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Callistosporium pinicola (Basidiomycota), a fungus species new to Poland

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Abstract

Basidiomata of *Callistosporium pinicola* were collected in years 2012–2013, on strongly decayed fallen trunks of *Picea abies* and *Abies alba* on two remote sites, localized in North-eastern and southern Poland. These localities represent the first records of this fungus in Poland. A full description and illustration of *C. pinicola* based on Polish specimens are provided and the distinguishing features and delimitation of the species are briefly discussed. The ecology of *C. pinicola*, its general distribution and threat are also presented. As all specimens of *C. pinicola* were collected only in recent years we suggest that this species is increasing its distribution in Poland. However, we cannot rule out the possibility that, because of its cryptic nature, *C. pinicola* has been much overlooked in the past.

Keywords: micromorphology; the Beskid Żywiecki Mountains; the Augustów Plane; Wigierski National Park; Polish mycobiota

Introduction

The genus *Callistosporium* Singer, which contains 13–16 species [1,2] belongs to the tricholomatoid clade and to the family Tricholomataceae within Basidiomycota [3,4]. However, members of this genus may have a relationship with the entolomatoid clade, and analysis of only rRNA data place *Callistosporium* in the Entolomataceae [3,5]. Members of the *Callistosporium* genus are generally characterized by the collybioid habit, the white spore deposit, smooth spores without iodine reactions, the presence of yellow pigment bodies inside the spores, and also often other elements of the hymenophore

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("necropigments" colorless in fresh condition but visible in exsiccates), the absence of true cystidia and clamp-connections as well as the lilac, violet to purple staining of tissues in KOH and $NH_3 \cdot H_2O$ [4,6]. The fungi live as saprotrophs on humus, litter and various kinds of decaying wood, also on *Sphagnum*, mostly in forests [4,6–9]. The genus *Callistosporium* is completely unknown in Poland and appears poorly investigated in other European and non-European countries. The most of known *Callistosporium* species are probably under-collected due to their somewhat cryptically colored basidiomata and superficial similarity to *Simocybe*, *Gymnopilus* or *Cortinarius* (*Dermocybe*).

During field studies, both authors of this paper independently found an interesting small-spored *Callistosporium* basidiomata growing on strongly decayed wood of conifers. In comparison with recent literature we identified these records as *C. pinicola* Arnolds, a species new to the mycobiota of Poland. This wood-inhabiting basidiomycote was recognized for the first time by Bon [10], but at the species level it was described and validly published first by Arnolds [11] on the basis of Dutch material collected on strong rotten stump of conifer in mature beech forest (Lheederzand, Dwingeloo) [12]. *Callistosporium pinicola* is generally known to prefer strongly decayed wood of conifers. It is reported from heterogeneous both deciduous and coniferous forests and from mixed stands. The known distribution of the species seems to be limited to the East-Central and Western Europe, and only exceptionally does it reach the southern and the northern part of the continent. This fungus is rather rare in all parts of its distribution [4,11,13].

The main aim of this paper is to describe morphologically the first collections of *C. pinicola* for Poland, and to compare their characters with published data. Furthermore, this paper aims to evaluate its ecology and distribution in Poland (Fig. 1) in regard to the previous records in Europe.

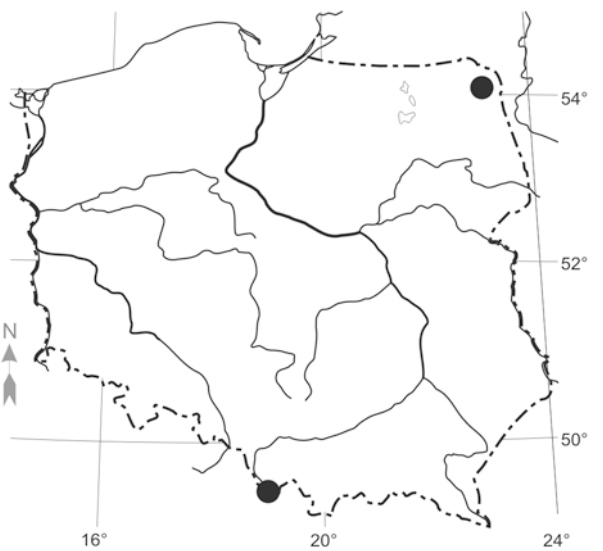

Fig. 1 Localities of *Callistosporium pinicola* Arnolds (circle) in Poland.

