Panaeolus bisporus - an adventitious fungus in central Europe, rich in psilocin

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This is the first record of *Panaeolus bisporus*, originally described from Morocco, in central Europe; and first evidence of psilocybin and psilocin in this species.

Regular visits to parks in urban areas result in surprising records. This was the case in early summer last year, when on the lawn of a churchyard, renewed the year before, a large production of a *Panaeolus* species was observed. It is a first record of this species for central Europe.

Taxonomy

Panaeolus bisporus (Bertault & Malençon) Gerhardt (Figs 1-8)
Copelandia papilionacea var. *bispora* Bertault & Malençon, Flore des Champignons Supérieurs du Maroc 1, p.301. 1970.
Copelandia bispora (Bertault & Malençon) Singer & R. A. Weeks in Weeks et al., J. Nat Products **42**: 472. 1979.
Panaeolus cyanescens var. *bisporus* (Bertault & Malençon) G. Moreno & Esteve-Raventos in Moreno et al., Cryptogamie, Mycol. **12**: 195. 1991.

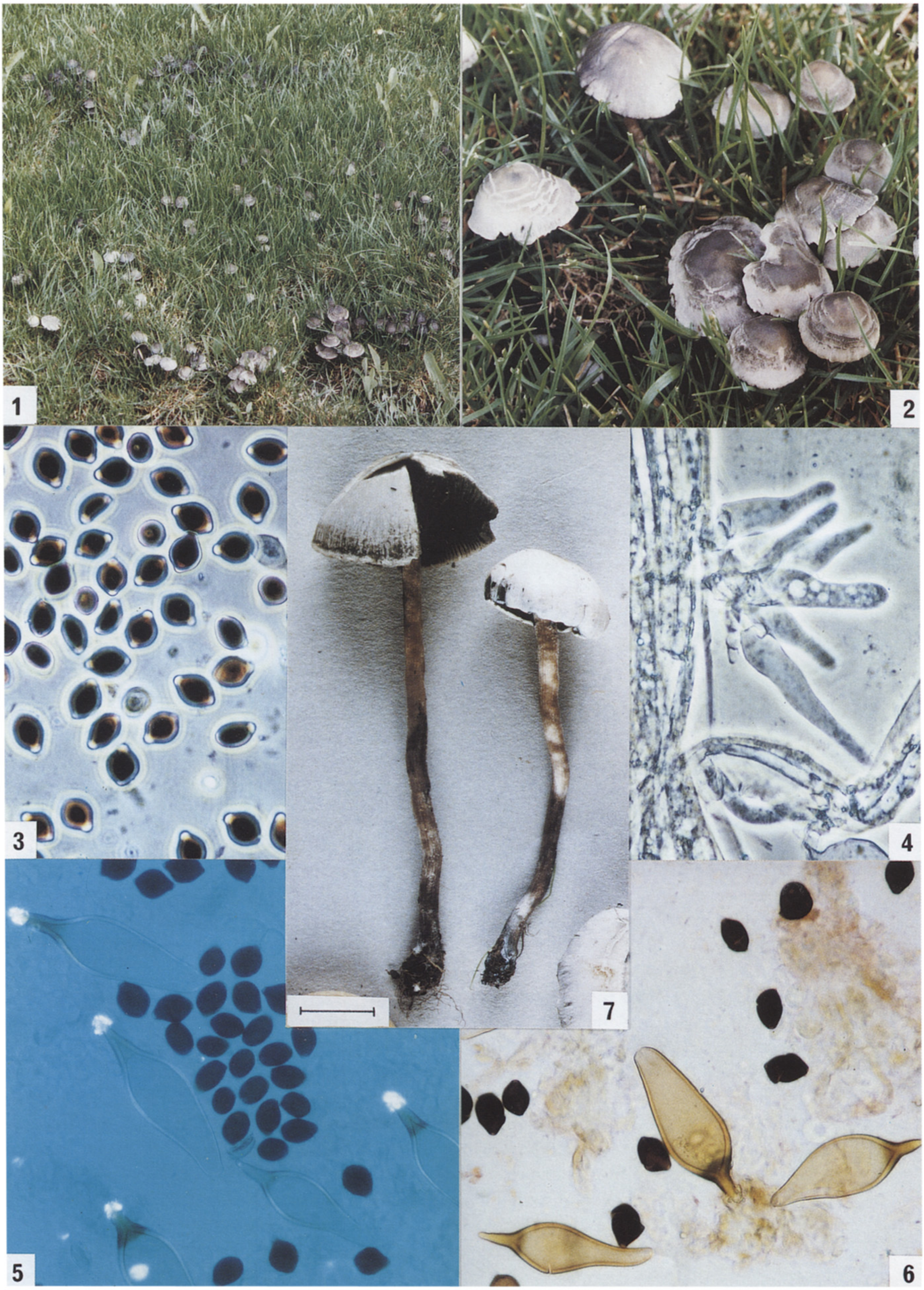
Cap 15-28 mm, semiglobate (Fig 7), campanulate to convex, hardly expanding, with margin first slightly involute later straight or slightly crenate and often getting torn, smooth, not viscid, towards margin slightly wrinkled (best seen half dry), hygrophanous and rather indistinctly translucently striate, dark grey-brown (Munsell 2.5 Y 3/3), with centre more brownish (10 YR 4/3), drying whitish (Munsell 10YR 7/3-8/3). Gills ventricose-adnate, up to 5 mm broad, crowded, grey to olivaceous black with distinct white edge. Stem 4-6.5 cm x 2-3 mm, cylindrical, fistulose, punctate-pruinose from apex to 1/3 towards base, downwards sparsely fibrillose, reddish brown (Munsell 10 YR 5/4-7.5 5/4), towards apex greyish, bluish tomentose at base. Smell of bakery, taste indistinct.

