180	Collection in the Institute of Botany, Třeboň (Czech Republic), 430 m a.s.l.

Table 2
Biometric measures, based usually on 50 pollen grains for each population. Abbreviations: P = polar axis; E = equatorial diameter; SO = suboblate; OS = oblate spheroidal; PS = prolate spheroidal; A/M = asymmetric/malformed.

Species	P (μm)	E (μm)	P/E	Shape	Sexin ornamentation	Number of colpori
U. australis	30.7 ± 0.16 (22.5–39.6)	31.6 ± 0.14 (26.1–40.5)	0.98	OS	Psilate–fossulate	(10)-11-15-(16)
U. bremii[a]	28.4 ± 0.99 (25.7–32.4)[a]	33.2 ± 1.17 (27.9–36.0)[a]	0.86[a]	A/M	Perforate	10-13(-14)
U. intermedia	30.5 ± 0.23 (22.5–37.8)	34.5 ± 0.20 (28.8–42.3)	0.88	SO	Psilate–fossulate	(11)-12-15(-16)
U. minor	28.1 ± 0.19 (21.6–34.7)	26.8 ± 0.14 (22.1–31.5)	1.05	PS	Psilate–finely fossulate	(10)-11-14(-15)
U. ochroleuca	28.5 ± 0.47 (17.6–38.7)	29.9 ± 0.32 (20.7–39.6)	0.95	OS	Psilate–fossulate	(11)-12-14-(15)
U. stygia[b]	30.2 ± 0.65 (26.1–35.1)[b]	29.7 ± 0.59 (26.1–36.0)[b]	1.02[b]	A/M	Psilate–fossulate	(10-11)-12-14-(15)
U. vulgaris	36.8 ± 0.21 (29.7–42.3)	35.6 ± 0.19 (30.6–42.3)	1.04	PS	Psilate–fossulate	15–19

[a] Over 95% of asymmetric/malformed (A/M) grains, based on the measurements of 6 normal grains in equatorial view.
[b] Over 95% of asymmetric/malformed (A/M) grains, based on the measurements of 15 normal grains in equatorial view.

heteropolar and malformed, with the sporadic presence of grains having anomalous dimensions i.e. gigapollen much larger than the average and micropollen much smaller. In our samples, micropollen (diameter ~ 20 μm) were observed in *U. ochroleuca* (Plate 1, fig. 13), while gigapollen (diameter ~ 45 μm) were quite numerous in *U. stygia* (Plate 1, fig. 16).

Normal pollen are characterized by colpori in which each aperture is associated with a colpus that is perpendicular to the equator; the mesocolpium appears thickened on the equator (Plate 1, figs. 1, 6, 7, 9, 11, 15, 17). The number of colpori is quite variable with a minimum of 10 in *Utricularia australis*, *Utricularia minor* and in the normal grains of *Utricularia bremii*, and a maximum of 19 in *Utricularia vulgaris* (Plate 1, fig. 18). Asymmetric pollen grains of *U. bremii*, *Utricularia ochroleuca* (from Beuren, Germany) and *Utricularia stygia* show a large number of anomalous colpori and in some cases the grains are spiraperturate (Plate 1, figs. 3–5, 16). The population of *U. ochroleuca* from Beuren showed a greater number of malformed pollen grains when compared with those from the other two populations from Ohlstadt (Germany) and the Nadějský fishpond (Czech Republic).

Many forms of syncolpism are present in all of the species, while the ones characterized as normal pollen have only sporadic anastomosing colpori approaching their polar region. The species with mainly deformed grains often have a very irregular pattern of colpori across the whole grain.

The pollen grains of *Utricularia* in polar view have a circular equatorial outline with the exception of some malformed grains of *Utricularia bremii*, *Utricularia ochroleuca* and *Utricularia stygia* which have an elliptical outline.

The shape (Table 2) of normal grains varies from suboblate (*Utricularia intermedia*) to prolate spheroidal (*Utricularia minor* and *Utricularia vulgaris*, Plate 1, fig. 17).

