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## Two new taxa of *Bolbitiaceae* (*Agaricales*) from Croatia

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**Abstract** — A new species *Conocybe caeruleobasis* and a new variety *Pholiotina mairei* var. *stercorea* are described from Croatia. The descriptions are accompanied by black and white photographs of fresh basidiocarps and microscopic elements. The new taxa are compared with closely related taxa.

**Key words** — *Basidiomycota*, biodiversity, mycobiota, taxonomy

### Introduction

The mycobiota of Croatia is poorly known due to the small number of mycologists who have researched the region. Some areas have never been mycologically explored. The first two authors of this paper made a survey of all species of the order *Agaricales* sensu lato recorded for Croatia until 2000 (Mešić & Tkalčec 2002, 2003; Tkalčec & Mešić 2002, 2003a,b). Only 11 species from family *Bolbitiaceae* were recorded (5 species of *Agrocybe*, 2 species of *Bolbitius*, 3 species of *Conocybe*, and 1 species of *Pholiotina*). In 1999, more intensive research started through the “Recording and Mapping of Croatian Fungi” project of Croatian Mycological Society. Thereafter, more than 40 species of *Bolbitiaceae* new to Croatian mycobiota have been found, as well as several taxa new to science. The first new species, *Conocybe thermophila* Hauskn. et al., is already published (Hausknecht et al. 2007). In the present paper we describe *Conocybe caeruleobasis* and *Pholiotina mairei* var. *stercorea* as new taxa.

### Materials and methods

The description of *Conocybe caeruleobasis* is based on one collection consisting of 10 basidiocarps, whereas description of *Pholiotina mairei* var. *stercorea*

is based on one collection consisting of 6 basidiocarps. Photographs of basidiocarps were taken in the field. Descriptions of macroscopic characters were based on observations of fresh basidiocarps. Basidiocarps were preserved by drying. Microscopic features were observed by a light microscope (brightfield) with magnification up to 1500× and photographed with a digital camera. Descriptions and photographs of microscopic characters were made from rehydrated dried specimens mounted in 5% potassium hydroxide (KOH) solution. Some hyaline elements are observed and photographed in Congo red (1% water solution) after pretreatment in 5% KOH. Spore color was also observed in water. Ammoniacal reaction was tested according to Hausknecht (1999). Spore measurements were made from the mounts of lamellae and based on calibrated digital photographs. Only mature spores were measured (chosen on the basis of their color and appearance). For each taxon, two mature basidiocarps were chosen and 100 randomly selected spores were measured (50 from each basidiocarp). Spore measurements (length, width) are given as: (min.) stat. min. – av. – stat. max. (max), where “min.” = minimum (lowest measured value), “stat. min.” = statistical minimum (arithmetic average minus two times standard deviation), “av.” = arithmetic average, “stat. max.” = statistical maximum (arithmetic average plus two times standard deviation), “max.” = maximum (highest measured value). Standard deviation (SD) of spore length and width is also given. The length/width ratio of spores is given as the “Q” value (min. – av. – max.). Holotypes and accompanied data are deposited at the Croatian National Fungarium in Zagreb (CNF), while isotypes are deposited at the Herbarium of University of Vienna (WU). Comparison of *Conocybe caeruleobasis* and *Pholiotina mairei* var. *stercorea* with similar taxa is based on descriptions in the following literature: Arnolds 2005a,b, Hausknecht 1996, 2001, 2002; Hausknecht & Enderle 1992, Hausknecht et al. 2004, Kühner 1935, Ortega et al. 2000, Orton 1960, Prydiuk 2006, Singer 1968, 1989; Watling 1982, 1983.