Material and methods

Material has been collected occasionally by the authors during several field trips in different regions of Poland in 2012 and 2013. Description of macroscopic features was based on fresh material comprising over 15 basidiomata in all stages of development, from three collections. Microcharacters were observed with a Nikon Eclipse E-400 light microscope equipped with a Nikon digital camera (DS-Fi1). For microscopic observations, dried materials were placed in 95% ethanol for about 1 min, and then transferred to 5% $NH_3 \cdot H_2O$ solution until they became pliable. Free-hand sections of the rehydrated pieces of basidiomata were examined in 5% $NH_3 \cdot H_2O$ and Congo red in ammonia. Image-grabbing and biometric analyses were done with NIS-Elements D 3.1 imaging software. Dimensions of microcharacters are given as (minimum) average ±standard deviation (maximum), and additionally in the form of the main data range (5–95 percentile values). *Q* value refers to the length/width ratio of basidiospores. For basidiospores size measurements, randomly selected mature spores were used, and measured without hilar appendix. Lengths of basidia were measured excluding sterigmata. Microphotographs were taken using Nikon DS-Fi1 digital camera. Statistical computations employed Statistica software (StatSoft). For morphological terminology see "Flora agaricina Neerlandica" [14,15]. The fungus nomenclature and its synonyms follow Arnolds [11] and Antonín et al. [13]. The nomenclature of forest communities follows Matuszkiewicz [16]. Specimens have been deposited in the Herbarium of the Museum of Natural History at the Wrocław University (WRSL), Poland.

Results and discussion

Diagnostic characters

This wood-inhabiting fungus was described and illustrated in details several times, e.g. by Arnolds [11] (p. 32, Fig. 2a–h), Arnolds and Becker [12] (p. 70, Fig. 1a–h, as *Calocybe cerina*), Antonín et al. [13] (p. 7, Fig. 1–2; p. 8, Fig. 3–6), Verbeken and Walleyn [17] (p. 4, Fig. 1, Fig. 2a–b, as *Callistosporium luteo-olivaceum* f. *minor*), and Wilhelm [18] (p. 63–64, as *Callistosporium luteoolivaceum* var. *minor*). Here are included merely some most distinguishable characters based on Polish materials (Fig. 2).

Callistosporium pinicola Arnolds in Arnolds, Acta Mycol. 41(1): 32. 2006. = *Callistosporium xanthophyllum* f. *minor* Bon ad int., in "Flore mycologique d'Europe", Doc. Mycol. Mém. hors Sér. 2: 95. 1992 (invalid name, ICBN Art. 32.1). = *Callistosporium luteoolivaceum* f. *minor* Verbeken & Walleyn, Doc. Mycol. 32(127–128): 3. 2003. = *Callistosporium minor* (Verbeken & Walleyn) Wilhelm, Schw. Z. Pilzk. 85(4): 137. 2007 (invalid name, ICBN Art. 52.1); misapplied names: *Calocybe cerina* (Pers.: Fr.) Donk sensu Arnolds and Becker in Coolia 36: 70. 1993, sensu Moser in Kleine Kryptogamenflora IIb/2: 135. 1983.

Basidiomata solitary, gregarious or in small groups. Pileus 5–30 mm, convex with narrow, distinctly involute margin when young, later plano-convex to applanate, plane to slightly depressed at center, finally inflexed or rarely straight at margin, initially with cuticle slightly exceeding the lamellae then restricted, surface not hygrophanous or weakly hygrophanous when moist (mature basidiomata), vividly yellow-brown when young, than orange-brown, rusty-brown to dark brown, in general with olivaceous tinge, finely white

Fig. 2 Characters of Polish findings of *Callistosporium pinicola* Arnolds: basidiomata (**a**–**d**); basidiospores (**e**). Photographs by Ryszard Rutkowski (**a**, WRSL-0510) and Marek Halama (**b**–**e**, WRSL-0512).