3.2. SEM survey (Plates 2-IV)

Pollen grains of *Utricularia* are usually radially symmetric and zonocolporate. A large amount of asymmetric grains is present in *Utricularia ochroleuca* (especially in the population from Beuren); *Utricularia bremii* and *Utricularia stygia* almost exclusively have asymmetric grains. The colpus membrane is smooth or rarely covered with sporadic granular elements (Plate 2, fig. 3, 9). The tectum is continuous and psilate with rare perforations on the mesocolpium (which can be thickened at the equator) or discontinuous and fossulate with the presence of irregular perforations on the apocolpium. The polar regions of *Utricularia australis*, *Utricularia intermedia*, *U. ochroleuca* (normal grains) and *U. vulgaris* can be slightly different in terms of their extension and the pattern of the fossulate ornamentation. *U. bremii* has a nearly continuous tectum with a prevalent perforate ornamentation (Plate 2, fig. 5-9).

3.2.1. U. australis (Plate 2, 1–4)

The grains are oblate spheroidal, radially symmetric, subisopolar and zonocolporate with (10)-11-15-(16) colpori. The profile of the colpus margin is regular and there are sporadic granules on the colpus membrane; anastomosing colpori are occasional near the polar region. The polar regions are slightly different: one pole has a wider surface and a more complex pattern of fossulae which are also more numerous if compared with the other pole. On mesocolpium (thickened on the equator), the ornamentation of the tectum is psilate with sporadic perforations (diameter ~0.1–0.3 μm). The number of perforations increases towards the apocolpium where the ornamentation becomes fossulate. Therefore, the whole ornamentation is psilate–fossulate.

3.2.2. U. bremii (Plate 2, 5–9)

The grains are from ellipsoidal to spheroidal, asymmetric, heteropolar and they often appear deformed. Irregular anastomosing colpori are very frequent on the whole surface and the grains often appear spiraperturate. Few normal grains have been observed and they are zonocolporate with 10-13(-14) colpori. The tectum is nearly continuous and the ornamentation is perforate (perforations ~0.1–0.3 μm).

3.2.3. U. intermedia (Plate 3, 1, 2)

The grains are suboblate, radially symmetric, subisopolar and zonocolporate with (11)-12-15-(16) colpori. The profile of the colpus margin is regular and there are sporadic granules on the colpus membrane; anastomosing colpori are occasional near the polar region. The polar regions are slightly different in size: one pole has a wider surface and as a consequences a greater number of fossulae than the other pole. The ornamentation of the tectum is psilate on the mesocolpium thickening at the equatorial region and fossulate on the apocolpium. In this species, the fossulate pattern of the ornamentation has a wide extension as it involves a part of the mesocolpium and ends just before the equator. Therefore the whole ornamentation is psilate–fossulate.

3.2.4. U. minor (Plate 3, 3, 4)

The grains are prolate spheroidal, radially symmetric, isopolar and zonocolporate with (10)-11-14(-15) colpori. The profile of the colpus margin is regular and there are sporadic granules on the colpus membrane; anastomosing colpori are rare. The ornamentation of the tectum is psilate on the thickened equator, perforate between the equator and the poles (perforation diameter 0.1–0.3 μm) and finely fossulate on polar regions. Therefore the whole ornamentation is psilate–finely fossulate.

3.2.5. U. ochroleuca (Plate 3, 5–7)

The grains are oblate spheroidal, radially symmetric, subisopolar and zonocolporate with (11)-12-14-(15) colpori. Asymmetric, spiraperturate and micropollen grains have been frequently observed, especially in the population from Beuren (Germany). The profile of the colpus margin is regular and there are sporadic granules on the colpus membrane; anastomosing colpori are very frequent near the polar region in normal grains and on the whole surface on malformed grains. The polar regions are slightly different in size: one pole has a wider surface and as a consequence a greater number of fossulae than the other pole. The ornamentation of the tectum is psilate with sporadic perforations (diameter 0.1–0.3

μm) on the mesocolpium (thickened on the equator), the number of perforations increases towards the apocolpium and the ornamentation becomes fossulate. Therefore the whole ornamentation is psilate–fossulate.

3.2.6. U. stygia (Plate 4, 1, 2)

The grains are from ellipsoidal to spheroidal, asymmetric, heteropolar and often malformed. Irregular anastomosing colpori are very frequent and the grains appear often spiraperturate; a large number of gigapollen grains have been observed. The rare normal grains are zonocolporate with (10-11)-12-14-(-15) colpori; the tectum is nearly continuous and the ornamentation is psilate on the mesocolpium (thickened on the equator) and fossulate on the apocolpium.