Spores 12-14 x 8-10 x 6-7.5 µm, Q (face-view) = 1.1-1.6, mean volume (calculated) = 560 μm^3, citriform, elliptic in side-view, broadly elliptic-ovoid to obscurely angled in face-view, thick-walled, smooth, almost black in water and alkali, with broad central germ-pore (Fig 3) and distinct apiculus. Basidia 18-21 x 7-9 µm, two-spored, clamped. Cheilocystidia 28-40 x 7-10 µm, fusiform with an obtuse apex, or narrowly utriform to lageniform, hyaline. Pleurocystidia 32-60 x 11-16 µm, as prominent fusoid metuloids (Fig 5-6), with ochre-yellowish thick walls, yellowish in water, hardly staining in congo red, with distinctly olivaceous - greenish amorphous material toward apex, with anisotropic crystals (see Fig 5) often only as amorphous crystalline material. Cap cuticle a hymeniderm of rounded, more or less pedicellate cells intermixed with some fusiform metuloids similar to those on the gills. Stem cuticle of parallel, filamentous hyphae supporting clusters of cystidia similar to those on the gill edge (Fig 4). Clamp-connections present.

Habitat: lawn of the churchyard of Belp near Bern, newly ploughed and sown. Hundreds of fruit bodies appeared in fairy rings (see Fig 1) during the second half of June 1997.

Panaeolus bisporus resembles strongly *Panaeolus (Copelandia) cyanescens (Bk. & Br.) Sacc.*; however, neither the olivaceous tints in the

