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An adventive *Panaeolus antillarum* in Poland (Basidiomycota, Agaricales) with notes on its taxonomy, geographical distribution, and ecology

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Abstract – Coprophilous fungus, *Panaeolus antillarum* rarely recorded in Europe, is reported here for the first time from the Augustów Plane, north-eastern Poland. This thermophilic species was found outdoors in August on horse dung mixed with straw. A chemical analysis did not confirm the presence of the psychoactive alkaloids in collected material. A complete description and illustration of the species based on Polish specimens are presented and notes on its taxonomy, ecology, world distribution and comparison with similar taxa – *P. semiovatus* var. *semiovatus*, *P. semiovatus* var. *phalaenarum*, and others are also provided.

***Anellaria antillarum* / coprophilous mushrooms / GC-MS / Polish mycobiota**

INTRODUCTION

The genus *Panaeolus* (Fr.) Quél. of the family *Psathyrellaceae* is characterized by small to rather medium sized basidiomata with usually coprophilous or nitrophilous habitat. According to Kirk *et al.* (2008) it is represented by *ca*. 15 species. However, Gerhardt (1996) mentions 27 species of the genus worldwide. Depending on the systematic treatment, hitherto 13-16 species of *Panaeolus* have been found in Europe (Gerhardt, 1996; Pegler & Henrici, 1998; Senn-Irlet *et al.*, 1999; Ludwig, 2001b). In Poland 9 species of this genus have been found until now: *P. acuminatus* (Schaeff.) Gillet, *P. alcis* M.M. Moser, *P. cinctulus* (Bolton) Sacc., *P. fimicola* (Pers.: Fr.) Gillet, *P. guttulatus* Bres., *P. olivaceus* F.H. Møller, *P. papilionaceus* (Bull.: Fr.) Quél., *P. semiovatus* (Sowerby: Fr.) S. Lundell sensu lato (Wojewoda, 2003; cf. Kujawa, 2012), and *P. subfirmus* P. Karst. (Halama, unpublished), and *P. acuminatus* and *P. papilionaceus* would seem to be the most widely distributed. Due to the fact that the name *Panaeolus leucophanes* (Berk. & Broome) Sacc. (*Agaricus leucophanes* Berk. & Broome) is treated as a *nomen dubium* (Gerhardt, 1996; Legon *et al.*, 2005), the identity of this taxon reported by Łuszczyski (2007, 2008) remains to us unclear for the moment.

During field studies in the vicinity of Sarnetki village (the Augustów Plane, NE Poland; Figs 1-2), the first author of this paper found an interesting and distinct large-spored *Panaeolus* species growing on horse manure. In comparison

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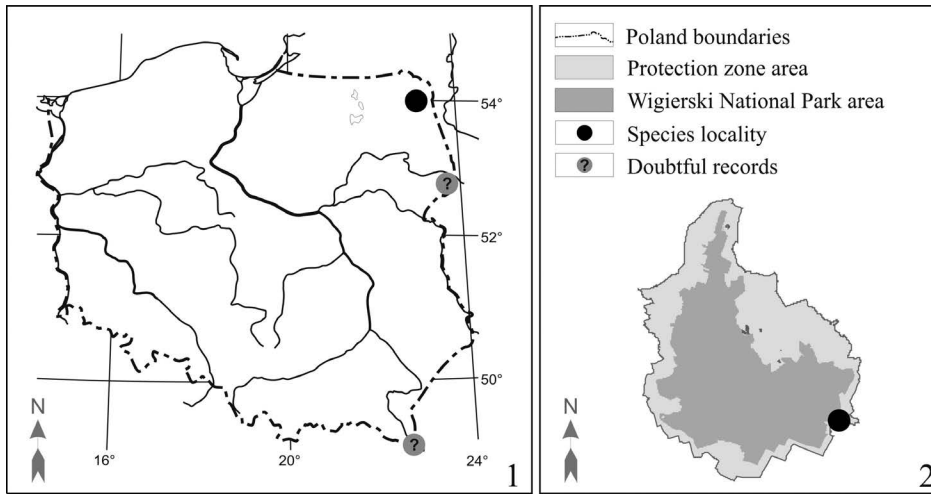


Figs 1-2. Distribution of *Panaeolus antillarum* in Poland (1) and in the Wigierski National Park (2).

with recent literature it was identified as *Panaeolus antillarum* (Fr.) Dennis, a species new to mycobiota of Poland. The main aim of the present work is to describe morphologically the first collections of *P. antillarum* for Poland, and to compare their characters with published data. Furthermore, this paper aims to evaluate world distribution and general ecology of the species in relation to the situation in Europe. In addition, collections of *P. antillarum* were analysed for psilocin, psilocybin and related active tryptamine derivatives content. The results of this investigation are reported below.

MATERIAL AND METHODS

Morphology: Collections of *P. antillarum* were made in 2013 during mycological investigations of deadwood *macromycetes* in the Wigierski National Park by the author. The description of macroscopic features is based on fresh material. Microcharacters of basidiomata were observed with a Nikon Eclipse E-400 light microscope equipped with a Nikon digital camera (DS-Fi1). All microscopic structures were observed in dried material. Free-hand sections of the rehydrated pieces of basidiomata were examined in 5% NH_3 H_2O and Congo red reagent. 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 = 100, 2, 2) means that 100 microelements from two basidiomata originating from two collections 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 lengths of basidia were measured

excluding sterigmata. Statistical computations employed Statistica software (StatSoft). For morphological terminology see Vellinga (1988). Details of the microcharacters were figured by freehand drawing, with exact proportions and general shapes traced from photographs. The collections studied have been deposited in Museum of Natural History, Wrocaw University, Wrocaw, Poland (herbarium WRSL).

Extraction procedure for chemical analyses: Basidiomata samples were dried (at 40ºC, for 24h), pulverized and extracted with methanol (by sonication). With this method 250 mg of mushroom specimen has been 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 concentrated under vacuum to 0.2 ml, then GC-MS analysis has been performed.

Apparatus and chromatographic conditions: The measurements were performed using HP 6890 Series gas chromatograph equipped with an HP 5973 mass selective detector. Helium was used as the carrier gas through the fused silica capillary column (RTX5-MS capillary, 30 m × 0.32 mm ID, 0.25 µm film thickness) at 2 mL/min. The GC oven conditions used for these experiments 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 into the gas chromatograph. Once the sample was loaded, the system was automatically controlled with the computer.