3.2.7. U. vulgaris (Plate 4, 3, 4)

The grains are prolate spheroidal, radially symmetric, subisopolar and zonocolporate with 15–19 colpori. The profile of the colpus margin is regular and there are sporadic granules on the colpus membrane; anastomosing colpori are occasional near the polar region. The polar regions are slightly different in size: one pole has a wider surface and as a consequence a greater number of fossulae than the other pole. The ornamentation of the tectum is psilate with sporadic perforations (diameter 0.1–0.3 μm) on the mesocolpium, the number of perforations increases towards the apocolpium and the ornamentation becomes fossulate. In this species the fossulate pattern of the ornamentation has a wide extension because it involves a part of the mesocolpium and ends just before the equator. Therefore, the whole ornamentation is psilate–fossulate. Table 2 summarizes the main features of the grains.

4. Discussion

In Europe, especially in lowland areas, *Utricularia* species have become rare due to habitat loss, degradation, eutrophication (caused mainly by agricultural activities) and drainage. These plants colonize endangered oligo-mesotrophic and dystrophic habitats which are nowadays protected by the European Commission (e.g. "Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora"). The formation of these habitats in the Holocene is investigated using pollen diagrams; the pollen of bladderworts can be found in the sediments of current wetlands in which plant succession has changed the habitats of the shallow water into a now unsuitable environment or, in some cases, former wetlands which have been drained. For all these reasons, improving the knowledge of the characteristic organisms of these habitats can lead to remarkable progress: our research is exhaustive in all of the palynological aspects of all seven European species of *Utricularia*; the previous data were incomplete and based mainly on light microscopy.

Our analysis of the investigated species confirmed the general typology of the pollen grains of *Utricularia* in terms of the number of colpori, the shape (P/E ratio), the exine ornamentations and described for the first time the pollen of *Utricularia stygia*.

Confirming the results of previous works (Erdtman, 1952; Erdtman et al., 1961; Thanikaimoni, 1966; Huynh, 1968; Casper and Manitz, 1975; Lobreau-Callen et al., 1999; Danylyk et al., 2007; Tsymbalyuk et al., 2008), the pollen grains of European *Utricularia* are released as free monads, which are sub-isopolar (isopolar in *Utricularia minor*), polyaperturate and zonocolporate; some species are characterized by having a large number of asymmetric and malformed grains. Observed anomalies of these grains may be in their size (presence of gigapollen and micropollen), their shape (P/E ratio cannot be calculated as normal) and in the patterns of the colpori.

In this work, pollen grains (less than 5%) with unusual size have been observed only in *Utricularia stygia* (gigapollen) and *Utricularia ochroleuca* (micropollen) while Casper and Manitz (1975) observed gigapollen in all the species that they studied except *Utricularia minor* and they observed micropollen also in *Utricularia australis* and *Utricularia bremii*.

The shape of the normal grains varies from suboblate in *Utricularia intermedia* to prolate-spheroidal in *Utricularia vulgaris* and *Utricularia minor*; the shape can also be irregularly spheroidal for the species with malformed grains. The number of colpori varies from 10 to 19, the lower value was observed in *Utricularia australis*, *U. minor* and in some rare normal grains of *Utricularia bremii* while 19 were observed only in *U. vulgaris* (Table. 2). The main ornamentation is psilate–fossulate, psilate on the thickened equator and fossulate on the polar regions; *U. minor* presents a more finely fossulate pattern than the other species investigated while *U. bremii* presents a perforate ornamentation.

Some authors reported the pollen of European bladderworts as being normal or, in some species, as having a wide range of malformed grains (Huynh, 1968; Casper and Manitz, 1975; Käsermann and Moser, 1999). In particular, Huynh (1968) stated that the phenomenon of syncolpism is quite frequent in the genus *Utricularia*, this has also been observed in our investigations for all species but it reaches a particularly high level of complexity in the species having malformed grains. Our observations show that in *Utricularia bremii* and *Utricularia stygia*, over 95% of the pollen has a large number of malformed and anomalous grains; the colpori anastomoses which characterize these grains are irregularly distributed across their entire surface making it impossible to distinguish between the polar and the equatorial regions. *Utricularia ochroleuca* has a lower rate of malformed grains for the populations

Plate I. LM micrographs (acetolyzed pollen). (see on page 26)

Scale bars: 1–12, 14, 15, 17, 18 = 10 μm; 13 = 25 μm; 16 = 100 μm.

