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Pholiotina cyanopus, a rare fungus producing psychoactive tryptamines

Abstract: *Pholiotina cyanopus* was collected from wood chips and other woody remnants of undetermined tree species. Its basidiomata were found in June within the area of closed sawmill in the central part of Żywiec city (SW Poland). Description and illustration of *Ph. cyanopus* based on Polish specimens are provided and its ecology, general distribution and comparison with similar taxa – *Pholiotina smithii*, *Pholiotina sulcatipes*, and others are discussed as well. The identity of the active compounds of *Ph. cyanopus* was additionally determined. Liquid chromatography–mass spectrometry (LC-MS) data sets were obtained to support the occurrence of psilocybin and its analogues – psilocin, baeocystin, norbaeocystin, and aeruginascin in air-dried basidiomata of the species. The content of psilocybin was found to be high ($0.90\pm 0.08\%$ of dry weight), besides, analysed samples contained lower concentrations of psilocin ($0.17\pm 0.01\%$), and baeocystin ($0.16\pm 0.01\%$). Additionally, the chemical analysis revealed small amounts of norbaeocystin ($0.053\pm 0.004\%$) and aeruginascin ($0.011\pm 0.0007\%$) for the first time in the species.

Keywords: *Conocybe cyanopus*, section *Cyanopodae*, wood-inhabiting fungi, hallucinogenic mushrooms, psilocybin, psilocin, baeocystin, norbaeocystin, aeruginascin, Polish mycobiota

DOI 10.1515/biol-2015-0005

Received February 27, 2014; accepted June 20, 2014

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1 Introduction

The section *Cyanopodae* Singer of the genus *Pholiotina* Fayod comprises three species in Europe, namely, *Pholiotina cyanopus* (G.F. Atk.) Singer, *Pholiotina aeruginosa* (Romagn.) M.M. Moser, and *Pholiotina atrocyanea* Esteve-Rav., Hauskn. & Rejos [1]. These fungi are rare or extremely rare on the continent, and are hardly ever found among lists of mushroom discoveries from European countries [1]. So far, none of them have been reported from Poland. During mycological investigations in Żywiec Basin (S Poland) in spring of 2012, unknown conocyboid basidiomata growing on woody remnants, and partly characterized by bluish base of stipe were found. This agaric could not be determined taxonomically with certainty in the field, but supplementary microscopic analyses confirmed our initial presumptions, and finally the recorded mushroom was identified as *Ph. cyanopus*, a species not yet known to grow in this country. Hence, the aim of the present study is to contribute to the knowledge on the variability of *Ph. cyanopus* by providing the description and figures of macro- and micromorphological characteristics based on the Polish specimens, to summarize current data on its morphology, ecology and general distribution, and to compare it with similar species to facilitate its identification.

Chemical investigations of the psychoactive properties of *Ph. cyanopus* conducted by various authors revealed the presence of at least three hallucinogenic components of the tryptamine type in the species, i.e. psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine), the main psychotropic compound, psilocin (4-hydroxy-N,N-dimethyltryptamine), and baeocystin (4-phosphoryloxy-N-methyl-tryptamine) [2-9, 7, 10]. Since psilocybin and psilocin are controlled compounds and considered narcotic drugs in Poland (Dz. U. 2005 Nr 179, poz. 1485) and in most European countries [10, 11] they must be reliably identified and quantified in mushroom samples. Therefore, the collected specimens of *Ph. cyanopus* were also subsequently screened for their psychoactive chemical properties using various methods of

separation, identification and quantification of naturally occurring tryptamine indoles. We have employed gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) to determine the active compounds. The results obtained were discussed and compared with previous findings about hallucinogens of *Ph. cyanopus*.

2 Material and Methods

2.1 Morphology

The description of macroscopic features was based on fresh material comprising over 100 basidiomata in all stages of development, from a single collection. Microcharacters were observed with a Nikon Eclipse E-400 light microscope equipped with a Nikon digital camera (DS-Fi1). For microscopic observations, dried material was placed in 95% ethanol for about 1 min., and then transferred to 5% NH₃ H₂O solution until they became pliable. Free-hand sections of the rehydrated pieces of basidiomata were examined in 5% NH₃ H₂O 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 (10 – 90 percentile values). The expression ($n = 301, 3$) means that 301 microelements from 3 basidiomata were measured. 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. The length of basidia was measured excluding sterigmata. Statistical computations employed Statistica software (StatSoft). Morphological terminology follows Vellinga [12] and Vellinga and Noordeloos [13]. The collections studied have been deposited in the herbarium of the Museum of Natural History, Wrocław University, Wrocław, Poland (WRSL).

2.2 Extraction procedures

The collection of *Ph. cyanopus* was divided into six representative samples used for further extraction and chromatographic analysis. Basidiomata samples were dried (at 40°C, for 24h), pulverized and extracted with methanol using ultrasound-assisted process. In this method 500 mg of each mushroom specimen was ground to a powder in a mortar with a pestle, transferred to a glass vial and, after addition of 50 ml methanol, placed

in an ultrasonic water bath for a period of 3 hours. The filtered extract was then evaporated to dryness under vacuum and dissolved in 0.2 ml methanol following which chromatographic techniques combined with mass spectrometry analysis were performed. The presented results of the hallucinogenic compounds content are given as mean with standard deviation value.