RESULTS

Panaeolus antillarum (Fr.) Dennis, Kew Bull. 15(1): 124. 1961.

Agaricus antillarum Fr., Elench. fung. (Greifswald) 1: 42. 1828. *Anellaria antillarum* (Fr.) Hlaváček, Mykologický Sborník 74(2): 52. 1997. = *Agaricus antillarum* var. *praelonga* Fr., Acta Regiae Soc. Sci. Upsaliensis, 3(1): 25. 1851. = *Agaricus sepulchralis* Berk., London J. Bot. 1: 452.1842. = *Panaeolus sepulchralis* (Berk.) Sacc. [as '*sepulcralis*'], Syll. fung. (Abellini) 5: 1119.1887. = *Anellaria sepulchralis* (Berk.) Singer, Lilloa 22: 475. 1951. = *Agaricus solidipes* Peck, Ann. Rep. Reg. N.Y. St. Mus. 23: 101. 1872. = *Panaeolus solidipes* (Peck) Sacc., Syll. fung. (Abellini) 5: 1123. 1887. = *Agaricus ovatus* Cooke & Massee, in Cooke, Grevillea 18(no. 85): 4. 1889. = *Panaeolus ovatus* (Cooke & Massee) Sacc., Syll. fung. (Abellini) 9: 147. 1889. = *Agaricus eburneus* Cooke & Massee, in Cooke, Grevillea 18(no. 85): 4. 1889. = *Panaeolus eburneus* (Cooke & Massee) Sacc., Syll. fung. (Abellini) 9: 147. 1891. = *Panaeolus bolombensis* Beeli, Bull. Soc. R. Bot. Belg. 61(1): 96. 1928.

Icones: Pegler, 1968: p. 510, Fig. 4.3a-e; 1977: p. 413, Fig. 4a-e; Yokoyama, 1979: p. 111, plate 1: Figs. 1-6, p. 114, plate 3: (photo) a-b; Pegler, 1983: p. 479, Fig. 95k-n; Gerhardt, 1987: (photo) by A. Schmidt between p. 224 & 225, p. 227, Fig. 2a-b; Young, 1989: p. 84, Fig. 4a-f; Robich, 1992: p. 642, Fig. 1a-f (as *Anellaria phalaenarum*); Ludwig, 2001a: p. 127, Fig. 59.12; 2001b: p. 488; Martinelli, 2001: front cover (photo); Doveri, 2004: pp. 222-225, p. 1093 (photo); Enderle, 2004: p. 326 (photo); Roux, 2006: p. 1106 (photo, as *Panaeolus phalaenarum*); Rommelaars & Arnolds, 2007: p. 144, plate. 7 (photo), p. 147, Fig. 1a-c.

Basidiomata generally scattered. **Pileus** 12-55 mm, first convex to conico-convex then obtusely conical, conico-convex or hemispherical to some extent, with margin exceeding gills, fleshy, not hygrophanous, initially pure white to gray-white, occasionally gradually darkening to alutaceous cream in central part, at first smooth, becoming slightly wrinkled, and rugulose or areolately cracked on dying, matt (with moisture sticky and shiny). **Lamellae** very crowded, L = 35-50, I = 3-6, ventricose to broadly ventricose, adnate, seceding to adnexed, first beige then dusky, and black marbled (clearly mottled), with conspicuously white pruinose edge. **Stipe** 30-155 × 2-8 mm, cylindrical or slightly evenly thickening towards the base and upwards, sometimes clearly broadened at base, and prolonged into a short pseudorrhiza, solid, straight to curved, white to off-white, at age usually with a brownish or flesh-colored tinge, smooth or especially in the upper half somewhat fibrillose-striate to fibrillose-ribbed, almost entirely whitish poorly pruinose when fresh, often holding drops of water near apex when wet (Figs 3-4). **Velum** absent even in young basidiomata. **Context** first quite firm, in pileus

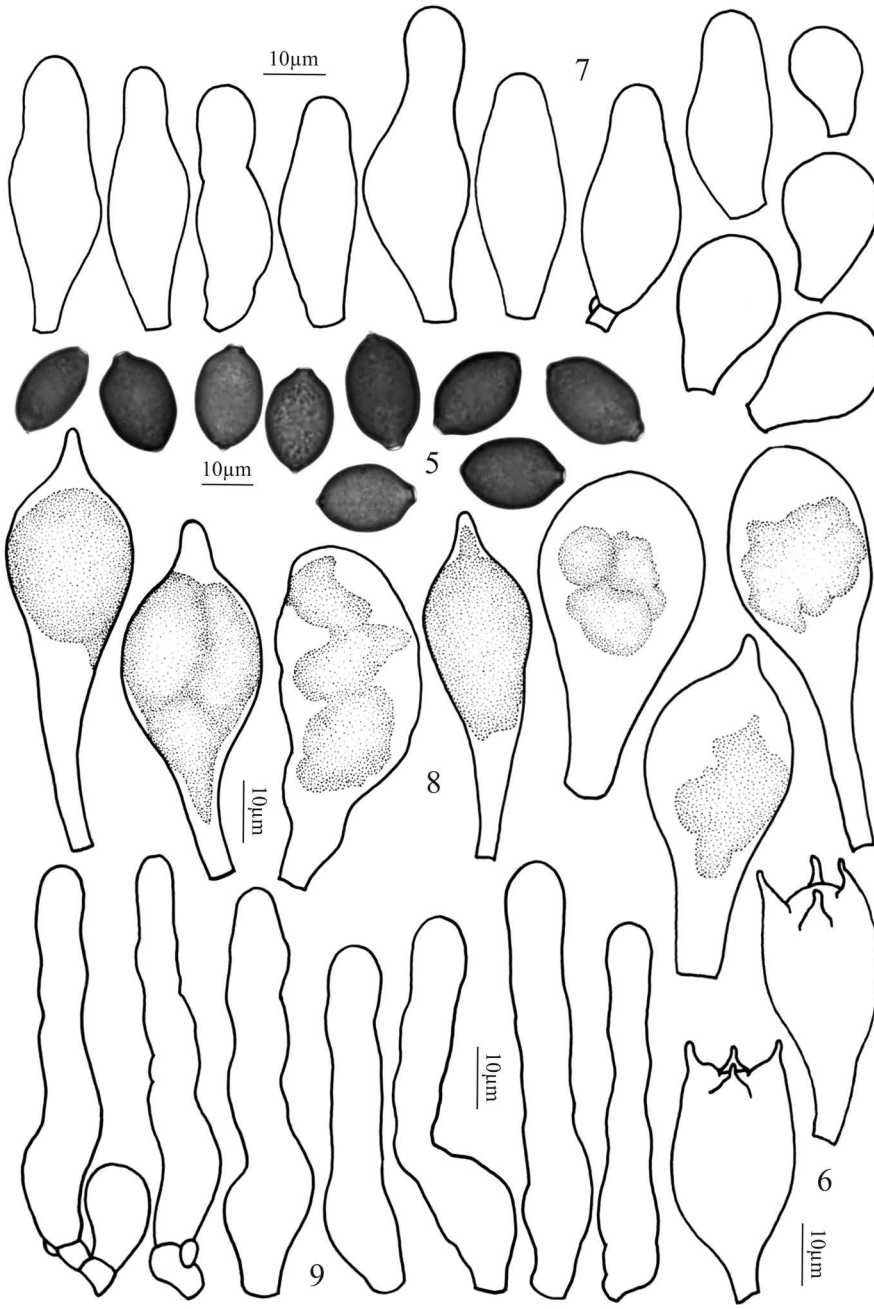
Figs 3-4. *Panaeolus antillarum* (WRSL-24082013.474). Side and bottom views of basidiomata (photo by M. Halama).