### Taxonomy

***Conocybe caeruleobasis*** Tkalčec, Mešić & Hauskn., sp. nov.

FIGS. 1, 3–9

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*Pileus* 8–14 mm *latus*, *hemisphaericus* vel *convexus*, *tenuiter hygrophanus*, *humidus rubello-brunneus*, *siccus dilute brunneus* vel *ochraceus*, *non vel ad marginem tantum translucido-striatus*, *glaber*. *Lamellae anguste adnatae* vel *adnexae*, *primum ochraceae*, *demum ferrugineo-ochraceae*. *Stipes* 12–24 × 1.8–2.5 mm, *subcylindricus*, *basi sensim dilatatus* vel *bulbiformis*, *1/4 ad 1/2 partis inferioris in arenam demersa*, *albicans* vel *dilute luteolo-brunneus*, *striatus*, *flocculosus*. *Contextus pallide* vel *obscure brunneus*, *basi stipitibus pallide caeruleus*. *Sporae* (7.0–)7.2–8.3–9.4(–9.7) × (4.8–)4.8–5.3–5.7(–5.9) μm, *Q* = 1.40–1.58–1.80, *ellipsoideae*, *subamygdaliformes* vel *amygdaliformes*, *non lentiformes*.



FIGS. 1–2. Basidiocarps in situ. 1. *Conocybe caeruleobasis*. 2. *Pholiotina mairei* var. *stercorea*.

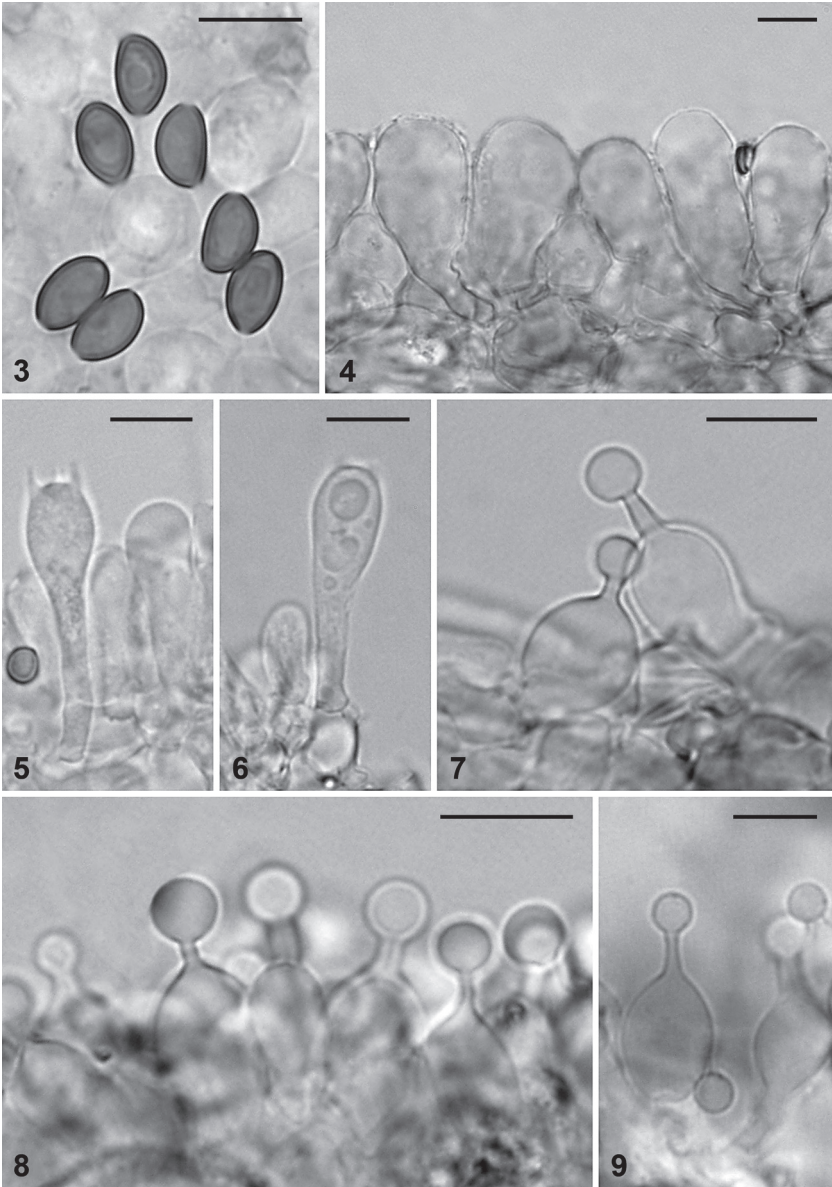
*laeves, porus germinativus centralis* (0.8–1.4  $\mu\text{m}$  latus), in KOH ferrugineo-brunneus. Basidia 27–51  $\times$  5–9  $\mu\text{m}$ , tetrasporigera, claviformia. Cheilocystidia lecythiformia, 19–29  $\times$  6–10  $\mu\text{m}$ , capitulo (2–)3–5(–5.8)  $\mu\text{m}$  lato. Pileipellis hymeniformis, elementis plerumque claviformibus. Pileocystidia desunt vel rara, cheilocystidiis similia. Stipitipellis est cutis. Caulocystidia lecythiformia, 15–33  $\times$  8–16  $\mu\text{m}$ , capitulo 3.5–8  $\mu\text{m}$  lato. Fibulae adsunt.