2.3 GC-MS (gas chromatography - mass spectrometry) analysis

The analysis was performed using HP 6890 Series gas chromatograph equipped with an HP 5973 mass selective detector. Helium was used as the carrier gas through a fused silica capillary column (Rtx 5-MS capillary, 30m×0.32mm ID, 0.25 µm film thickness) at 2 ml/min. The GC oven conditions used for these analyses were as follows: held at the initial temperature of 50°C for 1 min, ramped to 100°C at 15°C/min, held for 1 min and then ramped to 280°C, held at 280°C for 20 min. 1 µl of each solution was injected on-column into the gas chromatograph. Once the sample was loaded, the system was automatically controlled with a computer.

2.4 LC/ESI-MS (liquid chromatography/ electrospray ionization mass spectrometry) analysis

The UPLC system consisted of Dionex Ultimate 3000 series including a binary pump, a diode-array detector, an autosampler and a column compartment (Thermo Scientific, San Jose, CA). Methanolic extracts of *Ph. cyanopus* were separated on a Phenomenex Gemini C18 column (3µl, 150 × 3.0 mm I.D.; Phenomenex, Torrance, CA, USA) maintained at 35°C. The mobile phase consisted of a mixture 0.2% formic acid in water and a mixture 0.2% formic acid in acetonitrile. A constant flow of 0.2 ml/min was applied. The acetonitrile percentages were: 0-1.5 min, 5%; 1.5 – 12 min, linearly from 5% to 95%; 12-20 min, 95%; 20 – 25 min, linearly from 95% to 5%; 25-30 min, (equilibration step), 5%. The effluent from the chromatographic column was injected into microOTOF-Q-II time of flight mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) interface in the positive mode. The main mass conditions were: capillary temperature 200°C, spray voltage 4.5 kV. Mass data were collected in the product ion scan mode. The MS/MS conditions were: collision energy: 15%. For analyte identification the following precursor ions were used respectively: m/z 205.13 for psilocin, m/z 285.10 for psilocybin, m/z 271.08 for baeocystin, m/z 257.06 for norbaeocystin and 299.11 for aeruginascin.

3 Results

3.1 Taxonomy and morphology

Pholiotina cyanopus (G.F. Atk.) Singer [as “*cyanopoda*“], Trudy Bot. Inst. Akad. Nauk SSSR, ser. 2, Sporov. Rast. 6: 425. 1950. *Galerula cyanopus* G.F. Atk., Proc. Am. phil. Soc. 57: 367. 1918. *Conocybe cyanopus* (G.F. Atk.) Kühner [as “*cyanopoda*“], Encyclop. Mycol. 7: 128. 1935.



Illustrations: Hausknecht ([1], photo: p. 845-846, fig. 39 b-d: p. 926), Benedict *et al.* ([2], fig. 1 a-j: p. 153, as *Conocybe cyanopus*), Cetto ([14], photo 2663: p. 139), Stamets ([15], photo – left: p. 177, as *Conocybe cyanopus*), Kasperek ([16], photo 30: 1-2, as *Conocybe cyanopus*), Ludwig ([17], fig. 96.8 A-B: p. 154; [18], fig. 96.8: p. 522), Prydiuk ([19], fig. 2 a-e: p. 277), Arnolds ([20], fig. 200: p. 202), Einhellinger ([21], fig. 15.95), Kühner ([22], fig. 40: p. 130, as *Conocybe cyanopoda*); Figures 1-3 (this study).

Figure 1: *Pholiotina cyanopus* (G.F. Atk.) Singer (WRSL, ref. 0361). Top and side views of basidiomata (photo by R. Rutkowski).



Figure 2: Microcharacters of *Pholiotina cyanopus* (G.F. Atk.) Singer (WRSL, ref. 0361): A. basidiospores, B. cheilocystidia.