concolorous, in stipe white at apex and often darker (brownish) in lower part. **Smell** not distinctive, slightly fungoid. **Taste** not verified. **Spore print** colour black.

Basidiospores (13.3) 16.7 ± 1.4 (20.0) × (7.7) 9.5 ± 0.7 (11.4) × (9.4) 11.4 ± 0.6 (12.9) µm, 14.8-18.4 × 8.6-10.4 × 10.6-12.2 µm, Q_w = (1.3) 1.8 ± 0.2 (2.2), Q_w = 1.5-2.0, Q_b = (1.2) 1.5 ± 0.1 (2.0), Q_b = 1.3-1.6 (*n* = 224, 2, 2), oblong-ellipsoid in side-view, limoniform, angular-limoniform, and typically (sub)hexagonal in frontal view, flattened ventrally (adaxially), with dark, smooth, thickened wall, with ± prominent, central germ pore, (1.3) 1.9 ± 0.3 (2.6), 1.5-2.2 µm, very dark reddish brown in ammonia. **Basidia** (21.7) 29.5 ± 4.4 (40.8) × (12.6) 15.7 ± 1.7 (19.7) µm, 24.0-35.2 × 13.8-18.4 µm (*n* = 102, 2, 2), 4-spored, clavate to broadly clavate, clamped. **Lamella edge** sterile. Cheilocystidia (16.9) 26.6 ± 5.5 (45.6) × (7.0) 10.9 ± 1.9 (16.6) × (3.2) 5.8 ± 1.2 (10.4) µm, 20.1-34.3 × 8.6-13.1 × 4.4-7.4 µm (*n* = 203, 2, 2), variable: mostly lageniform, utriform to fusiform, typically with a broad, obtuse apex, thin-walled, colourless, sometimes flexuosus, abundant. Another type of ± short broadly clavate cheilocystidioid elements is rarely also present. **Sulphidia** 34.0) 43.7 ± 5.5 (57.9) × (12.4) 18.0 ± 2.4 (24.0) µm, 37.1-53.5 × 14.7-21.1 µm (*n* = 76, 2, 2), numerous on the gill-face, occasionally found on the lamella-edge, inflated clavate or fusiform, occasionally mucronate, thin-walled, hyaline but containing principally a single irregular, refractive body which appears pale yellow in ammonia. **Caulocystidia** (19.1) 46.3 ± 9.2 (60.8) × (5.9) 8.6 ± 1.4 (13.8) × (5.0) 7.2 ± 1.2 (12.1) µm, 32.6-56.9 × 7.2-10.4 × 5.8-8.5 µm (*n* = 110, 2, 2), in groups, present numerously at apex and more scattered downwards, similar to cheilocystidia, though most frequently cylindrical with an obtuse apex, hyaline, thin-walled. (Figs 5-10). **Pileipellis** an epithelioid hymeniderm, made up of broad clavate (pyriform) to globose elements, 12-40 µm wide, hyaline, smooth and thin-walled. Stipitipellis a cutis, made up of cylindrical, 4.0-13 µm wide, hyaline or yellowish hyphae, pigment invisible or parietal, and intracellular. **Clamp-connections** infrequently present in all tissues.

Specimens examined: POLAND, the Augustów Plane, in the vicinity of Sarnetki village: 1. 54.002703ºN 23.216006ºE, road verge adjacent to a paddock and close to a forest edge (142 m a.s.l.): on horse dung mixed with straw, 2013.08.24, *leg.* M. Halama, WRSL (ref. 474); 2. Ibid.: on horse dung mixed with straw, 2013.08.24, *leg.* M. Halama, WRSL (ref. 475).

Additional specimens examined: *P. semiovatus* var. *semiovatus*: POLAND, Littoral Kashubia, "Beka" nature reserve, salt meadow: on cattle dung, 2013.05.19, *leg.* M. Wantoch-Rekowski, WRSL (ref. 504).



Figs 5-9. Microcharacters of *Panaeolus antillarum* (WRSL-24082013.474): **5.** basidiospores, **6.** basidia, **7.** cheilocystidia, **8.** chrysocytidia, **9.** caulocystidia (drawings by M. Halama).

Fig. 10. Basidiospores of *Panaeolus antillarum* (WRSL-24082013.475; microphotographs by M. Halama).

