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New European records of basidiomycete *Burgoa anomala* from coniferous litter and sediment in underground tunnel

ONDŘEJ KOUKOL*, ALENA KUBÁTOVÁ

Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Praha 2, Czech Republic; ondrej.koukol@natur.cuni.cz
*corresponding author

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Burgoa anomala is a peculiar microscopic basidiomycete not forming any basidiocarps in its life cycle, but producing conspicuous multicellular structures (bulbils) and clamp connections on its mycelium. So far, this saprotroph has sporadically been found mainly on different woody substrates but the overall knowledge of its ecology and distribution is yet sparse due to its rarity. Our records from pine needle litter and sediment in an underground tunnel are only the second and third finds in Europe and the first from these habitats. The identification of this fungus was based on a combination of phenotypic and molecular (ITS rDNA sequence) data. Morphological characteristics and data on its growth between 5–30 °C on selected agar media are presented and discussed.

Key words: ecophysiology, anamorphic Agaricomycotina, *Cantharellales*, *Sistotrema*.

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Burgoa anomala je podivná mikroskopická stopkovýtrusná houba netvořící plodnice, avšak produkující na myceliu charakteristické mnohobuněčné útvary (bulbily) a přezky. Dosud byl tento saprotrof ojediněle nacházen především na dřevních substrátech, ale znalosti o ekologii a rozšíření tohoto druhu jsou vzhledem k jeho vzácnosti zatím nedostatečné. Naše nálezy z borového opadu a půdního sedimentu v podzemním tunelu jsou teprve druhým a třetím evropským nálezem a prvním z těchto habitatů. Identifikace byla založena na kombinaci fenotypických a molekulárních dat (sekvence ITS rDNA). V článku jsou uvedeny a diskutovány morfologické znaky a růstové hodnoty při teplotách 5–30 °C.

INTRODUCTION

The genus *Burgoa* Goid. (*Hydnaceae*, *Cantharellales*, Agaricomycotina) was established by Goidànich (1937) to accommodate microfungi producing multicellular spore-like structures with differentiated peridial and internal cells, i.e.

bulbils. Apart from the production of the bulbils, members of this genus were distinguished by the formation of clamp connections on their mycelium. This feature showed their affinity to members of the Agaricomycotina, but their position within the *Cantharellales* was recognised only recently (Lawrey et al. 2007). Goidànich (1937) described three new species (*Burgoa alutacea* Goid., *B. hutsonii* Goid., *B. verzuoliana* Goid.) and combined two more from the genus *Papulaspora* Hotson [*B. anomala* (Hotson) Goid. and *B. nigra* (Hotson) Goid.], a genus accommodating hitherto all known bulbilliferous microfungi. Later, *B. pisi* S.Q. He & D.Z. Tang was described from roots of *Pisum sativum* (He & Tang 1998). Most recently, three epiphytic or lichenicolous species *B. angulosa* Diederich, Lawrey & Etayo, *B. moriformis* Diederich, Ertz & Coppins and *B. splendens* Diederich & Coppins were described by Diederich & Lawrey (2007).

In 2013, two isolates of *B. anomala* were obtained from coniferous litter and from sediment in an underground tunnel. These substrates represent new habitats and only the second and third records of this species in Europe. Previously, *B. anomala* had only sporadically been recorded from live oak chips and an old paper in California and Massachusetts, respectively, in USA (Hotson 1912), from wood pulp in Italy (Goidànich 1937) and most recently from a stone chamber interior in Japan (Kiyuna et al. 2015). The main aim of this contribution is to present phenotypic characteristics of this peculiar species and compare them with previous findings.