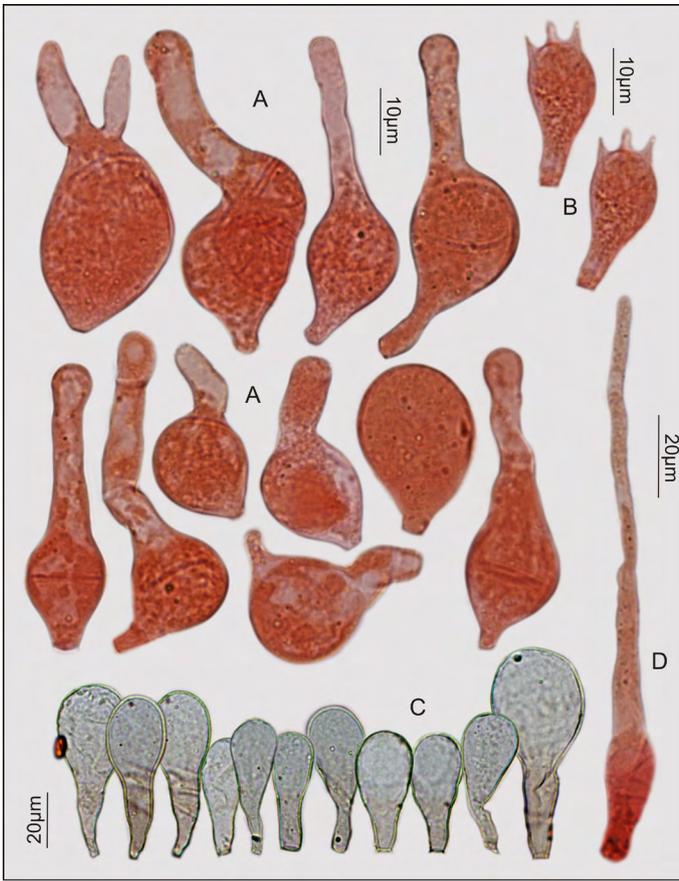


Figure 3: Microcharacters of *Pholiotina cyanopus* (G.F. Atk.) Singer (WRSL, ref. 0361): A. caulocystidia, B. basidia, C. pileipellis elements, D. pileocystidium.

Pileus (7.1) 14.7 ± 4.5 (26.8) \times (5.2) 15.5 ± 4.8 (25.2) mm, 9.5 – 20.6 \times 9.3 – 21.8 mm broad, ($n = 77$), campanulate-convex, conico-convex to plano-convex, hygrophanous, when moist rubiginous to reddish brown, translucently striate (from 3/5 up to centre), on drying orangey-brown, ochre-yellow to pale grey-orange; without veil remains. Lamellae, L (lamellae) = 16–26, l (lamellulae) = 1–3, narrowly adnate to adnexed, moderately distant, up to 2.5 mm wide, slightly to distinctly ventricose, young pale brown, mature orange-brown to rusty brown with inconspicuous (white flocculose) lamellar edge. Stipe (13.3) 22.8 ± 5.6 (37.6) \times (0.8) 1.4 ± 0.3 (2.1) (apex) \times (1.2) 2.4 ± 0.5 (3.8) (base) mm, 15.2 – 31.2 \times 1.0 – 1.8 \times 1.7 – 3.8 mm, ($n = 71$), cylindrical or more frequently faintly tapering upwards, occasionally slightly swollen at base, straight or nearly so, but more often irregularly bent, hyaline-white, white or greyish white at first then near base often with bluish grey hue, when bruised bluish colour sometimes more prominent (at least after a while), entirely white fibrillose striate lengthwise, pubescent, pruinose at apex. Context in pileus pale brown, in stipe whitish. Smell

absent or \pm acidulous. Taste not recorded. Spore print rusty brown.

Basidiospores (6.2) 8.0 ± 0.6 (9.3) \times (4.0) 4.7 ± 0.3 (5.6) μm , 7.1 – 8.8 \times 4.3 – 5.1 μm , Q = (1.5) 1.7 ± 0.1 (1.9), 1.6 – 1.8 ($n = 301$, 3), not or slightly flattened, ellipsoid oblong in frontal view, ellipsoid oblong to subamygdaliform in side-view, with slightly thickened wall and distinct, sometimes faintly eccentric germ-pore (20–27% of spores per collection), (0.5) 0.8 ± 0.1 (1.3) μm , 0.6 – 1.0 μm ($n = 301$, 3), rusty yellow to rusty brownish in ammonia. Basidia (15.0) 19.2 ± 1.9 (24.9) \times (8.4) 10.1 ± 0.9 (12.3) μm , 16.8 – 21.5 \times 9.0 – 11.3 μm ($n = 150$, 3) μm , 4-spored, clavate. Cheilocystidia (20.4) 33.9 ± 5.4 (50.6) \times (4.6) 9.5 ± 1.4 (14.0) μm , 27.0 – 40.9 \times 7.7 – 11.2 μm ($n = 301$, 3) μm , lageniform, often with more swollen body, and blunt, slightly broader or subcapitate apex, with neck (2.6) 4.3 ± 0.7 (6.4), 3.4 – 5.3 μm broad, thin-walled, hyaline, sometimes accompanied by scattered spheropedunculate cells. Pleurocystidia not observed. Pileipellis an epithelioid hymeniderm, made up of clavate and spheropedunculate elements, (15.9) 34.2 ± 7.0 (54.7) \times (10.5) 21.9 ± 6.0 (44.4) μm , 27.4 – 44.0 \times 14.9 – 30 μm

($n = 100, 3$), in between sporadically \pm cylindrical, nettle hair-shaped to narrowly lageniform pileocystidia, $(38.5) 93.8 \pm 29.2 (155.4) \times (8.1) 12.0 \pm 3.6 (21.4) \mu\text{m}$, $61.2 - 139.1 \times 8.8 - 18.4 \mu\text{m}$ ($n = 20, 3$). *Stipitipellis a cutis* composed of cylindrical, hyaline hyphae, $2.0 - 5.0 \mu\text{m}$ wide, with clusters of caulocystidia. Caulocystidia $(23.1) 42.8 \pm 9.3 (80.0) \times (3.0) 11.0 \pm 3.2 (27.1) \times (2.8) 4.9 \pm 1.0 (9.9) \mu\text{m}$, $31.5 - 54.5 \times 8.2 - 14.7 \times 3.9 - 6.0 \mu\text{m}$ ($n = 150, 3$), predominantly similar to cheilocystidia, but more irregular and also subcylindrical, often accompanied by scattered spheropedunculate cells. Clamp-connections present, but mostly rare (observed at the septa of some of the cheilocystidia, the caulocystidia and the hyphae of the stipe cortex).