DISCUSSION

Taxonomy and morphology

P. antillarum is accepted here in the sense of Gerhardt (1996). This species was originally described in the end of 1820s from the material collected in Saint Croix (United States Virgin Islands) by Danish pharmacist – Peder Eggert Benzon (Fries, 1828). The name '*Agaricus antillarum* Fr.' was not much used in the mycological literature from the time of E.M. Fries until the 1960s. Dennis (1961) was the first who recognized conspecificity of *A. antillarum* Fr. with later *Agaricus sepulchralis* (Berkeley, 1842) and *Agaricus solidipes* (Peck, 1872), after he had collected corresponding material from the Lesser Antilles (Trinidad). Then he also placed *A. antillarum* Fr. in the genus *Panaeolus*. *P. antillarum* is widely distributed in subtropical and tropical regions (see discussion below), and it has been given a lot of epithets (e.g. Cooke, 1881; Kalchbrenner, 1881; Cooke, 1889; Masee, 1902; Beeli, 1928). Its more complete list of synonyms is given by Pegler (1968, 1977, 1983); and Young (1989); Gerhardt (1996); Ludwig (2001b). Together with *P. semiovatus* (Sowerby: Fr.) S. Lundell var. *semiovatus* (syn. *Panaeolus separatus* (L.) Quél., *Anellaria semiovata* (Sowerby) A. Pearson & Dennis), and *P. semiovatus* var. *phalaenarum* (Fr.) Ew. Gerhardt (syn. *Panaeolus phalaenarum* (Fr.) Quél., *P. teutonicus* Bride & Métrod, *P. semiovatus* f. *exannulatus* A. Pearson), *P. antillarum* belongs to the subgenus *Anellaria* (P. Karst.) Ew. Gerhardt, which is marked by mostly robust and fleshy basidiomata with very little pigment, initially viscid and not hygrophanous pileus, stipe with or without traces of a veil, smooth and opaque basidiospores, presence of chrysocystidia-like sulphidia, and constant lack of thick-walled metuloids (Gerhardt, 1996). *P. antillarum* is a striking species and seems distinctive in subgen. *Anellaria* by virtue of complete absence of velum and by the differences in outline appearance of the spores and the size of them. In general there are

no problems to separate *P. semiovatus* var. *semiovatus* and *P. antillarum* macroscopically in the field due to the annulate stipe of the former. However, very closely related *P. semiovatus* var. *phalaenarum* lacking an annulus, and has incredibly similar colouration to *P. antillarum*, what is well illustrated by Cetto (2005: 2628). This species seems not to be restricted to herbivorous dung, since it also appears on the ground in manured pastures and meadows (Gerhardt, 1996, 2008). Nevertheless, *P. semiovatus* var. *phalaenarum* is said to differ externally from *P. antillarum* by having a dentate or bordered cap margin (Gerhardt, 2008). Atypical specimens may occur but are then easily determined microscopically (similarly as typical form) by the larger, more elongated, and less flattened basidiospores with clearly higher average length/breadth ratio (Q_b = (1.6)1.7-1.8(1.9)). In addition, a distinction is partly available whether the germ pore is central or oblique in side view. Namely, *P. antillarum* is described as having regularly a central germ pore. In comparison with this species, *P. semiovatus* has often slightly eccentric germ pore. However, the difference in pore locations between the two species is not always distinct, as in the case of *P. semiovatus* one can find the germ pore in both locations even in a single spore deposit (cf. Breitenbach & Kränzlin, 1995; Fig. 11). Furthermore, in the group of *Panaeolus* species with pale coloured basidiomata, *P. antillarum* can show sometimes more or less similarity to coprophilous representatives of subgenus *Copelandia* (Bres.) Ew. Gerhardt, e.g. *Panaeolus cyanescens* (Berk. & Broome) Sacc., *Panaeolus cambodginiensis* Ola'h & R. Heim, and *Panaeolus tropicalis* Ola'h. However, these species are characterized macroscopically by clearly more slender and normally more strongly pigmented basidiomata, and microscopically first of all by the presence of thick-walled metuloids instead of chrysocystidia-like sulphidia. Besides, these fungi are tropical and subtropical species indicating the ability to tolerate only a limited range of clearly higher temperatures.

The identity of *P. antillarum* has been in a state of misunderstanding for many years and therefore some distribution data may be uncertain, due to the confusion with similar species (mainly *P. semiovatus* var. *phalaenarum*). Apparently the confusion was a consequence of the fact that too much importance

Fig. 11. Basidiospores of *Panaeolus semiovatus* var. *semiovatus* (WRSL-19052013.504; microphotographs by M. Halama).

has been laid on the color and texture of pileus, and the presence of velum leftovers, characters which may be very variable owing to external circumstances. Whole basidioma of *P. antillarum* are pure white to gray-white when young, but may gradually turn dull in accordance with drought and with the deposit of mature basidiospores. The pileus cuticle is initially smooth or somewhat wrinkled radially, sticky and shiny with moisture, but it may become areolately-squamose or even covered with rounded warts in the central part or even all over on drying, and additionally may be streaked with sooty areas (Yokoyama, 1979; Young, 1989). Hlaváček (1997) reported *P. antillarum* with two types of basidiospores, both opaque, dark brown to black-brown, with a relatively thick wall and distinct central germ pore, i.e. a) ellipsoid to almost amygdaliform 17-22 (24) × 9-12 μm, and b) almost limoniform (14) 15-18 × 11-13 μm. The first may constitute a different species (most probably ringless form of *P. semiovatus*) while the second almost certainly represents *P. antillarum.* Furthermore, an interesting and valuable contribution by Yokoyama (1984) containing the interpretation of *P. antillarum* as a species with rather dark coloured basidiomata and elliptic – large basidiospores seems to correspond better with ringless form of *P. semiovatus* (spores 21-22.5 μm long, pileus creamy at margin, pale brown elsewhere, hollow stipe). In the literature there are also cases of the opposite possible misinterpretations. For example, a good description of a find of *P. semiovatus* var. *phalaenarum* (as *Anellaria phalaenarum*) from the area of Venice (Italy) is given by Robich (1992). However, there is hardly any doubt which species he had in view as he depicted ellipsoid to ovoid and usually subhexagonal basidiospores. Due to the published figures and given length of spores (16-19 (22) μm) this record surely relates to *P. antillarum* (cf. Hausknecht & Krisai-Greilhuber, 2003).