HOLOTYPE: CROATIA, Dalmatia, island of Brač, near village of Postira, 43°22'08"N, 16°40'01"E, alt. 5 m, on sandy soil mixed with gravel, 6 Dec. 2002, leg. Z. Tkalčec and A. Mešić, CNF 1/2927. ISOTYPE: WU 28460.

ETYMOLOGY: *caeruleus* (Latin) = blue, *basis* (Latin) = base; named for its blue context in the base of stipe.

PILEUS 8–14 mm broad, hemispherical to convex (sometimes with slightly depressed center), rarely  $\pm$  conical, moderately hygrophanous, reddish brown when moist, light brown to ochre brown on drying, not translucently striate or translucently striate at margin only, surface dull, glabrous, dry. LAMELLAE narrowly adnate to adnexed, moderately distant, ochre brown at first, then with rusty shade, with slightly paler to concolorous,  $\pm$  even edge. STIPE 12–24  $\times$  1.8–2.5 mm, subcylindrical with slightly swollen to bulbous base (up to 4 mm broad), its lower 1/4 to 1/2 part buried in the sand, seemingly more strongly bulbous as a result of adhering sand, whitish to light yellowish brown, fistulose, striate lengthwise, flocculose (especially in upper part), dry. VEIL absent. CONTEXT light brown in stipe (except in base) and rather dark brown in pileus when moist, pale brown on drying, in the base of stipe always pale blue. SMELL weak, not distinctive. TASTE not recorded.

SPORES (7.0–)7.2–8.3–9.4(–9.7)  $\times$  (4.8–)4.8–5.3–5.7(–5.9)  $\mu\text{m}$ , SD = 0.55  $\times$  0.23, Q = 1.40–1.58–1.80 (n = 100 spores), ellipsoid in frontal view, subamygdaliform to amygdaliform (rarely ellipsoid) in side view, not flattened, smooth, slightly thick-walled (0.5–0.7  $\mu\text{m}$ ), with distinct and central germ-pore (0.8–1.4  $\mu\text{m}$ ), rusty brown in KOH, yellow brown in H<sub>2</sub>O. BASIDIA 16–39  $\times$  7–11.5  $\mu\text{m}$ , 4-spored, clavate. LAMELLAR EDGE sterile to almost sterile. CHEILOCYSTIDIA 19–29  $\times$  6–10  $\mu\text{m}$ , lecythiform with globose, ellipsoid or clavate basal part, short to rather long neck and (2–)3–5(–5.8)  $\mu\text{m}$  broad globose capitulum, thin-walled, hyaline. PLEUROCYSTIDIA absent. PILEIPELLIS a hymeniderm made of clavate to broadly clavate, less often sphaeropedunculate, subglobose, utriform or subcylindrical elements, 21–55  $\times$  7–28  $\mu\text{m}$ , pale yellow brown to almost hyaline, pigment parietal (rarely minutely incrusting). Cells in subpellis and pileal trama yellow brown to rusty brown and pigment coarsely incrusting. PILEOCYSTIDIA absent to rare, similar to cheilocystidia. STIPITIPELLIS a cutis, made of 1.5–15  $\mu\text{m}$  wide, pale yellow brown to almost hyaline hyphae with incrusting pigment. CAULOCYSTIDIA similar to cheilocystidia but often somewhat larger, 15–33  $\times$  8–16  $\mu\text{m}$ , with 3.5–8  $\mu\text{m}$  broad capitulum, abundant in upper part, less frequent in lower part (along with some short clavate or



