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# Morphological characterisation and phylogenetic placement of the very rare species *Psathyrella sulcatotuberculosa*

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Battistin E., Chiarello O., Vizzini A., Örstadius L., Larsson E. (2014) Morphological characterisation and phylogenetic placement of the very rare species *Psathyrella sulcatotuberculosa* – *Sydowia* 66 (2): 171–181. A recent find of the very rare species *Psathyrella sulcatotuberculosa* (*Psathyrellaceae*) from Italy provided the authors with new data useful for improving the knowledge of an almost mysterious entity. Of this collection, a detailed morphological description, a statistical analysis of the spore size along with several photographs of the basidiomata in field and microscopical structures are provided and compared with the features of the type collection. Morphologically and/or molecularly allied species are discussed. Phylogenetic analysis of *Psathyrellaceae* based on sequence data from the nuclear ribosomal DNA internal transcribed region and partial large subunit placed *P. sulcatotuberculosa* in the *P. candolleana* clade with strong statistical support, together with *P. candolleana*, *P. badiophylla*, *P. leucotephra* and *P. typhae*. The result is also supported by morphology, where the species in the clade all lack pleurocystidia but have clavate, utriform and lageniform cheilocystidia with a rounded apex.

**Keywords:** *Basidiomycota*, *Agaricales*, ITS sequences, molecular phylogeny, taxonomy.

*Psathyrella* (Fr.) Quél. (*Psathyrellaceae* Vilgalys, Moncalvo & Redhead) may be considered as one of the most difficult agaricoid genera to study because of the fragile nature of the basidiomata, the high number of species (about 400 taxa worldwide according to Kirk *et al.* 2008) and the occurrence of often subtle macro- and micromorphological differences among taxa. The traditional infrageneric classifications and species circumscriptions relied on morphological characters such as: presence/absence of a pseudorhiza and veils; size, colour and shape of spores; the proportion of the two different types of cheilocystidia along lamella edge; presence/absence of pleurocystidia and clamp connections; size, and shape of hymenial cystidia and presence/absence of crystals on their apex; pileipellis structure; veil structure and element size; number of sterigmata on basidia (Smith 1972, Romagnesi 1975, 1982, Kits van Waveren 1985).

Recently, molecular studies revealed that *Psathyrella* is a polyphyletic genus split into many clades (Larsson & Örstadius 2008, Padamsee *et al.* 2008, Vašutová *et al.* 2008, Nagy *et al.* 2011).

During a fieldwork focused on the *Agaricales* of northern Italy the very rare *P. sulcatotuberculosa* was found. It is a particular species: in the field at first sight features like the overall colorations, size, presence of tiny velar remnants on the pileus margin, the lack of an annulus can lead to think it is even a *Pholiotina* species.

The taxonomic framework of *Psathyrella sulcatotuberculosa* is complicated, not only for the aforementioned reasons, but also for the scarcity of specific bibliographic references. The aim of the present study was to provide an emended morphological description of the species on the basis of the Italian collections and to infer its phylogenetic position within *Psathyrella*.

## Materials and methods

### Morphology

Macroscopic description and habitat notations were based upon detailed field notes of fresh basidiomata.

Micromorphological characters were observed using two Nikon Eclipse E200 light microscopes, one of them equipped with phase contrast. Mature spores were measured directly in water at 1000 × magnification or first displayed on a 22" Samsung led monitor by a DCM 510 camera (La Nuova Didattica, Milan, Italy) inserted into the top end of the eyepiece tube of the microscope and the ScopePhoto software (La Nuova Didattica,

Milan, Italy) and then measured through the Mycomètre program (Fannechere 2005). For each parameter, i.e. length, width and Q, the mean value  $\pm$  its standard deviation and the extreme values (in brackets) were calculated by the GraphPad Prism 5.0 program (GraphPad Inc., San Francisco, USA). The notation [n/m/p] indicates that measurements were made on “n” randomly selected spores from “m” basidiomata of “p” collections. For other microscopic structures than spores at least 20 individuals were measured. Technical terms used for describing the morphological characters refer to Vellinga (1998). Digital images were recorded with Nikon Infinity 2 and Nikon Coolpix 5400 digital cameras. Spores were mounted in distilled water and a solution of ammonia while cystidia and other elements were mounted in a solution of ammonia stained with Congo Red before capturing digital images. Colours were coded by the Munsell Soil Color Charts (1975). Authors of fungal names are cited according to the Index Fungorum partnership (2014). Voucher specimens were deposited in MCVE under nr. 27699. Herbarium acronyms follow Thiers (2014) except “LÖ” that refers to the personal herbarium of Leif Örstadius.