MATERIAL AND METHODS

Two isolates of *Burgoa anomala* were used in this study. Isolate CCF 4805 was obtained by the first author from needles of *Pinus nigra* in litter collected in Monte Negro, Cetinje, mountain forest of *P. nigra* and *P. sylvestris* below Mt. Lovćen (42°22'43.65'' N, 18°50'18.72'' E, elevation 1183 m, 11 September 2013, collected by Tijana Martinović). Needles were cultivated in damp chambers at room temperature and *B. anomala* was isolated in December 2013 as a fast growing dominant in the chamber (Fig. 1a). Mycelium with numerous bulbils was found to colonise the whole surface of the filter paper filling the bottom of the chamber and transferred to a pure culture on malt extract agar (MEA) (Pitt 1979). The other culture, CCF 4806, was isolated by the second author from soil sediment in the underground of Dobrošov fortress, Czech Republic (50°24'09'' N, 16°12'09'' E, elevation 600 m, 19 March 2013, collected by Jiří Flousek) in December 2013 using the soil dilution method. Soil suspension was spread onto yeast extract-glucose agar (5 g yeast extract, 15 g glucose, 0.1 g chloramphenicol, 15 g agar, 1000 ml water) and incubated at 10 °C for 1 month. Both isolates were

deposited at the Culture Collection of Fungi (CCF) of the Department of Botany, Faculty of Science, Charles University in Prague (Czech Republic).

For growth procedures, three agar media were used: malt extract agar (MEA), potato glucose agar (PGA) (Booth 1971) and oat agar (OA) (Samson et al. 2004). Incubation was made at 5, 10, 15, 20, 25, 28 and 30 °C. For each agar medium and temperature, three Petri dishes were used.

DNA was isolated from 7–14 day old cultures using a Zymo Research Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Orange, USA). Nuclear rDNA containing internal transcribed spacers (ITS1 and ITS2) and the 5.8S region (further referred to as ITS rDNA) were amplified with primer sets ITS1/ITS4 (White et al. 1990, O'Donnell 1993). The PCR products were viewed by means of electrophoresis on a 1% (w/v) TAE agarose gel, stained with ethidium bromide. The PCR products were purified with the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). Both strands of the PCR fragments were sequenced with primers used for amplification in the Sequencing laboratory (Faculty of Science, Charles University in Prague, Czech Republic).