U. australis: 1. pollen grain in equatorial view; 2. polar view of a 12-colporate pollen grain.

U. bremii: 3–5. asymmetric and malformed pollen grains; 6. a rare non-malformed pollen grain in equatorial view. *U. intermedia*: 7. pollen grain in equatorial view; 8. polar view of a 15-colporate pollen grain.

U. minor: 9. pollen grain in equatorial view; 10. polar view of a 13-colporate pollen grain.

U. ochroleuca: 11. pollen grain in equatorial view; 12. polar view of a 13-colporate pollen grain; 13. group of pollen grains, a micropollen with a normal pattern of colpori, is visible (arrow).

U. stygia: 14. polar view of a 14-colporate pollen grain; 15. pollen grain in equatorial view; 16. group of pollen grains, an asymmetric and malformed gigapollen is visible (arrow).

U. vulgaris: 17. pollen grain in equatorial view; 18. polar view of a 18-colporate pollen grain.

Plate II. SEM micrographs of acetolyzed pollen grains. (see on page 27)

Scale bars: 1, 2, 5–7 = 10 μm; 3, 8, 9 = 2 μm; 4 = 1 μm.

U. australis: 1. pollen grain in equatorial view; 2. polar view of a 16-colporate pollen grain; 3. polar region showing a fossulate ornamentation, perforations are also visible (arrows); 4. equatorial region near a porus, sporadic perforations can be present on the psilate ornamentation (arrow).

U. bremii: 5–7. asymmetric and malformed pollen grains; 8. polar region showing a perforate ornamentation; 9. pollen wall with a perforate ornamentation.

Plate III. SEM micrographs of acetolyzed pollen grains. (see on page 28)

Scale bars: 1–3, 5–7 = 10 μm; 4 = 1 μm.

U. intermedia: 1. pollen grain in equatorial view; 2. polar view of a 15-colporate pollen grain.

U. minor: 3. pollen grain in equatorial view; 4. finely fossulate ornamentation on polar region.

U. ochroleuca: 5. pollen grain in equatorial view; 6. polar view of a 14-colporate pollen grain; 7. malformed pollen grain from Beuren (Germany).