#### Molecular methods

The complete ITS region was sequenced for two specimens of *P. sulcatotuberculosa*, the Italian specimen MCVE 27699 and LÖ55-12 from Germany. We tried to get an ITS sequence from the holotype collection (HM 342) but failed. DNA extractions, PCR reactions, and sequencing were performed as described in Larsson & Örstadius (2008). Sequences have been deposited in Genbank (LÖ55-12, acc. nr. KJ138422, and MCVE 27699, acc. nr. KJ138423). The phylogenetic position of *P. sulcatotuberculosa* was inferred using parts of the sequence data set from Larsson & Örstadius (2008), and additional sequences of species belonging to the *P. candolleana* (Fr.) Maire clade available in GenBank, *P. leucotephra* (Berk. & Broome) P.D. Orton and *P. badiophylla* (Romagn.) Park.-Rhodes (FM163226, FM160683) from Nagy *et al.* (2011) and *P. multipedata* (Peck) A. H. Sm. (AM712279, AM712280) from Vasutová *et al.* (2008).

Heuristic searches for the most parsimonious trees were performed using PAUP\* 4.0 (Swofford 2003). All transformations were considered unordered and equally weighted. Variable regions with ambiguous alignment were excluded and gaps were treated as missing data. Heuristic searches with 1000 random-addition sequence replicates and TBR branch swapping were performed. Relative robustness of clades was assessed by the bootstrap method using 1000 heuristic search replicates with 100 random taxon addition sequence replicates and TBR branch swapping, the latter saving at most 100 trees in each replicate.

## Results

#### Phylogenetic analysis

The aligned complete dataset consisted of 54 taxa and 1820 characters. After exclusion of ambiguous regions, mainly from the ITS1 and ITS2, 1413 characters remained for the analysis. Of these, 949 were constant, 114 were variable but parsimony uninformative, and 350 were parsimony informative. The maximum parsimony analysis yielded 107 equally parsimonious trees (length=1659 steps, CI= 0.4400, and RI= 0.6065). One of the equally parsimonious trees is presented as a phylogram in Fig. 1. The bootstrap analysis recovered the two sequences of *P. sulcatotuberculosa* with 100 % bootstrap support in the *P. candolleana* clade, together with *P. candolleana*, *P. badiophylla*, *P. leucotephra* and *P. typhae* (Kalchbr.) A. Pearson & Dennis (Fig. 1).