Material examined: SW Poland, Żywiec Basin, Żywiec, in the vicinity of Żwirowa Street ($49.6762233^{\circ}\text{N } 19.22081^{\circ}\text{E}$, 360 m a.s.l. ; Figure 4), within the area of closed sawmill, terrestrial on soaked wood chips and other wet woody remnants of undetermined tree species, 14.06.2012, leg. R. Rutkowski, WRSŁ (ref. no 0361, 0362, 0363).

3.2 Psychoactive compounds

The LC-MS analysis of methanolic extracts of air-dried specimens of *Ph. cyanopus* indicated with sufficient reliability the presence of the tryptamine derivatives: psilocybin, the main psychotropic compound, psilocin and baeocystin. Additionally small content of norbaeocystin as well as aeruginascin was also determined in the analysed samples (Figure 5). Using GC-MS technique we estimated

cumulative content of psilocin and psilocybin in the studied material. Among the indentified compounds the psilocybin appeared in the largest quantities, and its content in dry matter was $0.90 \pm 0.08\%$. In contrast, the content of psilocin ($0.17 \pm 0.01\%$) and baeocystin ($0.16 \pm 0.01\%$) was similar and about five times lower compared to the level of psilocybin. Whereas the amounts of determined norbaeocystin ($0.053 \pm 0.004\%$) and aeruginascin ($0.011 \pm 0.0007\%$), turned out to be the lowest (Table 2).

4 Discussion

4.1 Delimitation and morphological variability

Pholiotina cyanopus was originally described in the beginning of the twentieth century from the Northeastern region of the United States (Ithaca, New York State) under the name *Galerula cyanopus* [23, cf. 24, 25]. Afterwards, with enhanced knowledge of microcharacters and their application as generic criteria, the species has been placed in *Conocybe* [22] and finally in *Pholiotina* [26], where currently it belongs to the section *Cyanopodae* Singer [1]. According to Hausknecht [1], distinctive features of *Ph. cyanopus* are: \pm brown, striate pileus, white stipe – changing to grey-blue at base when bruised (a reliable field character for the presence of the

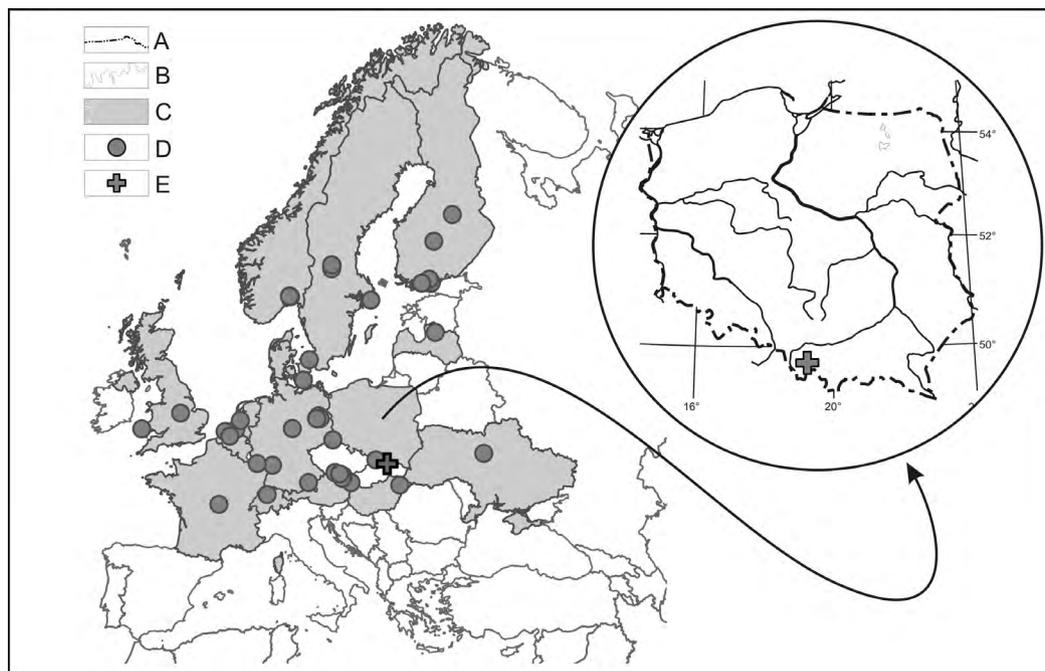


Figure 4: Known distribution of *Pholiotina cyanopus* (G.F. Atk.) Singer in Europe and in Poland: A-B – border of countries, C – countries in which the species has been observed (based mainly on literature records and supplemented by unpublished data), D-E– localities of the species.