FIGS. 3–9. *Conocybe caeruleobasis*. 3. Spores. 4. Pileipellis. 5, 6. Basidia. 7. Caulocystidia. 8, 9. Cheilocystidia. Bars = 10  $\mu$ m.

lageniform elements). CLAMP CONNECTIONS present. CHEMICAL REACTIONS: ammoniacal reaction negative.

HABITAT – Open sandy sea coast with a few trees of *Cupressus sempervirens*, on sandy soil mixed with gravel.

DISTRIBUTION – Croatia, known only from the type locality.

NOTES: On the basis of smooth spores, almost exclusively lecythiform caulocystidia and stipe deeply buried in the sand, *Conocybe caeruleobasis* belongs to the section *Conocybe*, series *Dunensis* Hauskn. & Krisai. It is characterized by sandy habitat, stipe deeply buried in the sand, blue color in the context of stipe base, not or hardly translucently striate pileus, medium sized and not flattened spores, cheilocystidia with a rather small capitulum and negative ammoniacal reaction. Hausknecht & Krisai-Greilhuber (2006) classified three species in the series *Dunensis*. The European species *Conocybe dunensis* T.J. Wallace differs by absence of blue color in the context of stipe base, longer stipe without bulbous base, rather slender habit and much larger spores ( $[9.5-]10.5-14.0[-15.0] \times [5.5-]6.0-8.5[-9.0]$   $\mu\text{m}$ ). The European species *C. sabulicola* Hauskn. & Enderle differs by absence of blue color in the context of stipe base, darker pileus, somewhat larger spores ( $[7.0-]9.0-11.0[-12.4] \times [5.0-]5.5-6.5[-6.7]$   $\mu\text{m}$ ), presence of non-lecythiform elements on the top of the stipe and positive ammoniacal reaction. The South American species *C. macrorrhiza* (Speg.) Singer differs mainly by larger spores ( $10.5-10.8-12.3(-14.2) \times (5.3-6.0-7.0(-8.0))$   $\mu\text{m}$ , absence of blue color in the context of stipe base and positive ammoniacal reaction. Singer (1968) placed *C. macrorrhiza* in section *Mixtae* based on its possession of numerous non-lecythiform caulocystidia. However, the third author of this article, who studied the type material (preserved in a very bad condition) and two additional Singer collections from similar habitats in Argentina (herbarium BAFC, S502 and S536), found nearly exclusively lecythiform caulocystidia in Singer's collections. Consequently, Hausknecht & Krisai-Greilhuber (2006) placed *C. macrorrhiza* in section *Conocybe*, series *Dunensis*, close to *C. sabulicola* with similar stipitipellis.