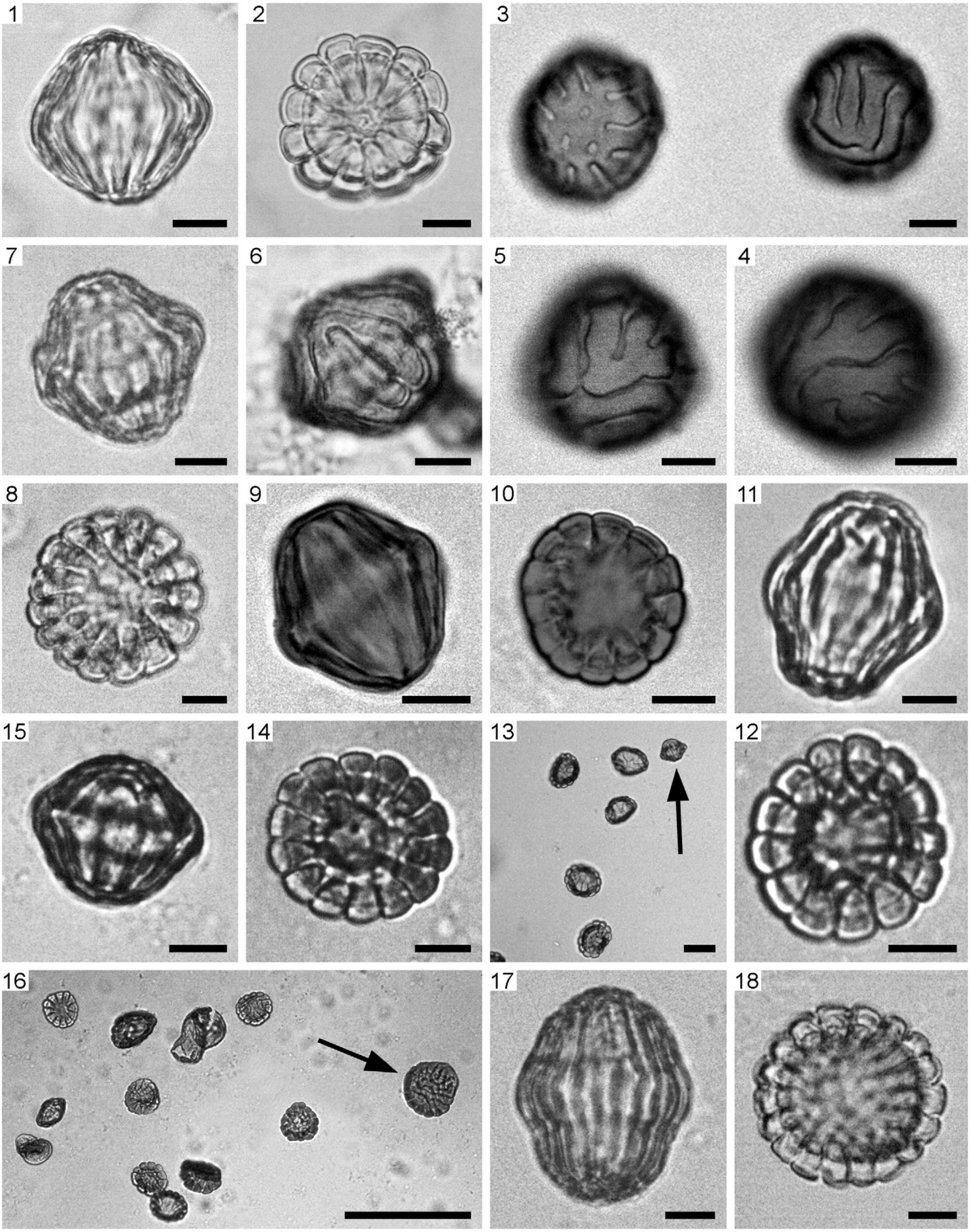

Plate I *(caption on page 25).*

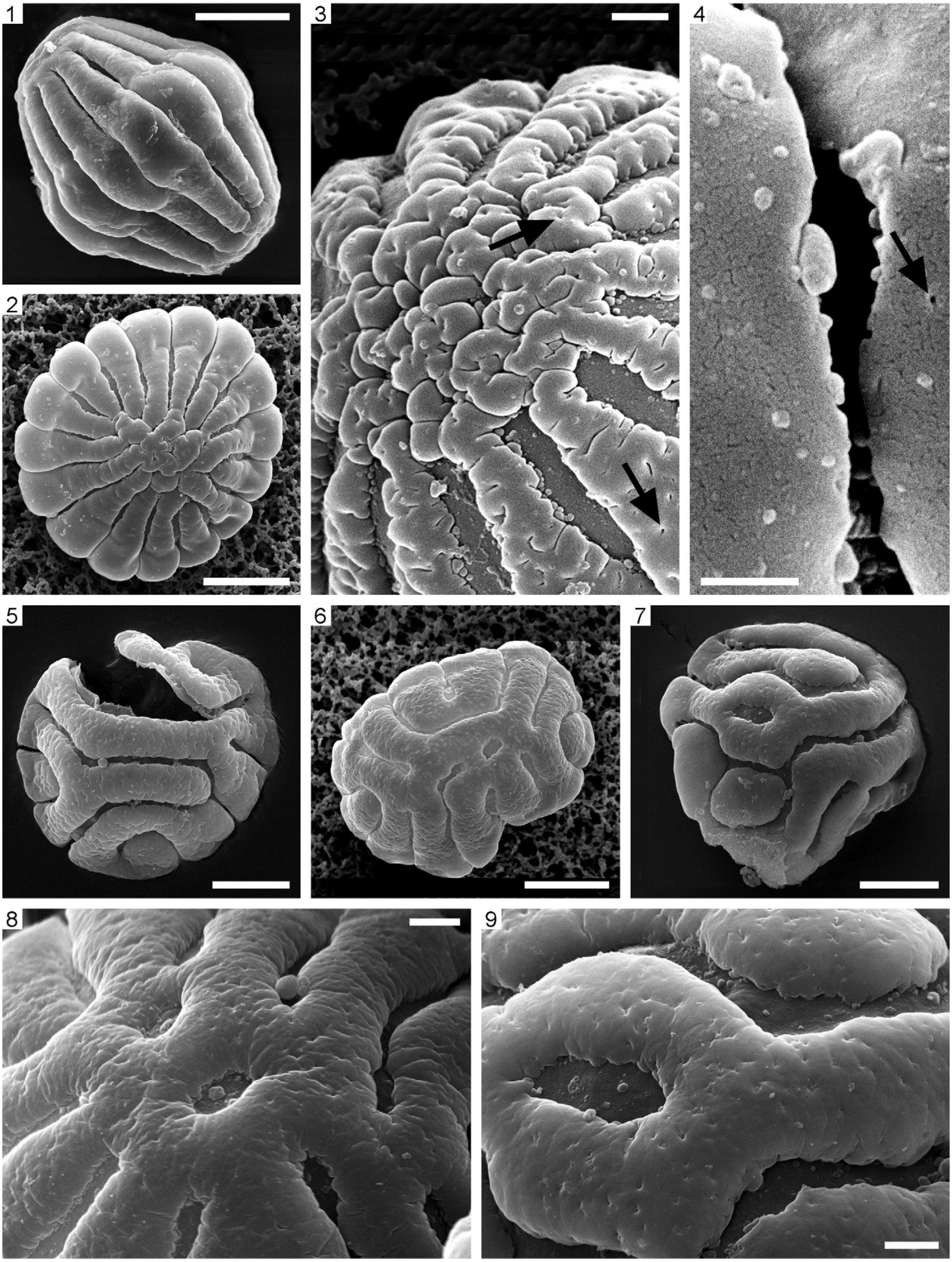

Plate II *(caption on page 25).*

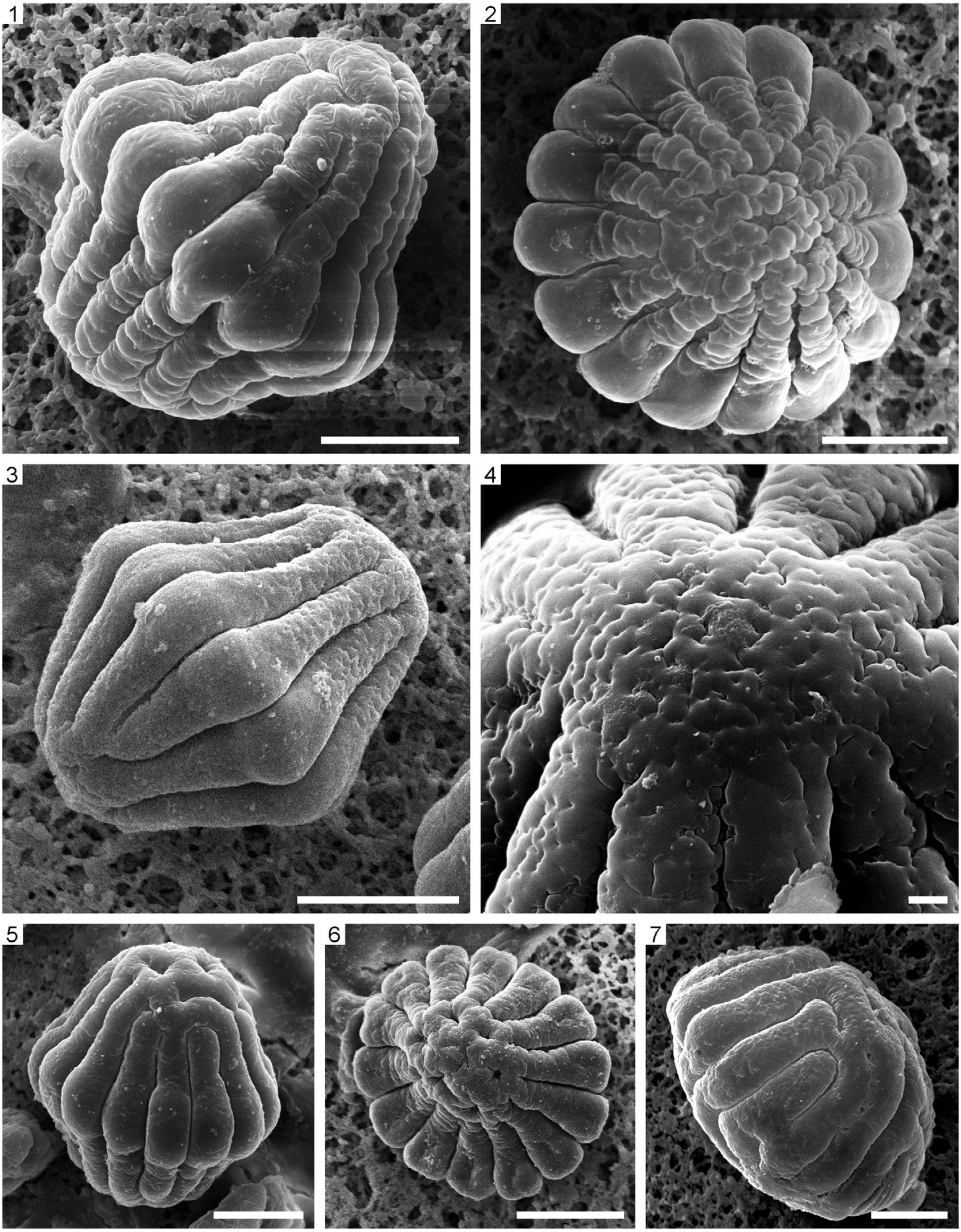

Plate III *(caption on page 25).*

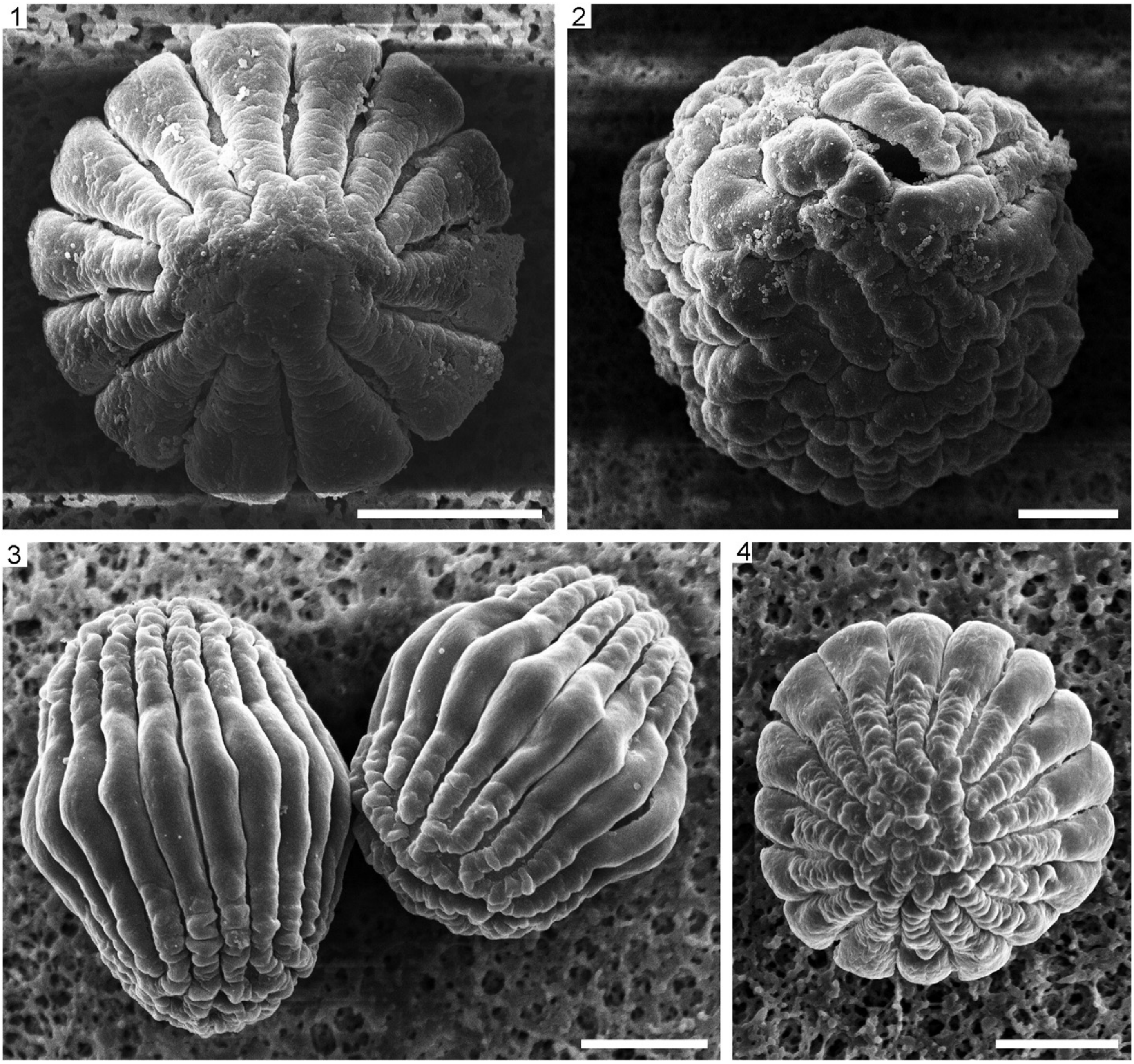

Plate IV. SEM micrographs of acetolyzed pollen grains.
Scale bars: 1–4 = 10 μm.
U. stygia: 1. polar view of a 13-colporate pollen grain; 2. asymmetric and malformed pollen grain.
U. vulgaris: 3. pollen grains in equatorial view; 4.
U. vulgaris: polar view of a 17-colporate pollen grain.

Table 3
Pollen key of European *Utricularia* species.

A: Prevalence of asymmetric and malformed grains	
Perforate ornamentation	*U. bremii*
Psilate-fossulate ornamentation	*U. stygia*
B: Presence of normal as well as asymmetric and malformed grains	*U. ochroleuca*