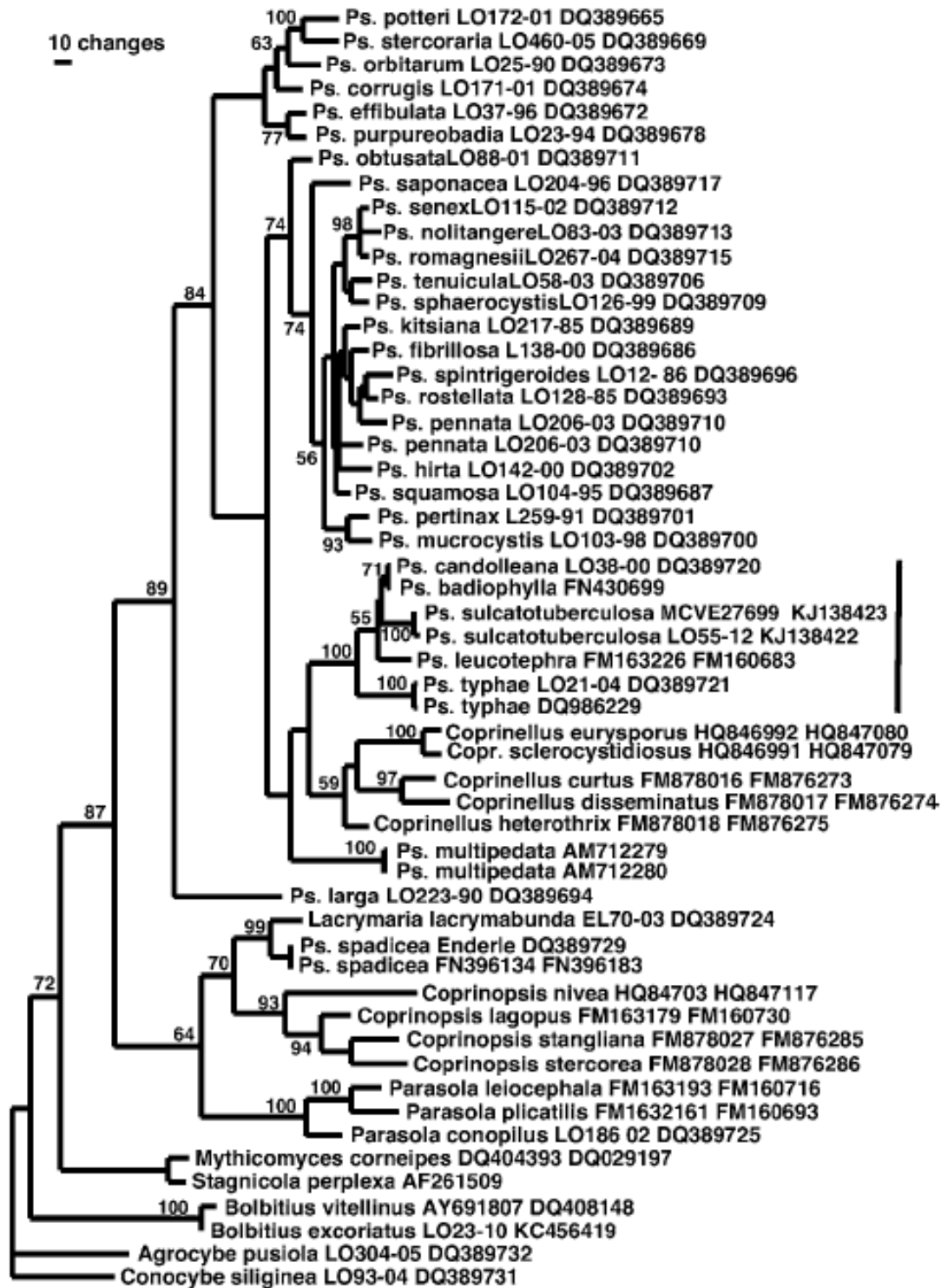


Fig. 1. One of the most parsimonious trees obtained from the maximum parsimony analysis based on ITS and LSU sequence data of *Psathyrellaceae*, showing the position of *P. sulcatotuberculosa*. The *P. candolleana* clade is marked with a scale bar. Bootstrap values are indicated on branches.

## Taxonomy

***Psathyrella sulcatotuberculosa*** (J. Favre) Einhell., Ber. bayer. bot. Ges. 47:123, 1976. – Figs. 2–9.

MycoBank no.: MB 321418

≡ *Psathyrella typhae* var. *sulcatotuberculosa* J. Favre, Mat. Fl. crypt. Suisse 10: 215, 1948 (MB 346363).

≡ *Drosophila typhae* var. *sulcatotuberculosa* (J. Favre) Kühner & Romagn., Fl. Analyt.

Champ. Super.: 366, 1953 (MB 346740) (comb. inval., basionym not given).

Selected descriptions. – Einhellinger (1976: 123); Favre (1948: 152–154); Kühner & Romagnesi (1953: 366).