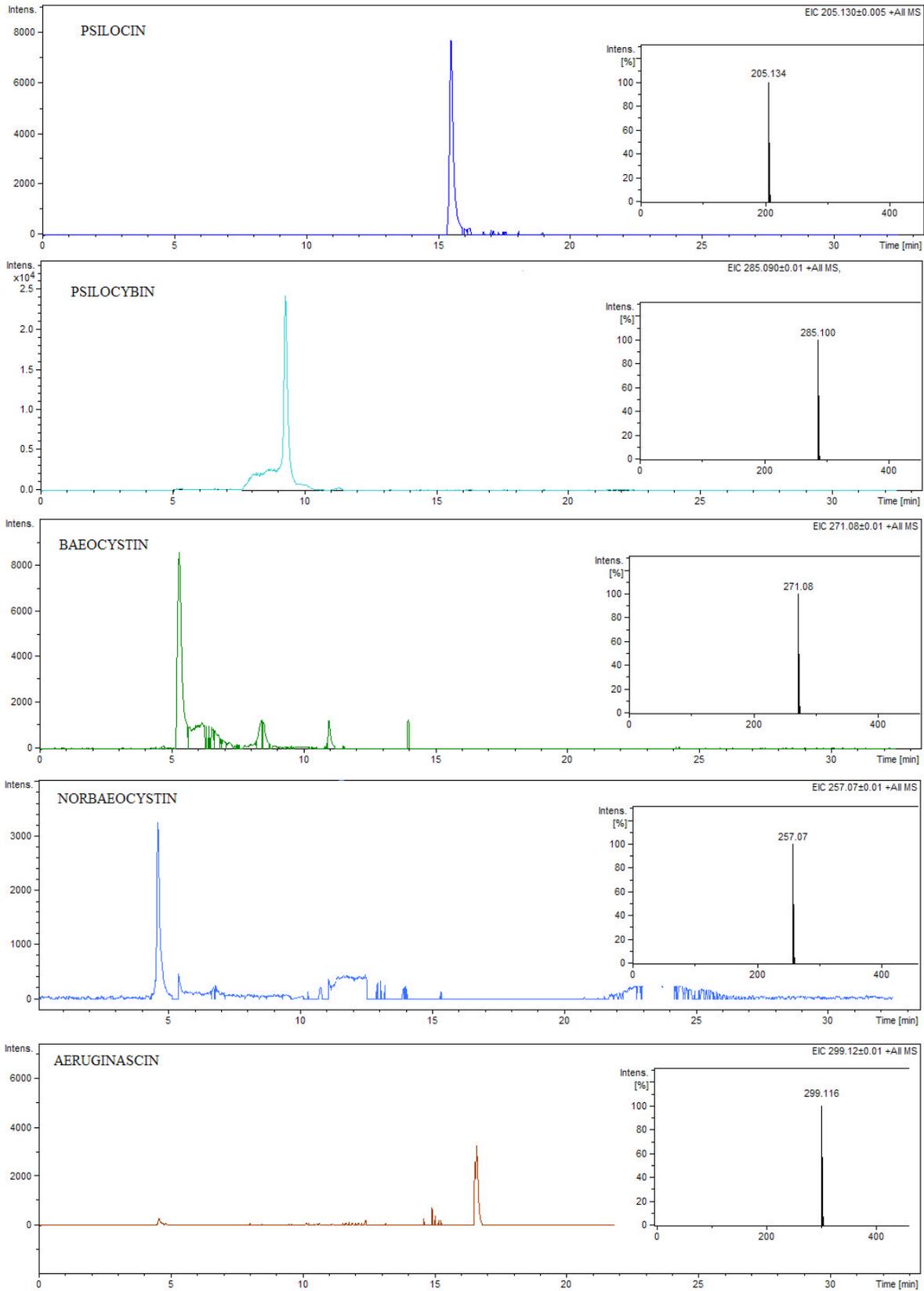


Figure 5: The extracted ion chromatograms of LC-MS analysis (A) and mass spectra of identified hallucinogenic compounds (B). $[M+H] = 205.13$ – Psilocin; $[M+H] = 285.10$ – Psilocybin; $[M+H] = 271.08$ – Baecocystin; $[M+H] = 257.07$ – Norbaecocystin; $[M+H] = 299.11$ – Aeruginascin.