(Across) Fig 1. Fairy ring of *Panaeolus bisporus.* Fig 2. Caps of *Panaeolus bisporus.* Fig 3. Spores as seen in water under phase contrast. Fig 4. Caulocystidia as seen in water under phase contrast. Fig 5. Metuloids wlth anisotropic crystals as seen in polarized light. Fig 6. Metuloids in water. Fig 7. Fruitbodies of *Panaeolus bisporus*, showing a bluish tint at the base of the stipe; bar=10µm.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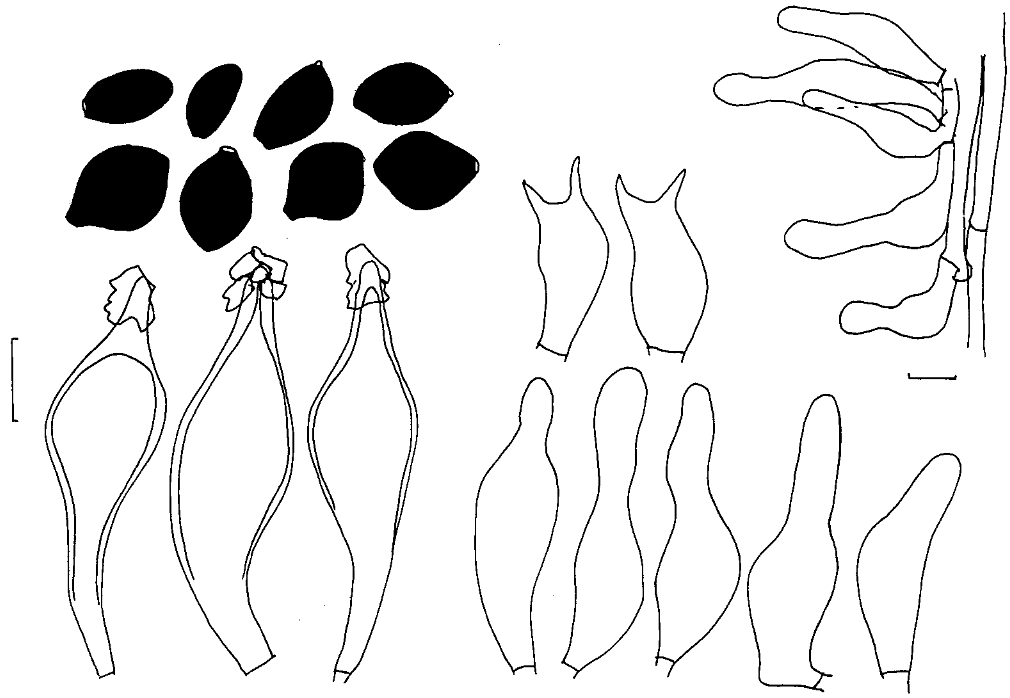

Fig 8. Line drawings of essential microscopic characters of *Panaeolus bisporus;* bar = 10µm.

metuloids nor the metuloids on the cap are mentioned as characters of *P. cyanescens* in the current literature (O'lah 1970, Watling & Gregory 1987, Gerhardt 1996). The basidia are exclusively two-spored, but the spore size is the same as *P. cyanescens* and therefore we agree with Gerhardt (1996) that *P. bisporus* deserves the status of a taxon at species level. The greenish tints in the metuloids are not mentioned for *P. bisporus.* They are a distinctive character of another - American - *Panaeolus* species, *P. chlorocystis.* However, *P. chlorocystis* is characterised by smaller spores ranging from 9 to 12 µm in length.

The bluish tints in our collection are not very obvious. Only very fresh, young fruit-bodies displayed faint blue tints on the cap and often more prominently on the stipe base.

Bresadola (1913) created the genus *Copelandia* for those species with yellowish, ventricose cystidia and an acute apex. In his monograph on the genus *Panaeolus* Gerhardt (1996) argues that no other agaric genus is exclusively based on an anatomical character of the cystidia, which furthermore is not a unique structure in agarics. Metuloids are found in all major families of agarics. Nor is the presence of psilocybin a unique character of *Copelandia* (see Stijve & Kuyper 1985), therefore *Copelandia* does not deserve generic status.

Panaeolus bisporus is mentioned as a typical Iberian-North African taxon by Moreno et al. (1991). Besides the Moroccan type locality this rare species has been found once in Spain, among the grass *Stenotaphrum americanum.* The site in Switzerland has been a favourite spot for fungi of one of us (A.N.) for years and we are sure that this fungus appeared here for the first time. As it grew on a newly ploughed and sown part of this churchyard we think that this fungus can be classified as adventitious, profiting probably from the milder winter months of the last years. Gerhardt (1987) mentions two other *Panaeolus* species rich in tryptamine derivatives with such a sporadic occurrence in Central Europe. As *Panaeolus bisporus* is not known to grow in pure culture nor to be sold on special markets, it can be assumed that the spores reached Central Europe from wild specimens.