## Description based on the Italian collections

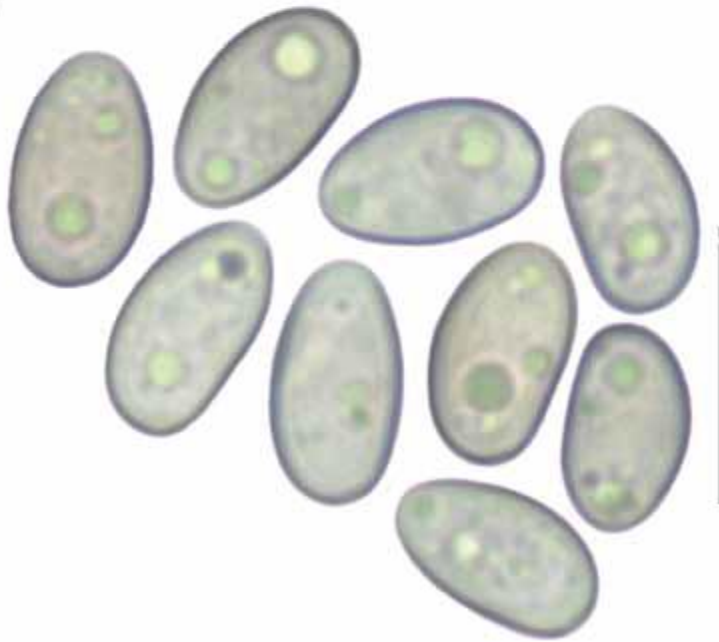
Basidiomata very fragile – Pileus: 8–35 mm wide, conical-convex to convex then more or less applanate, ochre-orange, orange-brown, brownish on drying (Mu. near 5YR 5/8, 7.5YR 5/4, 7.5YR 6/4); surface glabrous, mat, hygrophanous, translucently striate, sulcate-tuberclose up to 2/3 of the radius (similar to some species of *Russula* sect. *Pectinatae*) with small white velar remnants at margin in very young basidiomata. – Lamellae: distant to medium spaced, L= 13–28, interspersed with 1–2 lamellulae, adnexed or adnate, subventricose, up 4 mm broad, brownish (Mu. near 5YR 6/3, 7.5YR 6/4); edge paler and fimbriate. – Stipe: 16–40 × 0.5–2.5 mm, cylindrical, straight or flexuous, equal or thickened towards base, fibrillose, pruinose at apex, hollow, whitish to very pale brown. – Context: scanty, odourless, mild. – Spore print: pale reddish-brown.

Spores: [50/2/2] (6.2)7.4 ± 0.5(8.7) × (3.6)4.4 ± 0.3(5.0) μm, Q = (1.3)1.7 ± 0.1(2.1), oblong, ovoid, ellipsoid, in profile flattened on one side and rarely subphaseoliform, smooth, in water hyaline, very pale brown or yellowish (Mu. 10YR 8/4–8/6), germ pore indistinct to absent. – Basidia: 18.4–22 × 7.5–10 μm, (1, 2)4-spored. – Sclerobasidia: absent. – Cheilocystidia: 20–45(85) × 9–15(25) μm, numerous, polymorph, utriform, lageniform, clavate, pyriform, oblong, ellipsoid, hyaline, thin-walled, deprived of crystals. – Pleurocystidia: absent. – Caulocystidia: 31.7–67 × 8.4–13.4 μm, polymorphic, similar to cheilocystidia. – Pileipellis: an epithelium of subglobose to widely ellipsoid hyaline elements, 30–48 × 23–42 μm, rarely with apical projections and then racket-like and pileocystidioid; pileitrama rather pale. Veil elements 4–10 μm wide, cylindrical, subhyaline or pale brown. – Clamp connections: present. – Phenology: 3 and 7 May 2013.