N-methylated tryptamines), and small, quite strongly coloured basidiospores. Furthermore, lageniform and often slightly capitate cheilocystidia in combination with the presence of narrowly lageniform pileocystidia seems to be of great importance. Considering the basidiomata, *Ph. cyanopus* may resemble *Pholiotina smithii* (Watling) Enderle, in Enderle & Hübner (syn. *Conocybe smithii* Watling, *Galera cyanopes* Kauffman), which is rather common in North America and has not yet been reliably recorded in Europe. *Ph. smithii* is virtually identical except that it has more cinnamon-brown lamellae, more distinctly striate and paler pileus and somewhat wider cheilocystidia [cf. 2,25,27]. It probably favours mossy environments [15,28]. Also somewhat comparable but not enough recognized species known to exhibit a bluing or greening reaction in stem-base (and probably belonging to sect. *Cyanopodae*) is *Pholiotina sulcatipes* (Peck) Bon [cf. 18, 20]. This taxon was described from North America, where it was found growing on a bed of buckwheat bran [29]. In literature it has been probably erroneously synonymised by some authors with *Pholiotina filipes* (G.F. Atk.) Singer (syn. *Pholiotina aberrans* (Kühner) Singer; sect. *Piliferae*) [1, cf. 20,25,30]. It appears to have less macroscopic features in common with *Ph. cyanopus*, from which it differs in more thin-walled basidiospores and narrowly fusiform to narrowly lageniform cheilocystidia with less swollen body and not subcapitate apex. [29-31]. The other known members of the section *Cyanopodae* are characterized by never blueing stem base, however they can be distinguished by blue, pale greyish-blue to dark blue colours in pileus [1]. These are two closely related species, i.e. *Pholiotina aeruginosa* (Romagn.) M.M. Moser (syn. *Conocybe aeruginosa* Romagn.) and *Pholiotina atrocyanea* Esteve-Rav., Hauskn. & Rejos. The first of the mentioned species is easily recognised by the two-coloured pilei, blue-green in centre, strongly fading to ochre in the marginal zone, whitish stipe, slightly thick-walled, medium-sized spores, 4-spored basidia and lageniform to fusiform cheilocystidia. Similar species *Ph. atrocyanea* forms more intensive blackish blue to dark blue pilei and 2-spored basidia [1]. Ecologically, *Ph. aeruginosa* appears to be a species of humid and rather rich deciduous woods of Euro-Siberian character, whereas *Ph. atrocyanea* is only known from the type locality – a Mediterranean *Quercus faginea* forest on sandy, acid soil [32].

Although *Ph. cyanopus* is generally considered to possess the partial veil, all specimens we examined were devoid of this morphological feature. This is consistent with the observations of Arnolds [20]. However, it should be noted that Hausknecht [1,27] emphasised temporary and arachnoid character of *Ph. cyanopus* velum, which

in his opinion is visible only in young basidiomata. As we examined also very young specimens, it is likely that the inability to observe the velum in case of our collection could be caused by a fairly heavy rainfall that preceded and accompanied the field survey. Nonetheless, most of the remaining macro- and micromorphological features of the Polish collection clearly correspond to the most recent species-concept of *Ph. cyanopus* [1]. Microscopic examination of the collected material and survey of the available literature data led to recognize this species as not obviously heterogeneous in its selected morphological features. The macroscopical features of analysed basidiomata agree well with the description given by Hausknecht [1] and microscopical characters fall within the estimated range of variability reported in the literature (Table 1). Moreover, in most cases we did observe a bluish-gray discolouration at the stipe-base surface of our specimens, which is considered as characteristic on one hand [21,23,33,34], but appears to be not always observable feature on the other hand [1,14,27,35]. In our material the blueing of the stipe base was always delayed after bruising and started most frequently only after some time (from a few minutes up to several hours). In some specimens this discolouration was spread throughout the stipe. Nevertheless, in some specimens the gray-blue colour change was not noticeable or was absent completely even after a few hours. Unfortunately, as the blueing of the stipe base is sometimes delayed and starts only after some time, *Ph. cyanopus* can be also easily misidentified with a whole series of non-blueing species of the genus *Pholiotina* and *Conocybe*. For example, *Pholiotina sulcata* Arnolds & Hauskn occurs in similar habitats and it looks similar in appearance and comparable with microscopic features; however, it has distinctly sulcate pileus in the outer half, pelargonium-like smell and somewhat larger basidiospores. Very similar *Pholiotina filipes* (G.F. Atk.) Singer has larger basidiospores with clearly broader germ pore, and larger, subcylindrical to more elongate lageniform – cheilocystidia.

4.2 Habitat requirements, periodicity and distribution

Ph. cyanopus is considered as a saprophyte growing most frequently in grassy areas – in lawns and fields, in rather poor meadows, rarely in grassy clearings in forest, and under tree canopies. It has also been occasionally recorded from roadsides and paths, as well as from vineyard, gardens and timber yards. Basidiomata of *Ph. cyanopus* grow usually scattered in small groups, less often solitary or in large groups (e.g. this study),

Table 1: Comparison of selected morphological features of *Pholiotina cyanopus* (G.F. Atk.) Singer according to different studies (the abbreviation: N/D = no data; literature data represent range of minimum and maximum values; in case of this study dimensions of cheilo- and caulocystidia are given as: length × middle width × apical width).