Determination of psilocybin

The lyophylised and powdered fruit-bodies of *Panaeolus bisporus* were extracted by methanol and analysed by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) (experimental details are available on request). Psilocybin and psilocin could be identified by GC-MS. The HPLC quantification showed a content of 0.41% psilocin (calculated per dry weight), whereas interestingly only traces of psilocybin and no baeocystin (N-demethyl analogue of psilocybin) were detectable. Psilocin is thought to be a biogenetic or thermal dephosphorylation product of psilocybin, usually the dominating tryptamine alkaloid, formed enzymatically or more often by inappropriate drying and storage. As the fungi specimens were frozen at -20°C shortly after collection, the presence of psilocin is not due to the decomposition of psilocybin. In an earlier study of *Psilocybe semilanceata* (Fr.) Quél. we found that in no specimen was the psilocin content higher than 0.01% (Brenneisen & Borner, 1988).

References

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Mycological Dispatches

I recently subscribed to *Plant Talk* (P.O. Box 500, Kingston upon Thames, Surrey KT2 5XB, UK) and bought the back issue with *Amanita muscaria* on the cover (October, 1995). The cover references p 36-100 Plant Facts for Campaigning Conservationists - an ongoing compendium that, here, provides FACTs 19-30 about Fungi. It's a bit sketchy, and interested mycologists might wish to comment on or add to them. Also in this issue, p 14, the following paragraph appears in a report entitled "Potato Blight: an old problem lives on": "[T]he fungus that causes Potato Blight, *Phytophthora infestans*, continues to visit destruction on crops This summer a new strain of the fungus, believed to have come from Mexico, has swept through the potato fields of Idaho and other western states. It is feared that this strain may reach Europe. Recently, Swiss agronomists reported a strain of the fungus in their country that was able to reproduce sexually. Potato Blight has been a scourge in Europe for a century and a half, apparently on the strength of efficient sexual and parasexual reproduction by the fungus. What may be its potential after a phase of sexual recombination?"

A major article in *The Daily Telegraph* (12 March 1999) entitled "It's just a corker of a controversy", about wine corks vs. plastic closures, had the following statement: "The main issue is the little pest at the very root of this dispute. Produced by fungus within the cork, a chemical compound called TCA is responsible for the distinctive mouldy aroma and flavour that renders a wine 'corked' and therefore undrinkable." An e-mail to The Australian Wine Research Institute produced a reply from Dr Paul Henschke which led me to two articles: one by T. H. Lee & R. F. Simpson entitled "Microbiology and chemistry of cork taints in wine" (in G. H. Fleet, ed., *Wine Microbiology and Biotechnology*, 1993, pp 353-372) identified TCA as 2,4,6-Trichloranisole; Rae Blair, AWRI's Communication and Publicity Manager, also replied, outlining the Institute's extensive research programme on TCA and wine closures. The other reference by N. M. Daly, T. H. Lee & G. H. Fleet entitled "Growth of fungi on wine corks and its contribution to corky taints in wine" (*Food Technology in Australia* **36**, 1984, 22-24) found that the taint ('goût de bouchon') could be imparted by *Penicillium granulatum*, *P. glabrum* or two *Trichoderma* spp. The wine industry is by far and away the largest user of cork. According to *Plant Talk* 17, April 99, p. 18 "The move to plastic may have a knock-on effect upon the considerable stands of semi-natural oak [*Quercus suber*] woodland pasture- known as 'dehesas' in Spain and 'montados' in Portugal- across the Iberian Peninsula. ... These open woods on lime-poor soils support plant communities rich in endemics, including scrub, grassland and winter-wet pools, and are home to mammals, reptiles and birds, notably Imperial Eagle and other raptors. Woodland pasture, with well-spaced pollarded trees, is one of Europe's most ancient systems of land management." But what are the fungi of this unique ecosystem?

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