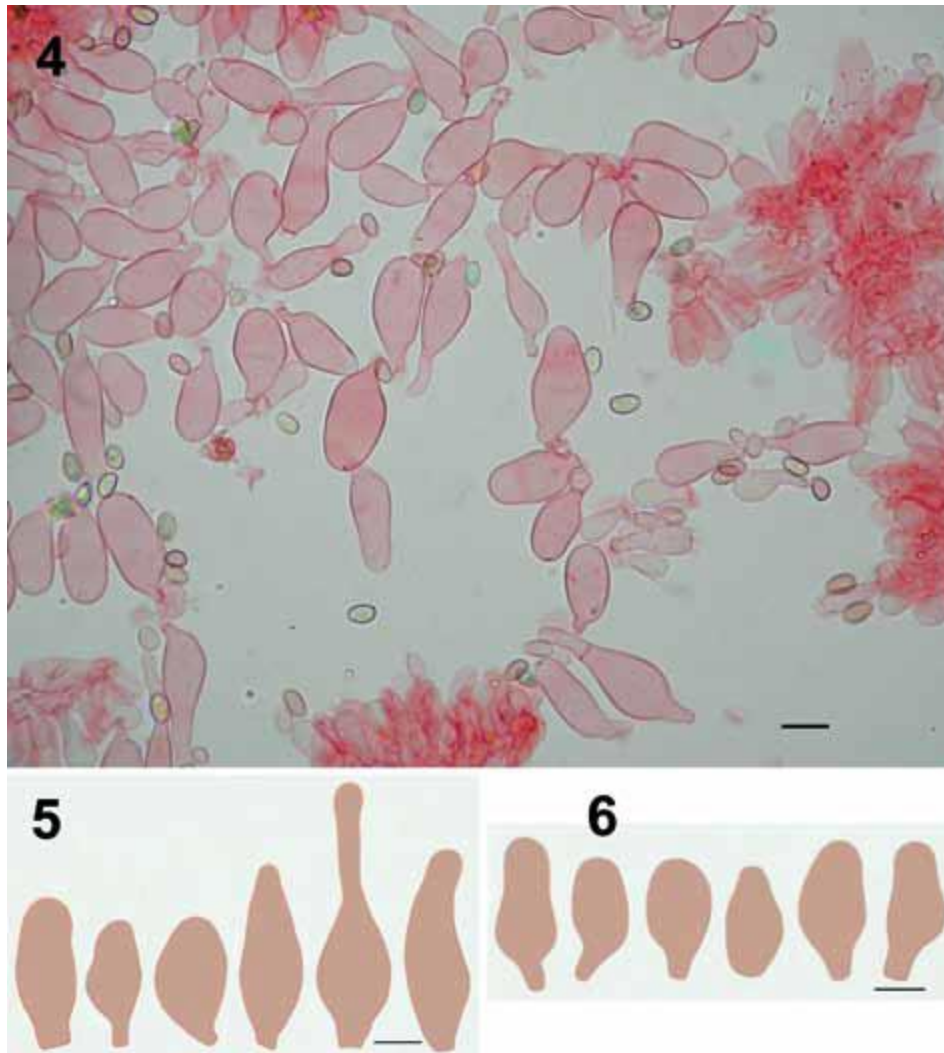
Habitat and distribution. – Solitary or in groups. “Bois des Tailles, à la base des pieds de grands *Carex* vivants, sur les feuilles extérieures pourrissantes” (Favre 1948); “Auf toten *Carex*-Blattspreiten am Rand eines kleinen Großseggenriedes im Übergang zum Kopfbinsenrasen. Hauptmoos an der Fundstelle war *Drepanocladus intermedius*” (Einhellinger 1976); “Auf Humus mit *Sparmannia africana* in einem Wintergarten” (Rave, see below); on soil and vegetal debris in a riparian wood made up of *Salix alba*, *S. triandra* and *Alnus glutinosa*. (Battistin & Chiarello, see below); dry, close to the sea on gravelly soil without vegetation (Dähncke, see below). Rarely reported from Germany, Italy, Switzerland and the Canary Islands (Spain).