Authors	Basidiospores		Cheilocystidia	Caulocystidia	Pileocystidia
	length × width	Q	length × width × width (apex)	length × width × width (apex)	length × width × width (apex)
[23]	8-10 × 5-6	N/D	30-40 × 10-17	N/D	N/D
[2]	6.5 - 7.5 (8.5) × 4.5 -5	N/D	7.5-10 × 20-25 × 3-5	N/D-90 × N/D-N/D × 4	18-26 × 7-8 × 4-5 (-6.5)
[33]	6.5-7.5 (8.5) × 4.5-5	N/D	20-25 × 7.5-10 × 4-5	N/D	N/D
[25]	7-9 × 4-5	1.55-1.85	14-30 × 4-12	N/D	not observed
[20]	7.0-9.0(10.0) × 4.5-5.5	1.5-1.9	20-39 × 6.5-11	24-39 × 5.5-10.5	not observed
[14]	6.5-9.5 × 4.5-5	N/D	N/D	N/D	N/D
[19]	6.5-9.0 × 4.3-6.0	1.4-1.8	19-31 × 7-16	24-52 × 12-19	N/D
[27]	6.5-9.5 × 4-5.5	1.55-1.8	20-45 × 6-11(-19)	N/D-40 × N/D-13	N/D-50 × N/D-6
[18]	7-8.5(10) × 4-5(6)	N/D	N/D-32(40) × N/D-14	N/D	not observed
[1]	6.5-9.5 × 4-5.5	1.55-1.8	20-45 × 6-11(19)	N/D-40 × N/D-13	N/D-50 × N/D-6
[35]	7-9 × 4-5	1.7-1.9	22-44 × 9-22	N/D	N/D-50 × N/D-5
This study (min. – max. values)	6.2-9.3 × 4-5.6	1.5-1.9	20.4-50.6 × 4.6-14 × 2.6-6.4	23.1-80 × 3.0-27.1 × 2.8-9.9	38.5-155.4 × 8.1-21.4
This study (10 – 90 percentile values)	7.1-8.8 × 4.3-5.1	1.6-1.8	27-40.9 × 7.7-11.2 × 3.4-5.3	31.5-54.5 × 8.2-14.7 × 3.9-6.0	61.2-139.1 × 8.8-18.4

mostly on ground among grass (and moss), rarely on bare soil among moss, on loessic or sandy soil, on grit ground, in humus enriched with woody debris, among leaf litter or directly on wood remains and sawdust [e.g. 1,2,9,15,16,18,19,27,36-38]. According to available data, the sporophore production by the fungus in Europe

mostly occurs in June-September. However, observations from May, October, and January have also been mentioned [27, 39]. The Polish specimens of the species have been collected from 360 m a.s.l., however the elevation of the European records ranges from ca. 150 to 600 m alt. in Austria, from ca. 5 to 60 m alt. in Denmark, from ca. 5 to

170 m alt. in Finland, from ca. 45 to 820 m alt. in Germany, and from ca. 20 to 350 m alt in Norway.

Ph. cyanopus is probably widely distributed and may be expected elsewhere across the north temperate and boreal regions of the world, but is easily overlooked because of its minute stature and its strong resemblance to a *Conocybe*. Nevertheless, it is nowhere characterized as common. According to our knowledge, hitherto *Ph. cyanopus* apart from temperate and boreal regions of North America (British Columbia, Colorado, Oregon, Washington, Québec, Michigan, New York) [2,3,9,15,23,39-42] and East Asia (the Russian Sikhote-Alin Mountains, Yakutia) [43,44] has been also found in fifteen European countries. It is known from ca. 60 collections gathered from: Austria [1,27], Belgium [45], Denmark [1,27,36,39], Finland [1, 15, 27, 36, 38, 46], France [1, 40, 47], Germany [1,7,15,16,18,21,22,27,39,40,48-51], Hungary [27,52], Latvia [27,37], Norway [6,15,27,35,36,39,40,53,54], Poland (this study), Sweden [36,39,55], Switzerland [56-58], Ukraine [1,19,27], and the Netherlands [20,27,59,60] (Figure 4). The species has also been mentioned from Great Britain (England: Leicestershire, Wales: Pembrokeshire), but due to the lack of authentic material, these data are accepted only with caution [2,33,61,62].

Polish collection appears to be a “missing link” in the European distribution area of *Ph. cyanopus*. Our record most probably does not reflect the distribution of the species in Poland. It is to be hoped that future collectors will be able to extend the present observation in this respect. This species, although wide-spread on the European mainland, occurs very sporadically and therefore it is regarded as rare [1]. Furthermore, it has been red-listed in several European countries, e.g. in the Netherlands [59], Denmark (http://www.dmu.dk/en/animalsplants/red_data_book), Switzerland [58], Hungary [52], and Latvia [63]. Because of its rarity it seems to be justified to place *Ph. cyanopus* on the Polish red list as well, at least in “vulnerable” species category.

4.3 Psychoactive properties

There are at least three species of conocyboid agarics worldwide that have been shown to contain hallucinogenic tryptamine-based alkaloids [cf. 10,64]. These are *Pholiotina cyanopus* [2-9], *Pholiotina smithii* [2,9,28], and *Conocybe velutipes* (Velen.) Hauskn. & Svrček (syn. *Conocybe kuehneriana* Singer) [8]. Regarding *Conocybe siligineoides* R. Heim – member of the section *Mixtae* Singer [25,65], reported by Wasson [66], Heim [67] and Heim and Wasson [68] as a sacred mushroom in Mexico used by the Aztecs for shamanistic purposes, no chemical study has been