tomentose-pruinose, at maturity sordid rusty brown with olive tinge to dark brown, not striate, smooth or slightly radially fibrillose, and rarely felted at center when dried up, apparently glabrous and slightly greasy-shining when moist. Lamellae, L = 22–36, l = 2–8, strongly emarginate, crowded to very crowded, segmentiform, up to 4 mm broad, yellow when young, then golden-yellow, rusty yellow, sordid olive-yellow to olive-brownish when old, with concolorous, entire than irregular (when old) edge, becoming dark red-brown to almost black in exsiccata. Stipe 15–35 × 1.5–4.0 mm, central or slightly eccentric, cylindrical or slightly compressed, sometimes faintly broadened upwards, solid or narrowly fistulose, ±concolorous with pileus, white longitudinally fibrillose-pruinose at first, then white striate lengthwise or partly glabrous, at apex slightly pruinose, at base often yellowish white tomentose. Context thin-fleshed (up to 2 mm thick), fragile, pale yellow or concolorous with surface in pileus and stipe (at maturity). Smell almost undetectable or weakly farinaceous; taste mild to slightly bitter.

Basidiospores (2.8)3.5 ±0.3(5.5) × (2.1)2.5 ±0.2(3.1) µm, 3.0–4.0 × 2.3–2.8 µm, Q = (1.1)1.4 ±0.1(2.1), Q = 1.2–1.6, n = 161, broadly ellipsoid, ellipsoid (sometimes with adaxial side less convex than abaxial one), rarely subglobose in side view, broadly ellipsoid, ellipsoid or ovoid, rarely subglobose in frontal view, with small hilar appendix, smooth, thin-walled, without iodine reactions, pale yellowish-grey in Melzer's reagent, colorless to pale yellowish-grey in ammonia with yellow-greenish refractive body and darker (brownish) intracellular pigment (necropigment). Basidia (13.4)16.5 ±1.9(19.6) × (3.6)4.7 ±0.5(5.6) µm, 13.4–19.2 × 4.0–5.5 µm, n = 50, narrowly clavate, 4- (very rarely 2-) spored, staining like spores. Cheilocystidia absent. Pleurocystidia absent. Hymenophoral trama regular to subregular, made up of rather narrow, ±thin-walled, non-dextrinoid hyphae with cylindrical or slightly inflated elements, 5–15 µm wide, with pale yellow membranal and incrusting pigmentation in ammonia. Pileipellis a poorly differentiated cutis, made up of repent, interwoven, cylindrical, 3.0–8.0 µm wide hyphae, with yellow-brown membranal and incrusting pigment, towards the center often with fascicles of ascending and erect hyphae (intricate trichoderm). Stipitipellis a cutis, made up of cylindrical, parallel, smooth or incrusted, up to 6 µm wide hyphae, with regular (clavate, lageniform) or irregular terminal elements. Clamp-connections absent in all tissues. Chemical reactions: all parts of the fresh basidiomata turning immediately dark red to red-brown with 5% ammonia (macroscopically); preparations of all tissues turning immediately reddish in 5% ammonia.

Specimens examined

POLAND. (***i***) Beskid Żywiecki Mountains, "Śrubita" nature reserve (49.407483°N 19.012633°E), alt. 926 m, a natural fir-beech forest: phytocoenosis of *Dentario glandulosae-Fagetum*, on strongly decayed, almost disintegrated lying trunk of *Abies alba*, 27 Jun 2013, leg. R. Rutkowski, WRSL (ref. No. 0510). (***ii***) Augustów Plane, Wigierski National Park (54.002089°N 23.201850°E), alt. 143 m, a natural oak-hornbeam forest: phytocoenosis of *Tilio cordatae-Carpinetum betuli*, on the inside surface of strongly decayed, hollow stump and (fallen) trunk of *Picea abies*, 25 Jul 2013, leg. M. Halama, WRSL (ref. No. 0511). (***iii***) ibid. a natural oak-hornbeam forest: phytocoenosis of *Tilio cordatae-Carpinetum betuli*, on the inside surface of strongly decayed, hollow (fallen) trunk of *P. abies*, 3 Oct 2012, leg. M. Halama, WRSL (ref. No. 0512).

Additional collections examined

POLAND. (***i***) near Drezdenko, maple forest, mixed litter, leg. W.G. Lasch, WRSL [as *Agaricus* (*Tricholoma*) *cerinus* Pers.], verified as *Rugosomyces naucoria* (Murrill) Boffelli. (***ii***) ibid. pine forest, mixed litter, autumn 1854, leg. W.G. Lasch, WRSL [as *Agaricus* (*Tricholoma*) *cerinus* Pers.], verified as *R. naucoria* (Murrill) Boffelli.