3



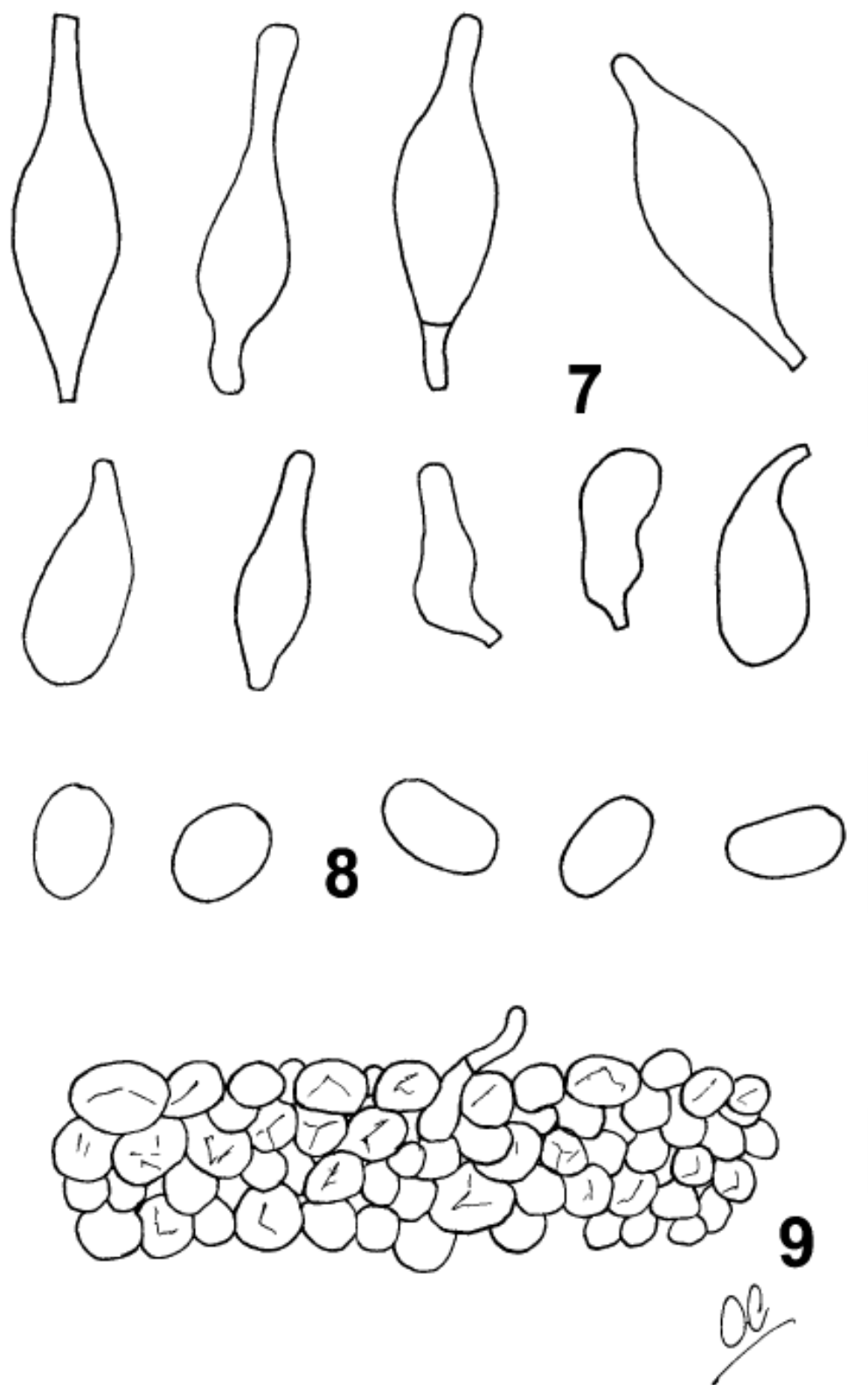
**Figs. 2–3.** *Psathyrella sulcatotuberculosa* (MCVE 27699). **2.** Basidiomata **3.** Spores. Bars: 2 = 20 mm, 3 = 10  $\mu$ m



**Figs. 4–6.** *Psathyrella sulcatotuberculosa*, cheilocystidia. **4, 5.** MCVE 27699. **6.** Holotype (HM 342). Bars: 4–6 = 10  $\mu$ m

**Material examined.** – SPAIN, Canary Islands: La Palma. Cancajos, 10 Dec. 2010, R. M. Dähncke 3163 (herb. Dähncke); GERMANY, Baden-Württemberg. Heidelberg, July 2012, M. Rave (part in herb. LÖ 55-12); ITALY, Vicenza. Trissino, 3 and 7 May 2013, Battistin & Chiarello (herbarium Battistin 20130507/02, and MCVE 27699 ); SWITZERLAND, Canton de Vaud. Bois des Tailles, 4 and 20 June 1941, J. Favre HM 342 (G, holotype).





Figs. 7-9. Line drawings of the microscopical characters (MCVE 27699). 7. Cheilocystidia. 8. Spores. 9. Pileipellis. Bars: 7-8 = 10  $\mu$ m; 9 = 40  $\mu$ m