In Poland *P. antillarum* is an adventive species, probably firstly found on horse dung by Ryszard Rutkowski (pers. comm.) in 2009 in the Pogorzelce village near Biaowiea National Park (Bielsk Plain, E Poland). Unfortunately, this finding has not been supported by any voucher specimen. When revising the available photograph documentation of this collection, we found that there is no trace of velum neither in the stipe nor on the pileus edge. However, photographed specimens have a clearly yellowish tint on the cap surface, what makes somewhat dubious if this finding is identical with the present concept of *P. antillarum*. Further collections of this species have allegedly been made the same year on old horse manure in Woosate village (Western Bieszczady, SE Poland). This location was mentioned by Kujawa *et al.* (2009) in the document of the environmental management plan of Bieszczady National Park. However, this finding was issued with any critical notes and illustrations of key morphological characters, so it remains to be seen whether these are in fact *P. antillarum.* Since, as we had no ability to verify these collections, what you see on the figures presented here is the first substantiated occurrence of the species in Poland. In general, the specimens of *P. antillarum* collected in this country are quite typical for the species and agree closely with the description and drawings provided by Gerhardt (1987, 1996, 2008, 2012) and supplemented by other authors (e.g. Pegler, 1968, 1977, 1983; Ludwig, 2001a,b; Rommelaars & Arnolds, 2007). Microscopic examination of the collected material and survey of the available literature data let to recognize *P. antillarum* as not obviously heterogeneous in its selected morphological features (Tab. 1). Besides, we found that in the case of basidiospores, little attention has been paid to the details of their germ pore dimension, length/width, as well as length/breadth ratios, so far. Therefore, this study completes these issues.

Table 1. Comparison of selected morphological features of *Panaeolus antillarum* according to different studies.

Authors	*Basidiospores*		*Sulphidia*	*Cheilocystidia*	*Caulocystidia*
	length × *width* × *breadth*	Q_w × Q_b	*length* × *width*	*length* × *width* × *width (apex)*	*length* × *width* × *width (apex)*
Pegler, 1968	15.5-21.5 × 8.5-11.5 × 10-14 (18 × 12 × 9.5)	N/D	34.0-40 × 11-17	28-35 × 8.5-11.5	14-70 × 4.5-8
Pegler, 1977	15.5-21.5 × 8.5-11.5 × 10-14 (18 × 12 × 9.5)	N/D	34.0-50 × 11-17	28-35 × 8.5-11.5	14-70 × 4.5-8
Pegler, 1983	15.5-21.5 × 8.5-11.5 × 10-14 (17 ± 1 × 11.2 ± 0.7 × 8.8 ± 0.4)	Q = 1.9	34.0-50 × 11-17	28-35 × 8.5-11.5	14-70 × 4.5-8
Gerhardt, 1987	17.5-21.5(23) × 9-10.5 × 10.5-12.5(14)	N/D	32 × 9	N/D	N/D
Young, 1989	15-19(22) × 10-14 × 8-12	N/D	35-55 × 12-20	20-40 × 8-11	32-55 × 5-11
Gerhardt, 1996	15-20(21) × 8-10(11) × 10-14	N/D	25-50 × N/D-N/D	30-45 × N/D-N/D	N/D
Hlaváček, 1997	17-22(24) × 9-12 (14)15-18 × 11-13	N/D	N/D-N/D × N/D-20(22)	N/D-N/D × N/D-20	N/D
Bon & Courtecuisse, 2003	16-20 × 9-12	N/D	N/D	35-60 × 5-12	N/D
Rommelaars & Arnolds, 2007	13.5-18 × 8-10 × 10-13	N/D	34-57 × 15-31	27-55 × 6-16	N/D-70 × N/D-15
Gerhardt, 2008	15-20 × 8-10 × 10-14	N/D	25-30 × N/D-N/D	30-45 × N/D-N/D	N/D
This study (minium-maximum values)	13.3-20.0 × 7.7-11.4 × 9.4-12.9	1.3-2.2 × 1.2-2.0	34.0-57.9 × 12.4-24.0	16.9-45.6 × 7.0-16.6 × 3.2-10.4	19.1-60.8 × 5.9-13.8 × 5.0-12.1
This study (10-90 percentile values)	14.8-18.4 × 8.4-10.4 × 10.6-12.2	1.5-2.0 × 1.3-1.6	37.1-53.5 × 14.7-21.1	21.1-34.4 × 8.6-13.1 × 4.4-7.4	32.6-56.9 × 7.2-10.4 × 5.8-8.5

Distribution and ecology

Judged from the literature (Guzmán & Pérez-Patraca, 1972; Young, 1989; Gerhardt, 1996; Pegler, 1997; Ludwig, 2001b) it would seem that *P. antillarum* is thermophilic, widespread pantropical fungus (cf. also Arnolds, 1996), although a dozen reports of its outdoors occurrence at higher and lower latitudes (see below) may, in part, suggest that it is not today solely confined to tropical and subtropical zones (cf. Singer, 1969). *P. antillarum* has been treated hitherto as an adventive species in temperate regions, that can hardly be expected to be observed outdoors where it appears almost exclusively during especially favourable conditions, i.e. hot and humid years (e.g. Gerhardt, 1987, 1996; Rommelaars & Arnolds, 2007). However, its distribution seems to clearly extend into northern and southern temperate areas at present (Watling & Richardson, 2010; this study) (Fig. 12). In South America *P. antillarum* is supposed to be common throughout the warm and temperate parts of the lowlands whereas *P. semiovatus* seems to occur only in the Andes and at high latitudes, as in Tierra del Fuego (Singer, 1952; Dennis, 1961). It has been reported from Colombia (Guzmán & Pérez-Patraca, 1972; Pulido, 1983; Franco-Molano & Uribe-Calle, 2000; Vasco-Palacios *et al.*, 2005; Betancur *et al.*, 2007), Venezuela (Dennis, 1961,1970), Brazil (Cooke, 1881; Pegler, 1983, 1990; Stijve & de Meijer, 1993; Alves & Cavalcanti, 1996; Gerhardt, 1996; Pegler, 1997; Sobestiansky, 2005; de Meijer, 2006; Forzza *et al.*, 2010), Uruguay (Gerhardt, 1996), Argentina (Guzmán & Pérez-Patraca, 1972; Gerhardt, 1996), and Chile (Singer, 1969). It is also known from Falkland Islands (Watling & Richardson, 2010; Niveiro & Albertó, 2012), where the southernmost record is at about 51°48'S.