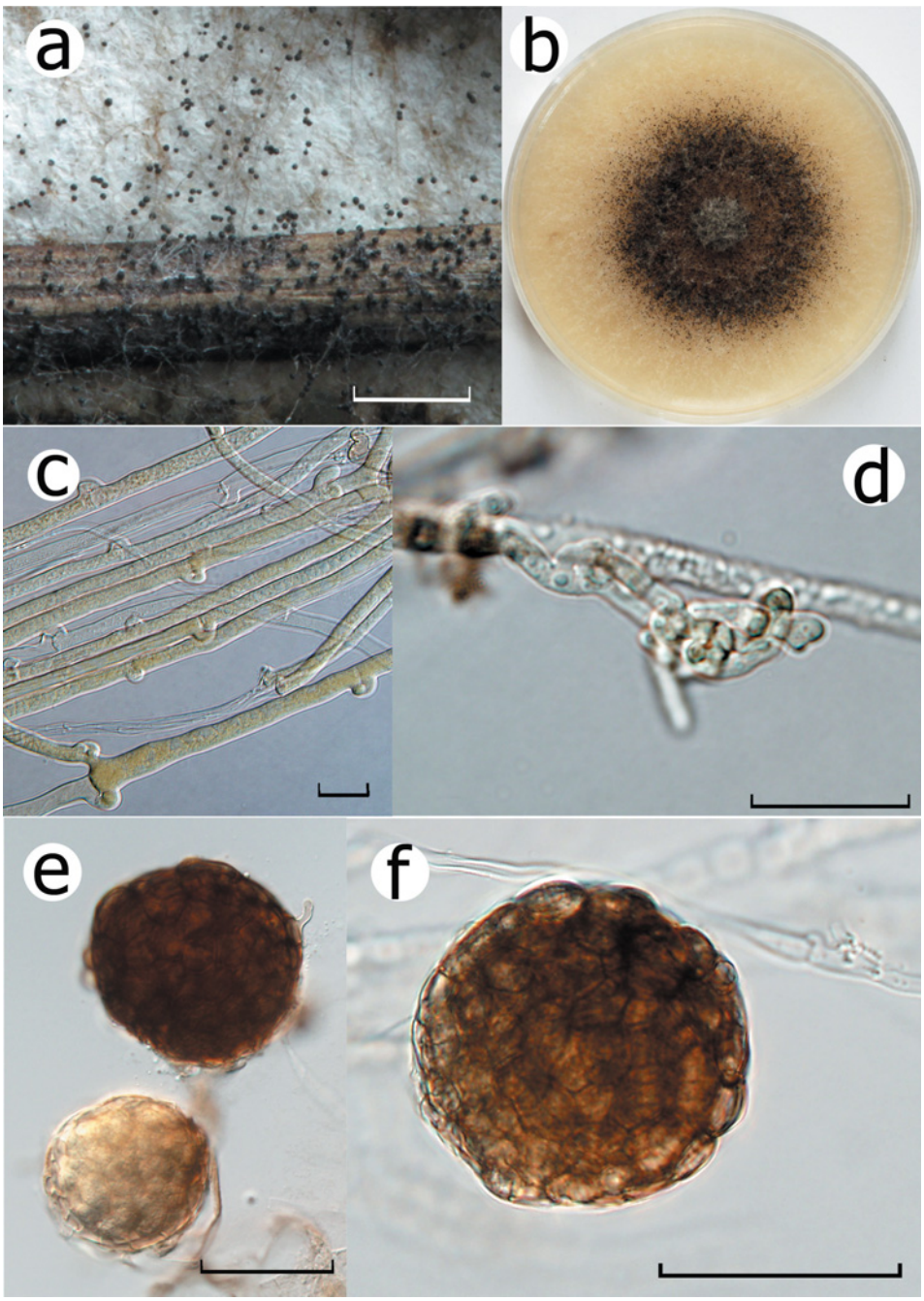
RESULTS AND DISCUSSION

Phenotypic description

Colony features of *Burgoa anomala* (Fig. 1b). Colonies at first whitish, becoming brown to brownish black along with the production of bulbils. Colony margins relatively broad, thin, inconspicuous, whitish. Reverse uncoloured, becoming dark in the central part where the bulbils are produced.

Fungal growth was recorded at 5–28 °C. No growth occurred at 30 °C. Colony sizes of our *B. anomala* isolates on MEA, OA and PGA at different temperatures after 7 and 14 days are presented in Tab. 1. The growth at 25 °C was comparable to the strains of Kiyuna et al. (2015). Nevertheless, differences were observed at 28 °C. Although strain CCF 4805 originated from a locality exposed to direct sunlight and thus to higher temperatures, it showed a slower growth compared to CCF 4806 obtained from a cold underground environment.

Micromorphological features of *Burgoa anomala* (Fig. 1c–f). Substrate mycelium loose, composed of hyaline to pale brown hyphae with thin wall, c. 2–5 µm in diam. Mycelium septate, septa with clamp connections. Bulbils spherical or almost spherical, produced from primordia composed of interwoven branched hyphae, originally hyaline, turning pale brown and later dark brown in maturity, (50)74–106(180) µm in diam. Cells composing bulbils angular, reaching up to 20 µm in diam., tightly packed together, some slightly projecting outside the bulbil, superficial cells germinating in new hyphae. The development and the


a
b
c
d
e
f

morphology of mature bulbils are identical to those described by Goidànich (1937) and Kiyuna et al. (2015).

Molecular data

Sequences of ITS rDNA were identical to multiple sequences originating from the study by Kiyuna et al. (2015). The phylogenetic analysis by Kiyuna et al. (2015) based on this region showed affinity of *B. anomala* to the basidiomycetous genus *Sistotrema*. *Burgoa anomala* formed a monophyletic lineage basal to the *Sistotrema eximum-octosporum* clade defined by Moncalvo et al. (2006). *Burgoa verzuoliana*, the type species of *Burgoa*, was also placed in this clade, but *B. angulosa* and *B. moriformis* were not, making *Burgoa* polyphyletic. *Sistotrema* is also polyphyletic with the type species *Sistotrema confluens* Pers. forming a distinct clade. Therefore, the species of *Sistotrema eximum-octosporum* clade might adopt the name of *Burgoa* in the future.