## Discussion

*Psathyrella sulcatotuberculosa* is distinguished by rather small basidiomata, rudimentary veil, sulcate-tuberculate pileus margin, habitat, pale spores and absence of pleurocystidia. It prefers growing in moist soils even though a collection is reported on dry gravelly soil from Canary Islands (see above). Traditionally, it is included in section *Spintrigerae* (Fr.) Konrad & Maubl. as emended by Kits van Waveren (1985), a section which encompasses species without pleurocystidia and with spores usually shorter than 10  $\mu\text{m}$ . The most prominent character of *P. sulcatotuberculosa* is the very pale brown to hyaline colour of the spores under LM, a rare phenomenon in genus *Psathyrella*. The sulcate pileus was emphasized by Favre (1948) and Einhellinger (1976). We cannot exclude that this feature could be connected with the drying process sometimes occurring in the hygrophanous species of the genus, for example in *P. corrugis* (Pers.) Konrad & Maubl. The features of the Italian collections fit well with those of the type collection (G, J. Favre HM 342) and the other examined specimens, with some exceptions. Pilei 5–20 mm wide and cheilocystidia mainly utriform to clavate, 20–45  $\times$  9–15  $\mu\text{m}$  in size were measured for all known collections of *P. sulcatotuberculosa* except for the Italian ones that differed in having a pileus up to 35 mm wide and larger, versiform cheilocystidia (with numerous fusiform to lageniform elements), 25–85  $\times$  9–25  $\mu\text{m}$ . Spores of the Italian specimens are slightly shorter than those of the other collections. In particular, the statistical comparisons between the spores of the holotypus of *P. sulcatotuberculosa* and the ones from the Italian collections, carried out by the Student's t-test ( $\alpha = 0.05$ ), revealed that the spores of the Italian collection were on average shorter than those ones of the holotypus [(7.6)8.23 $\pm$ 0.35(9.0)  $\times$  (4.4)4.85 $\pm$ 0.21(5.2)  $\mu\text{m}$ ,  $Q = (1.57)1.69\pm 0.07(1.86)$ ] [20/1/1]. Our data highlight that *P. sulcatotuberculosa* can show some variability in morphological features even though the ITS sequences obtained from the Italian and German specimens are identical. Globally more than 100 *Psathyrella* species are described lacking pleurocystidia (Dennis 1961, Singer 1962, 1969, 1978, Smith 1972, Romagnesi 1975, 1982, Kits van Waveren 1985). About one third of them has a spore length of 8  $\mu\text{m}$  on average.

Among the morphologically closely related species, there are *P. typhae*, *P. lacuum* Huijsman and *P. rubiginosa* A. H. Sm. *Psathyrella typhae* differs mainly by the not sulcate pileus margin and the larger spores [(9–11.5)(12.5)  $\times$  (5)5.5–7  $\mu\text{m}$ , Kits van Waveren 1985] [(9)10.2–11.8(12.5)  $\times$  (5.5) 5.9–6.8(8)  $\mu\text{m}$ , [360/18/18], pers. obs. by L. Örstadius]; *P. lacuum* can be separated by a pileus colour being white with a pale grey to brownish grey centre, a veil with dispersed white arachnoid fibrils or flocci and darker spores (L. Örstadius, pers. obs.; Kits van Waveren 1985); *P. rubiginosa* is distinguished by the presence of pleurocystidia and darker spores (L. Örstadius, pers. obs.; Smith 1972). Among recently described species of sect. *Spintrigerae*, *P. halophila* Esteve-Rav. & Enderle was described from Majorca, Spain (Esteve-Raventós & Enderle 1992), where it grew among rests of *Cladium mariscus* in salt-marshy areas. The type collection was examined by L. Örstadius who found the spores slightly larger than in *P. sulcatotuberculosa*, i.e. 8.6–11.4  $\times$  4.8–6.2  $\mu\text{m}$ , mean-value 9.8  $\times$  5.4  $\mu\text{m}$ . Also *P. halophila* lacks pleurocystidia and shares most characters with *P. sulcatotuberculosa* and *P. typhae*. The species should be sequenced in order to ensure its status. *P. submicrospora* Heykoop & G. Moreno, growing on calcareous soil with *Quercus rotundifolia* and *Juniperus thurifera* is distinguished from *P. sulcatotuberculosa* by the amygdaliform to ellipsoid spores which are not hyaline or extremely pale under microscope but reddish yellow (Heykoop & Moreno 2002; Mu. 5YR 6/8, pers. obs. by L. Örstadius). *Psathyrella microsporoides* Heykoop & Moreno (= *P. badiophylla* var. *microspora* Kits van Wav.) has brownish-yellow spores (Mu. 7.5YR 6/6) with a well-developed germ pore (Kits van Waveren 1985, as *P. badiophylla* var. *microspora*; Heykoop & Moreno 2002). The type of *P. badiophylla* var. *microspora* was recently examined by L. Örstadius who consider the taxon to be synonymous with *P. candolleana*.

The phylogenetic analysis (Fig. 1) placed *P. sulcatotuberculosa* in the well-supported *P. candolleana* clade, together with *P. candolleana*, *P. badiophylla*, *P. leucotephra* and *P. typhae* (all are species of sect. *Spintrigerae*). The species in this clade can be characterized by absence of pleurocystidia and presence of clavate, utriform to lageniform cheilocystidia with obtuse apex making the placement, as suggested by the molecular data, in congruence with the morphology.

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