In Central America *P. antillarum* is reported from Lesser Antilles (Barbados, Grenada, Guadeloupe, Martinique, Trinidad and Tobago) (Dennis, 1961; Pegler, 1983; Gerhardt, 1996), United States Virgin Islands (Saint Croix) (Gerhardt, 1996), Greater Antilles (Jamaica) (Pegler, 1983; Gerhardt, 1996), Cuba (Gerhardt, 1996), Puerto Rico (Gerhardt, 1996), Panama (Gerhardt, 1996; Guzmán & Piepenbring, 2011), Honduras (Guzmán & Pérez-Patraca, 1972), and

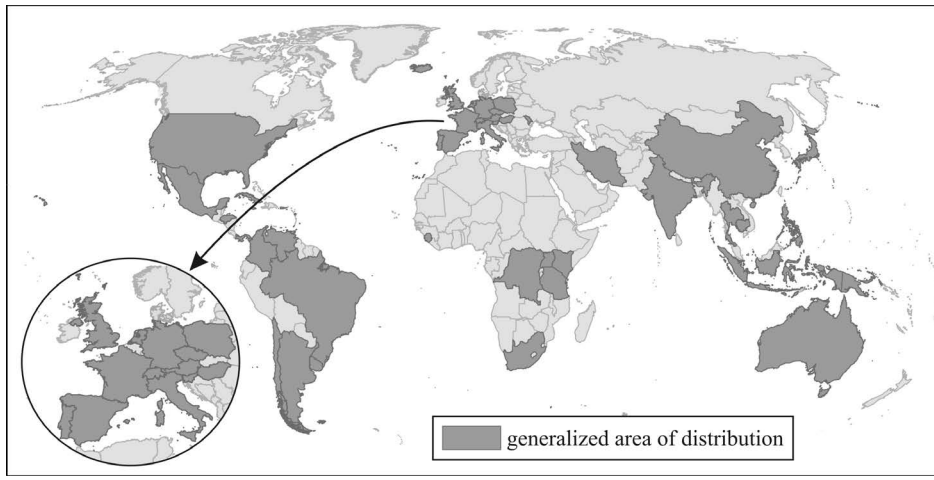


Fig. 12. Map showing the known world distribution of *Panaeolus antillarum* (based mainly on literature records and supplemented by unpublished data).

Mexico (Guzmán & Pérez-Patraca, 1972; Guzmán, 1973; Pérez-Silva *et al.*, 2006). In the last mentioned country, *P. antillarum* is very common in tropical and subtropical regions, where it mostly occurs on meadows – on cattle excrements, while it is rarer in temperate areas. In North America *P. antillarum* is known from USA (Alabama, Arizona, California, Florida, Kansas, Louisiana, Nevada, New Jersey, New York State, Pennsylvania, South Carolina, West Virginia) (Peck, 1872; McIlvaine & Macadam, 1912: as *Panaeolus solidipes*; Smith, 1948; Ola'h, 1969: as *Panaeolus phalaenarum*; Guzmán & Pérez-Patraca, 1972; Guzmán, 1973; Gerhardt, 1996; Bates, 2006). Additional records from the Bermudas (Gerhardt, 1996) and Hawaii (Merlin & Allen, 1993; Gerhardt, 1996; Desjardin & Don, 2001) further extends the range of distribution of the species. Basing on data of Miller Jr. (1968; as *Panaeolus solidipes*), *P. antillarum* seems also to be recorded in summers in the Yukon (Canada). However, Miller's interpretation differs in several aspects from that of Gerhardt (1996), especially by its greater spores (20-22 × 11-12.5 μm). Therefore, we think that his record from horse dung, followed by McAdoo (2011), appears to represent a different taxon, either atypical *P. semiovatus* or other, possibly undescribed one. The known African distribution of *P. antillarum* seems to be restricted to the area of the continent that lies south of the Sahara Desert, including Western, Southern, Central, and Eastern Africa. In this continent the species is reported from Sierra Leone (Pegler, 1968), South Africa (Kalchbrenner, 1881; Doidge, 1950; Pearson, 1950; Pegler, 1983; Gerhardt, 1996; Reid & Eicker, 1999), DR Congo (Beeli, 1928: as *Paneolus bolombensis*; Gerhardt, 1996), Kenya (Pegler, 1977; Williams, 1991), Tanzania (Pegler, 1977), and Uganda (Pegler, 1977). However, *P. antillarum* may be more widely distributed in Africa than it appears from the map, because of neglected recording. According to this survey the distribution of *P. antillarum* in Asia is somewhat scattered, although Southeastern Asia clearly shows a certain concentration of finds. It is apparently not known from Western Asia, Central Asia, and according to our knowledge no finds have been registered so far also from North Asia (Russian territory). *P. antillarum* is reported in this continent from Iran (Gerhardt, 1996), India (Manimohan *et al.*, 2007), China (Hebei, Jiangsu, Zhejiang, Guangdong, Yunnan, Tibet, Quinghai, Taiwan) (Zhishu *et al.*, 1993), Thailand (Masee, 1902; Gerhardt, 1996), Cambodia (Allen *et al.*, 2012), Japan (Guzmán & Pérez-Patraca, 1972; Gerhardt, 1996; Imazeki *et al.*, 2002), Indonesia (Java, Celebes) (Berkeley, 1842; Pegler, 1977,1983; Gerhardt, 1996), and Philippines (Gerhardt, 1996). Further distribution of *P. antillarum* includes Australia, where the species is known from the following states: New South Wales, Victoria, Queensland, and South-Australia, (Cooke, 1889; Pegler, 1977, 1983; Young, 1989; Gerhardt, 1996; Young *et al.*, 2001), and Oceania, with finds from New Guinea (Yokoyama, 1979).