C: Normal grains	
P and E > 35 μm; prolate-spheroidal grains, colpori number usually ≥ 15	*U. vulgaris*
P and E < 35 μm; colpori number usually ≤ 15	
P and E < 30 μm; prolate-spheroidal grains	*U. minor*
30 < (P and E) < 35 μm; suboblate or oblate-spheroidal grains	
Oblate-spheroidal grains, fossulate ornamentation only on the apocolpium; psilate elsewhere	*U. australis*
Suboblate grains, psilate ornamentation only on the equatorial region of the mesocolpium; fossulate elsewhere	*U. intermedia*

from Ohlstadt (Germany) and the Nadějský fishpond (Czech Republic) while the population of Beuren (Germany) has almost the same rate of malformation observed in *U. bremii* and *U. stygia* (Table. 2; Plate 1, 3-5, 13, 16; Plate 2, 5-7; Plate 3, 7; Plate 4, 2). Furthermore, *Utricularia australis*, *U. bremii*, *U. ochroleuca* and *U. stygia* are documented to be sterile as they do not produce seed (Thor, 1988; Taylor, 1989). Fruiting plants of *U. bremii* have been observed in only one ex situ population of plants which came from Lake Onega, Russia (Adamec, 2002). The sterile species present anomalous grains with the exception of *U. australis*, which presents morphologically normal grains. The sterility of these species needs further investigation based on the stages of sporogenesis and gametogenesis. Experiments in which sterile *U. australis* plants were crossed with a fertile species (*Utricularia vulgaris*) as a pollen donor failed. In a greenhouse, pollen of ripe *U. vulgaris* flowers (from Doubrava, Czech Republic) was transferred onto 71 ripe flowers of *U. australis* (Třeboň, Czech Republic). After 3–4 weeks, seed-set was analyzed. Of the 71 pollinated flowers, only 11 flowers from 10 inflorescences set seed with 24 seeds in total collected. However, these seeds were smaller than those of *U. vulgaris* and have never germinated, indicating that the embryos were not developed (Adamec, unpubl. res.).

Finally, we provided the diagnostic characteristics (size, shape, number of colpori and exine ornamentation) necessary to draw up a pollen key for the identification of the different species (Table 3). This pollen key is an important tool for pollen diagrams and consequently for the reconstruction of paleo-wet oligotrophic-environments through the Holocene.

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