Occurrence and ecophysiology

Burgoa anomala has hitherto been recorded from habitats differing in substrate and climate showing a rather wide adaptability. Woody substrates dominate, which may be attributed to the delimitation of the genus *Sistotrema* as wood saprotrophs (Moncalvo et al. 2006). The isolate obtained from pine needles in litter strongly colonised filter paper in the damp chamber (Fig. 1a), which suggests its utilising lignocellulose. Rather surprising are two records from the underground environment, the soil sediment in this study and the plaster of a mural painting on the walls and ceiling of a stone chamber in Kiyuna et al. (2015). These habitats are poor in any nutrients (especially lignocellulose) and also have a specific climate. They particularly have a high humidity [100% relative humidity in Kitora Tumulus (Kigawa et al. 2009)] and constant, low temperature throughout the year. Previously reported substrates and the coniferous litter recorded in this study are typical for their temperature changes in a circadian rhythm and between the seasons. Interestingly, our growth test did not show any tendency to psychrophily, as the fastest growth was observed at 25 °C on all three media tested (Tab. 1). The occurrence of *B. anomala* in an underground environment thus seems to be rather accidental and does not point to specific habitat requirements of this fungus.

◀ **Fig. 1.** *Burgoa anomala* in damp chamber and in culture (CCF4805 a, d–f; CCF4806 b–c): **a** – loose mycelium with numerous bulbils produced on pine needle and surrounding filter paper (scale bar = 2 mm); **b** – colony on MEA (Petri dish 9 cm in diameter), 14 days old; **c** – mycelium with clamp connections (scale bar = 10 µm); **d** – early stage in bulbil formation (scale bar = 20 µm); **e, f** – mature bulbils (scale bars = 50 µm). Photo O. Koukol (a–b, d–f), A. Kubátová (c)

Tab. 1. Growth of two *Burgoa anomala* isolates on malt extract agar (MEA), oat agar (OA) and potato glucose agar (PGA) at seven different temperatures after 7 and 14 days. Values represent means of three measurements (diameter of colony in mm) and standard deviations.

Temperature	Isolate	MEA		OA		PGA	
		7 d	14 d	7 d	14 d	7 d	14 d
5 °C	CCF 4805	8.7 ± 0.6	26.3 ± 1.2	7.0 ± 1.0	31.3 ± 1.5	7.3 ± 1.2	31.7 ± 2.1
	CCF 4806	7.3 ± 0.6	22.0 ± 1.0	6.3 ± 0.6	27.7 ± 0.6	6.0 ± 1.0	24.7 ± 0.6
10 °C	CCF 4805	9.3 ± 0.6	31.0 ± 1.0	10.0 ± 1.0	33.3 ± 1.2	13.0 ± 1.0	35.7 ± 2.1
	CCF 4806	8.7 ± 0.6	27.3 ± 2.1	8.7 ± 0.6	30.7 ± 0.6	9.0 ± 1.0	29.0 ± 1.7
15 °C	CCF 4805	19.7 ± 0.6	52.0 ± 2.0	22.3 ± 0.6	59.7 ± 1.5	23.0 ± 1.0	63.3 ± 0.6
	CCF 4806	16.3 ± 0.6	48.7 ± 1.2	19.7 ± 0.6	59.7 ± 0.6	18.7 ± 1.2	57.0 ± 1.0
20 °C	CCF 4805	29.7 ± 0.6	70.0 ± 0.0	33.3 ± 1.2	69.7 ± 3.1	32.0 ± 0.0	74.5 ± 0.7
	CCF 4806	28.3 ± 0.6	69.3 ± 1.2	31.7 ± 0.6	75.0 ± 1.0	31.0 ± 1.0	76.3 ± 0.6
25 °C	CCF 4805	24.7 ± 1.5	60.3 ± 0.6	26.3 ± 0.6	64.7 ± 0.6	31.3 ± 0.6	70.7 ± 0.6
	CCF 4806	26.0 ± 0.0	68.0 ± 1.0	29.7 ± 1.5	72.7 ± 1.2	32.7 ± 0.6	76.7 ± 1.5
28 °C	CCF 4805	8.7 ± 0.6	18.7 ± 0.6	9.3 ± 0.6	21.7 ± 0.6	9.3 ± 0.6	21.7 ± 1.5
	CCF 4806	15.7 ± 0.6	43.7 ± 0.6	14.7 ± 0.6	37.3 ± 0.6	15.3 ± 0.6	40.0 ± 1.0
30 °C	CCF 4805	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	CCF 4806	0.3 ± 0.6	1.7 ± 2.9	0.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	2.3 ± 0.6

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