In Europe, *P. antillarum* was apparently published by Rald (1984) from Denmark for the first time. This record from the second half of the 1970s comes from indoors, from a horse stable in Tåstrup (Sjælland), where the taxon was found on horse manure mixed with straw for animal bedding. However, a careful review of the description and revision of the mentioned collection by Gerhardt (1996) showed that *P. antillarum* ss. Rald is identical with *Panaeolus semiovatus* var. *phalaenarum*. By the way, this Danish collection has been subsequently used to designate an epitype of the latter taxon. Nevertheless, studies of historical materials conducted by Bon (1987); Gerhardt (1996); and Hlaváček (1997) revealed that *P. antillarum* was collected outdoors in the European area as early as the forties of 20th century from France (Lusigny-sur-Barse, Champagnole) and the Czech Republic (Kladno, Košetice). In France, *P. antillarum* was also later

recorded in the 1960s, 1970s and 1980s (Bon, 1987; Durand, 2009), whereas in the Czech Republic – in 1960s, and again only in 1990s (Hlaváček, 1997; Antonín *et al.*, 2006). Furthermore, the species has also been found outdoors in the 1980s in Germany (Gerhardt, 1987; Krieglsteiner, 1991; Gerhardt, 1996; Enderle, 2004), Switzerland (Gerhardt, 1987,1996) and Slovenia (Jurc *et al.*, 2004, 2005). Afterwards it was recorded from Italy (Robich, 1992: as *Anellaria phalaenarum*; Doveri, 2010, 2011), Spain (including Balearic Islands; Cortés & Montón, 2002; Rubio *et al.*, 2006; Mir & Melis, 2008), Austria (Hausknecht & Krisai-Greilhuber, 2003), the Republic of Moldova (Roux, 2006: as *Panaeolus phalaenarum*), Iceland (Richardson, 2004), and probably Faroe Islands (Richardson, 2005, uncertain identification according to the author). It has recently been found also in the Netherlands (Rommelaars & Arnolds, 2007; Arnolds & Veerkamp, 2008), Hungary (Kaposvári, 2010) and Portugal (Azul *et al.*, 2011), and was confirmed from Britain (Legon *et al.*, 2005; B.M.S., 2010; Smith, 2013). Thus, this study represents record from the fifteenth European and fifth Central-European country.

An interesting aspect concerns the substrate utilized by *P. antillarum*. This fungus seems to behave as facultative coprophyte clearly able to grow on dung from a wide range of herbivores. According to literature, it has been reported most frequently on dung of horses and cattle, but also on unidentified dung, and rarely that of buffalo, hippo, elephant, and rhino. It has been also observed on manure and manured soil (Tab. 2). The preferences of the species seem to be not closely connected with the structure of dung, as mentioned above substrata differ between themselves in many ways, e.g.: compaction, nutrient composition, and content of poorly decayed material. The absence of *P. antillarum* to the north of Europe and at higher elevations is distinct when

Table 2. Comparison of substrat preferences of *Panaeolus antillarum* according to different studies.

Substrate	*Authors*
cattle dung	Doidge, 1950; Dennis, 1961; Pegler, 1968, 1977; Yokoyama, 1979; Pegler, 1983; Young, 1989; Zhishu *et al.*, 1993; Gerhardt, 1996; Reid & Eicker, 1999; Sobestiansky, 2005; Doveri, 2010; Watling & Richardson, 2010; Doveri, 2011
buffalo dung	Masee, 1902; Pegler, 1977
horse dung	Yokoyama, 1979; Pegler, 1983; Arora, 1986; Gerhardt, 1987; Young, 1989; Robich, 1992; Stijve & de Meijer, 1993; Gerhardt, 1996; Hlaváček, 1997; Cortés & Montón, 2002; Hausknecht & Krisai-Greilhuber, 2003; Richardson, 2004, 2005; Antonín *et al.*, 2006; de Meijer, 2006; Rubio *et al.*, 2006; Rommelaars & Arnolds, 2007; Mir & Melis, 2008; Kaposvári, 2010; Watling & Richardson, 2010
hippopotamus dung	Beeli, 1928; Gerhardt, 1996
rhinoceros dung	Gerhardt, 1996
elephant dung	Pegler, 1977; Reid & Eicker, 1999; Manimohan *et al.*, 2007; Smith, 2013
unidentified dung	Peck, 1872; Cooke, 1889; McIlvaine & Macadam, 1912; Pearson, 1950; Stijve & de Meijer, 1993; Vasco-Palacios *et al.*, 2005; Betancur *et al.*, 2007
(manured) soil	Berkeley, 1842; Cooke, 1881; Pegler, 1983, 1990
rotting hay, straw, manure	Fries, 1828; Cooke, 1889; Arora, 1986; Roux, 2006; Rommelaars & Arnolds, 2007

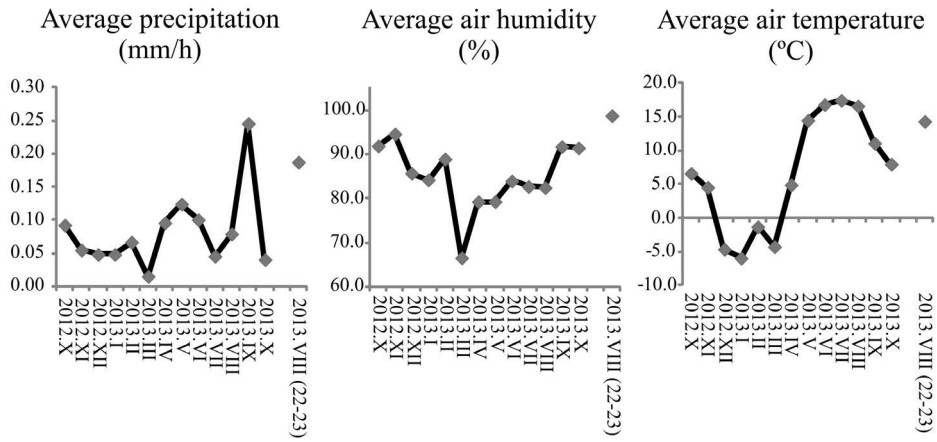


Fig. 13. Meteorological data from October 2012 until October 2013 at 'Sarnetki' weather station (NE Poland).

hypothetically compared with the much wider distribution of the suitable habitats. This tends to indicate climatic factors as being decisive for the distribution (cf. above) of the species. It appears that our find hardly belongs to the natural distribution of the species, but it indicates and confirms, however, that *P. antillarum* is greatly favoured by relatively high humidity and temperature (cf. Rommelaars & Arnolds, 2007). Although the relation between fruiting of fungi and weather is usually not direct and simple (Arnolds, 1981; Moore *et al.*, 2008) clear convergence between favourable weather conditions and fructification of *P. antillarum* can be observed. In figure 13 data are summarized on the rainfall, air humidity, and air temperatures per month between October 2012 and October 2013. All measurements were made by automated weather station operated by the institution of Wigierski National Park and located on the forest hill near the collecting place, about 500 m north-east of the locality. In order to compare the weather conditions with *P. antillarum* appearance we indicated in addition to the month range data sets also the two-day period preceding the collection date.