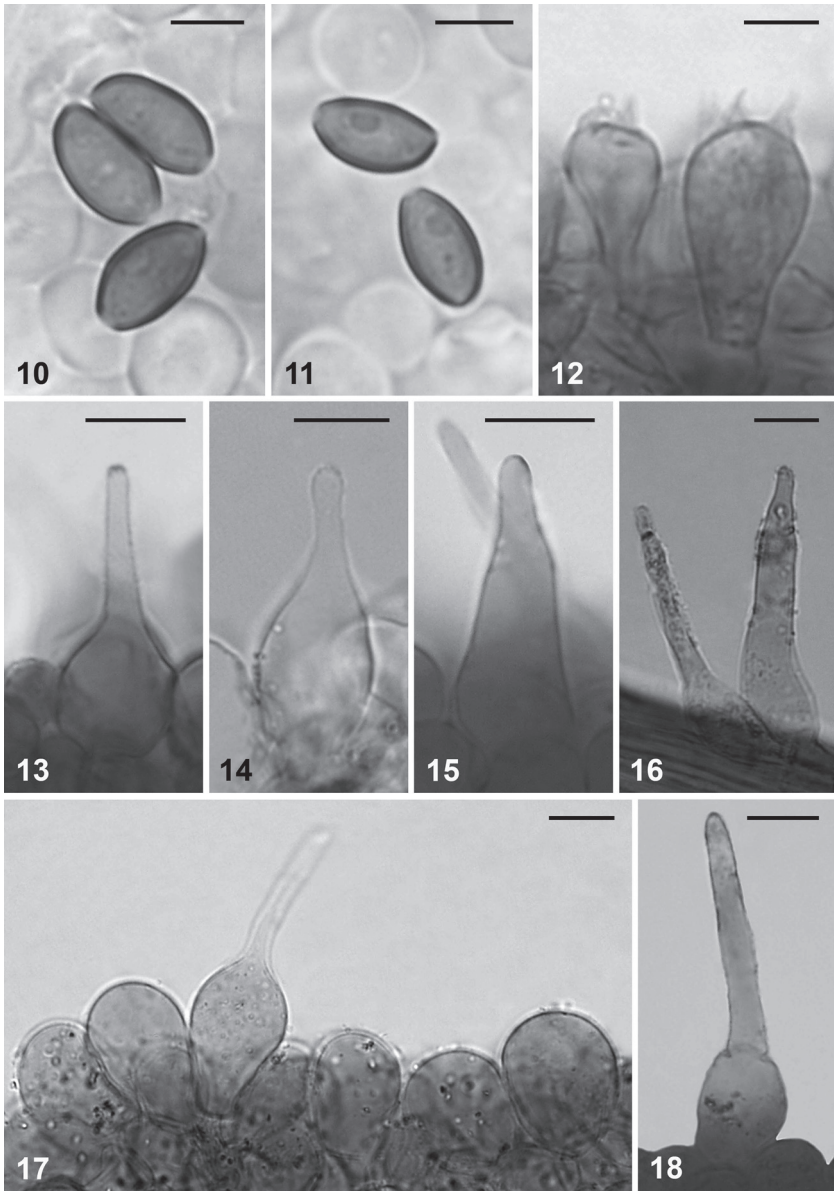
***Pholiotina mairei* var. *stercorea*** Tkalčec, Mešić & Hauskn., var. nov. FIGS. 2, 10–18  
MYCOBANK MB 511692

*A varietate typica cheilocystidiis latioribus, sporis paulum maioribus et in stercoribus crescendo differt.*

HOLOTYPE: CROATIA, Žumberak mountain, near Kostanjevec Podvrški village, 45°49'50"N, 15°35'17"E, alt. 240 m, 14 Oct. 2006, leg. M. Čerkez, CNF 1/4148.

ETYMOLOGY: Named for its growing on dung.