undertaken on this fungus to date. However, this species is suspected of active properties [10,15,69], and according to Allen [64] it should contain psilocybin. After reviewing the available literature, it can be stated that there is little mention about this species in a taxonomical context. *C. siligineoides* was collected only one time in 1955 by Robert G. Wasson and Valentina P. Wasson on rotting wood in the State of Oaxaca, Mexico, and no additional report of this (endemic) taxon has probably been made since then. Furthermore, Gastón Guzmán has not been able to re-collect this fungus in Mexico even after several years of extensive field-work [40,70]. According to our knowledge, no chemical studies for the presence of indole compounds have been made on *Conocybe caeruleobasis* Tkalčec, Mešič & Hauskn., a recently described species with pale blue context in stipe base [71] – as well as on the above mentioned members of the genus *Pholiotina*, i.e. *Ph. aeruginosa*, *Ph. atrocyanea*, and *Pholiotina sulcatipes*, related to *Ph. cyanopus*. Although, *Ph. aeruginosa* and *Ph. atrocyanea* (as representatives of the *Cyanopoda* group) are reported as being psilocybin positive [1,20,72], it is important to observe that these species have been listed as being psilocybian (producing these compounds) because of the blue, pale greyish blue to dark blue colours exhibited naturally by their pileus, rather than after a direct chemical examination. Although the presence of the blue tints in pilei of members of the *Cyanopoda* section seems to be quite a reliable field characteristic for detecting the N-methylated tryptamines, the blue pigment and its intensity are not always infallible indicators of the presence of psilocybin and psilocin in case of other mushrooms [2-4].

The occurrence of the psychoactive compounds: psilocybin, psilocin and baeocystin found in *Ph. cyanopus* amply confirm earlier published data (Table 2). However, it should be stressed that known investigations for psilocybin analogues in the species have been unrewarded with finding of norbaeocystin and particularly aeruginascin so far. Hitherto, the presence of aeruginascin seemed to be restricted only to *Inocybe aeruginascens* Babos [73-76]. Thus, it is noteworthy and interesting that it is the first time these analogues of psilocybin – one of them (aeruginascin) being closely related to the frog skin toxin bufotenidine – have been found in *Ph. cyanopus*. *Ph. cyanopus* appears to be moderately to highly active [15]. Beug and Bigwood [4] using high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) found merely a significant quantity of psilocybin (0.93%) in dried material from North America. European samples of the species analysed by comparable techniques showed diverse levels of psilocybin (0.11-1.01%) and traces

Table 2: Occurrence of psilocybin, psilocin, baeocystin, norbaeocystin, and aeruginascin (mg/kg dry weight if not otherwise stated) in *Pholiotina cyanopus* (G.F. Atk.) Singer according to different authors (the abbreviation: n.d. = non detectable level; + = detected, but not quantified; * = mg/kg fresh weight; an empty field implies that this compound was not analysed for; literature data represent range of minimum and maximum values or values coming from a single studied sample; in case of this study the hallucinogenic compounds content are given as: mean \pm standard deviation).

Geographical region where it was collected	Analytical method	Psilocybin	Psilocin	Baeocystin	Norbaeocystin	Aeruginascin	Reference
Europe (Finland)	HPLC	4500*	700*				[8]
Europe (Norway)	HPLC	3000-6000					[9]
North America (Canada)	TLC			300-1000			[9]
North America (USA)	PC	+	n.d.				[3]
North America (USA)	PC	+	n.d.				[2]
North America (USA)	HPLC/TLC	9300	n.d.				[4]
North America (USA)	TLC			500			[9]
Europe (Norway)	HPLC	3300-5500	40-70				[6]
Europe (Germany)	HPLC/TLC	7800-10100	n.d.	1200-2000			[48]
Europe (Poland)	LC-MS	9000 \pm 800	1700 \pm 100	1600 \pm 100	530 \pm 40	110 \pm 7	This study

of psilocin (0.004-0.07%) [6,9,48]. Hitherto, baeocystin was found infrequently both in American and European samples of *Ph. cyanopus* at levels from 0.03 to 0.20%. It seems that the content of psilocybin does not make the Polish *Ph. cyanopus* a potent narcotic drug. A typical recreational dose of pure psilocybin is 6 to 20 mg. It results in mild psychotic effect, usually experienced as a pleasure (so called 'good trip') [77, 78]. If we even consider that the content of active indolic compounds that makes up to 1% of the weight of the dry basidiomata and that a dry *Ph. cyanopus* weights ca. 40 mg, then, this species bears approximately 0.4 mg of psilocybin. Thus, ingestion of fifteen to fifty dried specimens of the species is necessary for similar effects. However, due to the large variation in potency within individual specimens, the same dosages based on a weight basis or on the number of specimens ingested may generate a potent hallucinogenic impression as well as almost no effect at all. Furthermore, the species, being rare and growing usually single or in small groups, can hardly be considered at present as a significant source of psychoactive substances for potential consumers.

Acknowledgements: The research was supported by Wrocław Research Centre EIT+ under the project "Biotechnologies and advanced medical technologies" – BioMed (POIG.01.01.02-02-003/08) financed by the European Regional Development Fund (Operational Programme Innovative Economy, 1.1.2). The authors wish to express their sincere gratitude to Dr. Egil Bendiksen

(Trondheim, Norway), Dr. Anton Hausknecht (Wien, Austria), and Mrs Bernadeta Pawlik (Cracow, Poland) for their kind help with completing mycological literature.

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