Notes

Though the holotype of *C. pinicola* from the Netherlands was not studied in this case, we consider the macro- and microcharacters exhibited by a number of Polish specimens to be consistent in principle with those described by Arnolds [11] and supplemented by others (e.g. [13]). The new findings presented below are characterized by rather variable shape of basidiospores. Although analyzed spores are ellipsoid in majority, they are also nearly amygdaliform (with adaxial side less convex than abaxial one) and subglobose in side view. In the protologue *C. pinicola* is described as having only four-spored basidia. However, the Polish material of the species revealed also a small proportion of two-spored cells. The presence of two-spored basidia in *C. pinicola* was also reported by Antonín et al. [13]. Moreover, the dominant four-spored basidia are smaller in Polish material in accordance with those described in the protolog.

Callistosporium pinicola is a small, in time cryptically colored species that can be easily overlooked. When it is wet, its basidiomata are nearly dark brown and almost unavailable to see on the rotten wood. This species belongs to subgenus *Callistosporium* in view of the crowded lamellae, small, ellipsoid spores, short basidia and the pigment not turning blue in ammonia [10,11]. It has a closest phenetic affinity to *C. luteo-olivaceum* (Berk. & M.A. Curtis) Singer [syn. *C. xanthophyllum* (Malençon & Bertault) Bon]. Vesterholt and Holec [4] showed that in general both species differ in the size of basidiomata, and primarily in the size of basidiospores. *Callistosporium luteo-olivaceum* forms somewhat larger and more often fasciculate basidiocarps and its spores are also greater (4–7 × 3–4.5 µm vs. 3–4 × 2–3 µm in *C. pinicola*) [4,19]. Considering the basidiomata, *C. pinicola* may also resemble *C. elaeodes* (Ramagn.) M. Bon., another member of the genus described from a wood of broadleaved tree [20,21]. Both taxa can be distinguished by the yellow-green or olive-green pileus, and clearly larger basidiospores (6.5–8.5 × 3.5–5.0 µm) in the latter [22,23]. Furthermore, Wilhelm [19] remarked on the green-yellow and olive-brown lamellae of *C. elaeodes* and their negative reaction with ammonia solution. Other related European taxa comparable to *C. pinicola* are *C. olivascens* (Boud.) Bon, *C. donadinii* (Bon) Contu, and *C. foetens* E. Ludw. *Callistosporium olivascens* occurs in Southern Europe especially under cedars (occasionally also growing with pines and cypresses), and can be distinguished in general by darker colors, larger, amygdaliform basidiospores (8–13 × 5.0–7.0 µm), and forming of ±cylindrical marginal pseudocystidia [22–24]. *Callistosporium donadinii* seems to be restricted to the Mediterranean coastal regions in Europe. It has ±vinaceous to dark red-brown pileus, camphor-menthol smelling odor, larger – amygdaliform basidiospores (6–9 × 4.0–5.0 µm), and presence of sparsely distributed ±cylindrical marginal pseudocystidia [8,23]. *Callistosporium foetens* is characterized by a strong unpleasant smell. It has a hygrophanous, (in wet) dark olive brown-blackish to umbra-blackish pileus, greyish-olive stipe, and larger – ellipsoid to somewhat amygdaliform basidiospores (6–8.5 × 4.0–5.0 µm) [23]. Furthermore, occasionally one may find it difficult to distinguish *C. pinicola* from a species of *Calocybe*, *Simocybe* or *Gymnopilus*.