PILEUS 4–5 mm broad, obtusely conical to conico-convex, hygrophanous, pale to light brown when moist, pale ochraceous to cream on drying, translucently



**FIGS. 10–18.** *Pholiotina mairei* var. *stercorea*. 10, 11. Spores. 12. Basidia. 13–15. Cheilocystidia. 16. Caulocystidia. 17. Pileipellis. 18. Pileocystidium. Bars: 10–12 = 5  $\mu$ m, 13–18 = 10  $\mu$ m.

striate when moist, surface minutely pubescent to pruinose under a hand-lens, dry. LAMELLAE adnexed, broad, ventricose, moderately distant to distant, pale brown to ochraceous with rusty brown tinge at maturity, edge white, flocculose. STIPE 15–24 × 0.4–0.6 mm, cylindrical or slightly swollen at base, whitish to pale brown, entirely minutely pubescent under a hand-lens, dry. VEIL absent. CONTEXT concolorous. SMELL AND TASTE not recorded.

SPORES (7.6–)7.6–8.6–9.5(–10.1) × (4.3–) 4.4–4.7–5.1(–5.3) μm, SD = 0.47 × 0.16, Q = 1.62–1.80–2.06 (n = 100 spores), ellipsoid in frontal view, ellipsoid to amygdaliform in side view, not flattened, smooth, thin- to slightly thick-walled (0.4–0.6 μm), with distinct, central to slightly eccentric germ-pore (0.7–1.3 μm), light rusty brown in KOH, pale yellow brown in H<sub>2</sub>O. BASIDIA 13–23(–29) × 7–9(–10) μm, 4-spored, clavate. LAMELLAR EDGE sterile. CHEILOCYSTIDIA 20–45(–56) × (5–)7–14(–17) μm, mostly lageniform with long or less frequently short, mostly thin neck, 1.5–3.5 μm broad at apex, but also conical or fusiform, thin-walled, hyaline, intermixed with some clavate, sphaeropedunculate, subglobose or ellipsoid elements. PLEUROCYSTIDIA absent. PILEIPELLIS a hymeniderm made of broadly clavate, sphaeropedunculate, ellipsoid to subglobose elements, 12–30 × 8–21 μm, hyaline. PILEOCYSTIDIA 28–60 × 8.5–20 μm, lageniform, hyaline, thin-walled, abundant. STIPITIPPELLIS a cutis, made of 1.5–10 μm wide, hyaline hyphae. CAULOCYSTIDIA 21–80 × 5–15 μm, narrowly lageniform to conical, hyaline, present in the whole length of stipe, abundant (especially in upper part). CLAMP CONNECTIONS absent.

HABITAT – Alongside the stable, on heap of horse dung.

KNOWN DISTRIBUTION – Croatia, known only from the type locality.

NOTES: *Pholiotina mairei* var. *stercorea* differs from the typical variety, *P. mairei* (Watling) Enderle var. *mairei*, by growing on dung (typical variety grows on soil in deciduous forests, parks and gardens), broader cheilocystidia (5–8 μm broad in the typical variety) and slightly larger spores (6.0–8.5[–9.5] × 3.5–4.5 [–5.5] μm, average 6.7–7.8 × 3.6–4.3 μm, in the typical variety). The most similar species are *P. parvula* (Døssing & Watling) Bon, which is differentiated by longer (46–66 × 9–11 μm) cheilocystidia, smaller (6.0–7.3 × 3.8–4 μm) spores and growth on soil; *P. filipes* (G.F. Atk.) Singer (= *P. aberrans* (Kühner) Singer, = *P. sulcatipes* (Peck) Bon ss. Hausknecht 2005), which is differentiated by presence of clamp connections, somewhat longer (up to 60[–75] μm) and often subcylindrical cheilocystidia, slightly larger (7.5–11 × 4.5–6 μm) spores and growth on soil; and *P. maireiaffinis* Singer, which is differentiated mainly by presence of clamp connections, narrower (5.5–8.5 μm diam) cheilocystidia that are variably shaped (cylindrical to slightly lageniform with broader apex), somewhat smaller (6.5–8.3 × 4–5 μm) spores, and growth on soil in deciduous forests.



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