Although the summer of 2013 was relatively dry, average precipitation and average air humidity during the two days preceding the collection time were both extremely high. It is also noticeable that this mentioned damp period was preceded by a relatively warm period.

It can be assumed that *P. antillarum* finding in north-eastern Poland may be early indicator for ecological responses to climatic changes, or simply emphasize our incomplete knowledge of its natural distribution. It is also possible it should be taken into account both of these factors at the same time. Nevertheless, these hypotheses are preliminary and should be carefully tested in future. The phenology, ecology and distribution of this curious species in Europe deserve further investigation.

Psychoactive properties and practical importance

P. antillarum was regularly incorrectly identified in past as one of the neurotropic representatives of the subgenus *Copelandia* (Bres.) Ew.Gerhardt by those people who use the fungi as a drug (Guzmán *et al.*, 2000). This confusion

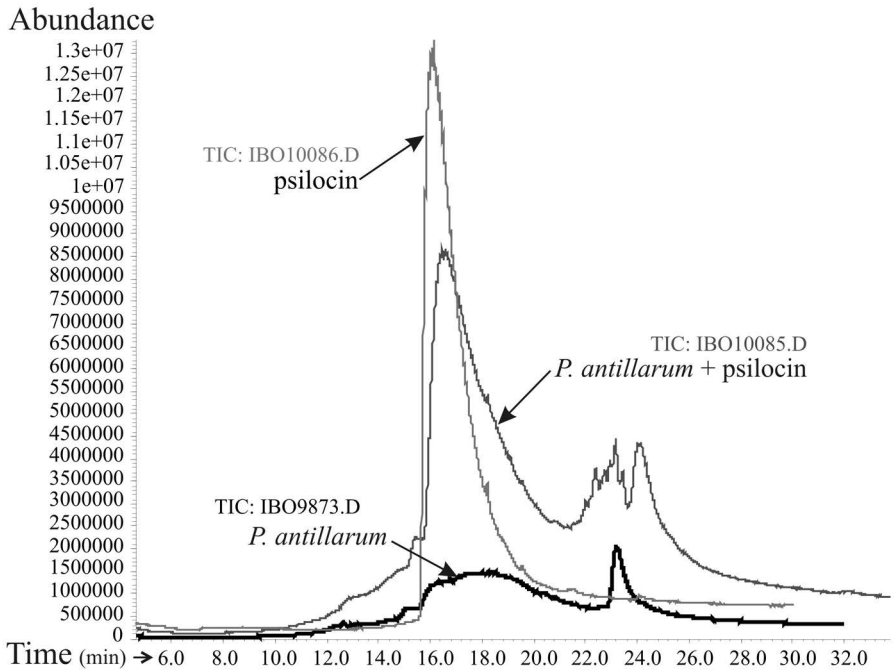


Fig. 14. Total Ion Chromatogram of an extract from *Panaeolus antillarum* and synthetic psilocin using methanol.

occurs because some members of *Copelandia* (e.g. *Panaeolus cyanescens* and allies) may look generally similar to the subtropical form of *P. antillarum* except they bruise bluish and are usually frailer in stature. Moreover, in warmer regions both taxa are mostly capable of growing together in the same dung of various ruminants (Allen *et al.*, 2012). Therefore, *P. antillarum* was suspected to contain hallucinogenic compounds or at least to be responsible for the infamous "hysteria fungus" symptoms (cf. Heim, 1978; Allen, 1999). In addition, Allen & Merlin (1992) based on specimens of *P. antillarum* collected in Thailand and Hawaii reported erroneously the alleged presence of trace amounts of psilocybin, psilocin, and baeocystin in this species. However, as they later showed using thin-layer chromatography (Merlin & Allen, 1993), *P. antillarum* does not contain any such compounds (cf. Young, 1989; Ott, 1996; Guzmán *et al.*, 2000; Ratsch, 2005; Allen *et al.*, 2012). The absence of the mentioned above active indole derivatives in basidiomata of *P. antillarum* was also demonstrated by Stijve (1987) and Stijve & de Meijer (1993). Our studies based on GC-MS analytical method also confirmed unambiguous deficiency of such compounds in the species (Fig. 14). By this technique we can only determine the presence of psilocin in mushroom samples as a sum of psilocin and psilocybin level because psilocybin is thermally labile and does not survive the conditions of GC/MS intact (Kikura-Hanajiri *et al.*, 2005). Figure 14 shows the Total Ion Chromatogram (TIC) of methanolic extract of *P. antillarum* (IBO9873.D). This TIC is overlapped by TIC of the synthetic psilocin standard (IBO10086.D) with retention time at 16.46 min, and by the TIC of *P. antillarum* with addition of psilocin standard (IBO10085.D). The identity of peak at 16.46 min was also conrmed as psilocin by MS (mass spectra) detection.

This detection was carried out using full scans (50-400 m/z) of the single quadrupole mass spectrometer. As we can see the characteristic pick of psilocin is absent in the *P. antillarum* extract. The characteristic molecular ion of psilocin (m/z 204) is also absent in the MS (mass spectra) of the species (data not shown) what confirms the deficiency of active indole derivatives.

P. antillarum is not included in Ammirati *et al.* (1985); Fischer & Bessette (1992); and in Hall *et al.* (2003), however, it is mentioned by Christensen (1972), who stated that the species (*P. solidipes*) is edible but not recommended. Yokoyama (1979) reported some married couple from Japan, who tried to eat many cooked basidiomata of *P. antillarum*. Afterward male had no trouble, but female suffered from diarrhoea for several days. However, McIlvaine & Macadam (1912) report *P. antillarum* to be excellent, while Arora (1986) claims its relatively large size makes it the only non-hallucinogenic *Panaeolus* worth eating, and considers it as an edible fungus. Moreover, edibility of *P. antillarum* is also cited by and Singer (1949), Singer (1986), Guzmán *et al.* (2000) and Allen (2012).

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