Arnolds [11] provides very interesting information on the affinities of *C. pinicola* with *Calocybe cerina* (Pers.: Fr.) Donk [the taxon is currently treated as *Calocybe chrysenteron* var. *cerina* (Pers.: Fr.) Arnolds or *Rugosomyces chrysenteron* (Bull.: Pers.) Bon]. He noticed that *C. pinicola* was sometimes misidentified as *C. cerina* in the past and are occasionally considered under this name in the literature and herbaria. Although similar at first glance, mainly because their comparable size and color at early stages, *C. cerina* is unlikely to be mistaken for *C. pinicola* when studied in more detail, mainly in view of the thicker, sulphur-yellow context, a taste becoming bitter after a while, and the substrate on soil and litter (rarely on very rotten wood). Its additional distinguishing characters are a pileipellis in the form of a cutis with transitions to a trichodermium, presence of clamp-connections, and lack of reddish discoloration of their tissues in alkaline solutions [11,25,26]. We checked the collections of the WRSL herbarium where few specimens of *C. cerina* are stored (as *Tricholoma cerinum* and *Agaricus cerinus*). However, all of them represent another taxon, namely *Rugosomyces naucoria* (Murrill) Boffelli (syn. *R. fallax* Bon, *Calocybe fallax* (Sacc.) Redhead & Singer invalid name, ICBN Art. 35.1).

Distribution and ecology

Apparently widespread but rare in Europe, *C. pinicola* is hitherto known from Austria [25], the Czech Republic [13], France [10,11,18], Germany [18,19], Italy [11], the Netherlands [11,12], Belgium [17], Switzerland [25], Slovakia [27], Ukraine [13], and Sweden [4]. *Callistosporium pinicola* is considered a saprophyte. Although, the species is associated with various kinds of woody substrates, it is most often found on decayed wood of conifers. In literature the most frequent records are on strongly decayed (disintegrated) fallen trunks and dead stumps of *Abies alba*, *Picea abies* and *Pinus sylvestris* [11,13,18,25]. There are also records from softwood of *Pinus pinea* [11], and hardwood of *Fagus sylvatica* [13,17]. In Poland *C. pinicola* has been found on strongly decayed, lying trunks of conifers. It was growing in cavities of the side part of fallen trunk of *Picea abies* covered by mosses, and on naked piece of almost disintegrated trunk of *Abies alba*. Associated fungi at the Wigierski National Park locality include, for example, *Chrysomphalina grossula* (Pers.) Norvell, Redhead & Ammirati, *Galerina triscopa* (Fr.) Kühner, *Heterobasidion annosum* (Fr.) Bref., *Mycena purpureofusca* (Peck) Sacc., and *M. viridimarginata* P. Karst.

Antonín et al. [13] have discussed the ecological preferences of *C. pinicola* in detail. They have tentatively concluded that one should take into account a strongly rotten wood (of mostly conifers) and humid climate as well, when considering the environmental requirements of the species. In spite of these factors, various relations between the occurrence of *C. pinicola* and kind of habitat have been also noticed. The typical habitats of the species in the Czech Republic and the Ukraine are old-growth forests (include a wide range of forests with natural occurrence of *Abies alba* and/or *Picea abies*), in rather cold and humid stands (montane or submontane forests and stream valleys or gorges in highlands), most frequent between 200–500 and 800–1100 m altitude. However, according to Antonín et al. [13] *C. pinicola* was also found in a man-influenced stand and cannot be considered a species confined exclusively to natural and virgin forests in the area. In The Netherlands *C. pinicola* grows in first-generation coniferous (*Pinus sylvestris*, *Picea abies*) and mixed forest plantations [11–13]. In France this species is reported from coniferous and mixed stands, with *Quercus*, *Carpinus*, *Fagus* and *Pinus*, in the colline belt [13,19]. In Poland, *C. pinicola* has been found in highly natural forest communities

(oak-hornbeam forest and fir-beech forest), possessing a natural tree species composition and multi-aged structure, and situated in planar and montane belt respectively. Thus, Polish findings seem to confirm a strong linkage of *C. pinicola* to old-growth forests in Central Europe. The humid meso- and microclimate of these forests and their rich supply of various decaying woody substrates (as measured by a diversity of diameters and decay classes) creates suitable conditions for this species. All specimens of *C. pinicola* in Poland were collected only in recent years (in the period 2012–2013), which may suggest that this species is increasing its distribution for unknown reasons. However, we cannot rule out the possibility that, because of its cryptic nature, *C. pinicola* has been much overlooked in the past. As an uncommon fungus restricted to threatened natural habitats (protected as nature reserve and the area under strict protection), *C. pinicola* should certainly be classified at least as a rare and included into the new edition of the red list of fungi of Poland.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: field studies, photographs: MH, RR; determination of the specimens, laboratory works, bibliography studies, writing the manuscript, preparing of the plate of photographs, final corrections: MH.

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