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AS: collected and examined the fungal material; AO: carried vegetation survey and interpretation; both authors conceived the research idea. interpreted the results and prepared the manuscript

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# **ORIGINAL RESEARCH PAPER**

# Poronia punctata (L.: Fr.) Rabenh. (Xylariales, Ascomycota) in Poland: a threatened, rare, or overlooked species?

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# Abstract

Poronia punctata is regarded as a threatened, rare coprophilous fungus species, especially in Europe. Lately, the fungus has been noted again in Poland after a century of absence. Micro- and macrotraits of P. punctata have been given on the basis of specimens from two collections: the first, contemporary one, found in the field in 2010, and the second and most probably oldest Polish one dating back to the years 1819-1845, from the collections of prof. Michał Szubert, found by us in the Herbarium of the University of Warsaw. The distribution of *P. punctata* localities within the present boundaries of Poland is presented. The occurrence conditions of P. punctata in Poland, especially the new locality, are characterized. Possible reasons why the species has not been noted within 1905–2009 in Poland are indicated. The threat status of the species according to the IUCN criteria is assessed and the threat category VU is proposed.

# **Keywords**

nail fungus; coprophilous fungi; morphology; ecology; distribution; dry grasslands; conservation; threat assessment

# Introduction

The threat assessment of species is often a difficult task. Among many reasons for this are non-equivalence of population size and its occupied area; the relativeness of accepted spatial scales; and non-equivalence of rarity and decline terms [1-3]. Therefore, definitions of a threat degree have been made more and more detailed, including: the number of individuals, the number of localities, size of an occupied area, geographical range, and rate of changes of population size and areas [4-6]. For fungi, this assessment is especially difficult because, firstly, fungi remain invisible for most of their life cycle; secondly, there are not enough specialists to note their actual occurrence; and thirdly, due to long-distance dispersion of their spores, fungi can appear suddenly in places far from each other [7,8]. One fungus species that exemplifies nearly all of the problems mentioned would be Poronia punctata (L.: Fr.) Rabenh. - nail fungus (sitniczek kropkowany in Polish) - a coprophilous fungus, found typically on older dung of horses, and less often cattle [9-15].

Poronia punctata is characterized by slow growth in culture and is included among species in the late stages of coprophilous fungal succession as a "late colonist" of herbivore dung, as it was noted on cattle dung lying for 1.5 to 4.5 years. To compete with fungi already present on dung, P. punctata produces antibiotics which are able to inhibit severely other mycelia [16-18].

The species has been found on the dung of horses, ponies, donkeys, mules, cows, sheep, and elephants (e.g., [11,13,15,19-24]). It has a worldwide range, however,

almost everywhere it is rare. It occurs mainly in Europe, Africa, Central, North, and South America, and Asia. The reports on the P. punctata occurrence in Australia actually refer to another closely related species - P. erici Lohmeyer & Benkert that was also recorded in some European countries [13,21,25]. In Europe, P. punctata is reported from 21 countries [26] and in most of them it is put on the red list, e.g., in Belgium -EN [27]; Bulgaria VU B2ab (i,ii,iv) [28]; Croatia – CR, C1, D [29]; Czech Republic – ? EX [30]; Estonia - VU [31]; Finland - RE [32]; Germany (Bavaria) - RE/VU [33]; Germany (Saxony) - Ex [34,35]; Great Britain - NT [36]; Sweden - VU, A1c, B1+2cd [37]; Netherlands - VU [38]; Norway - RE [39]; Romania - NT [40]; Slovakia - EX?/ VU [41]; Switzerland - CR D1 [42]. In Croatia, this fungus is strictly protected and is listed in the Crvena knjiga gljiva Hrvatske (Red book of Croatian Fungi) [29]. In Poland, in the first and second edition of the List of threatened plants in Poland it was included in the I threat category [43,44]. In the up-to-date third edition of the List... it has been located in the fungi group of the E category [45]. Ing [46] mentions it among 16 species of the highest A category of threat in Europe. Lately, it has been proposed on the preliminary global red list with the LC threat category [26].

Due to the fact that *P. punctata* was found again after more than a hundred years in Poland, this study aims at comparison of macro- and microtraits of specimens found in Poland with the traits of specimens described in the literature, characteristics of the occurrence conditions of the species, and assessment of its threat status in Poland on the grounds of the history of its records.

# Matherial and methods

The collected material was studied according to standard methods used in the taxonomy of fungi [47]. The microscopic structures were examined using dried material mounted in Congo Red (1% solution in 10% ammonia), in Melzer's reagent, and in water, with the aid of a Nikon Eclipse E 400 microscope. All measurements were made directly through the microscope under an oil immersion objective. The spore dimensions were established from measurements of 30 well-formed spores. For asci, the extreme size values were presented. For these structures, dimensions were obtained after measuring 20 elements. The collected material was deposited in the Fungarium of the Division of Mycology and Forest Phytopathology, Warsaw University of Life Sciences – SGGW (WAML).

The description of vegetation was made based on the field survey in 2015. The names of vascular plants were given according to Mirek et al. [48], mosses – Ochyra et al. [49], lichens – Fałtynowicz [50]. The nomenclature of plant communities was given according to Matuszkiewicz [51]. The names of physical geographical units were given in accordance with Kondracki [52].

The composition of horse dung was determined using the "point frame" method [53] after rinsing it on a 1-mm sieve. From each of the samples, 100 fragments of plants were collected which were identified in a stereoscopic microscope, at a magnitude of 25 times and classified into functional groups such as grasses, perennials, tree leaves, wooden shoots, grain etc.

# Results

Description of specimens

Poronia punctata (L.: Fr.) Rabenhorst (1844) Deutsch. Krypt. -Fl. I: 223 [54].

**Synonyms.** Peziza punctate Linné, Hypoxylon punctatum (L.: Fr.) Greville., Poronia fimitaria Persoon, Poronia gleditschii Willdenow, Sphaeria nivea Haller, Sphaeria poronia Persoon, Sphaeria punctata (L.) Fr.: Fries, Sphaeria punctata (L.) Sowerby, Sphaeria truncata Bolton, Xylaria punctata (L.) J. Kickx f.



Fig. 1 Stromata of the Poronia punctata (L.: Fr.) Rabenh. (photo by A. Szczepkowski, 2010).

Specimens examined. WAML 835, (Fig. 1); WA 0000051540.

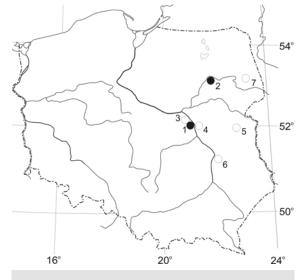
**Description.** Ascomata consisting of a stroma shaped like a nail, with a stalk that is straight or curved, dark grey-blackish, tapering downwards, and embedded in horse dung and a flat cup or more or less circular whitish disc, 2.5–12.0 mm across, dotted with black ostioles of the perithecia submereged in stroma. Perithecia located beneath the upper surface of the stroma, in a single layer, oviform to pyriform, ca. 0.3–0.6 mm in height and ca. 0.2–0.5 mm diam. Asci hyaline walled, elongated-cylindrical, broadest at the middle part, 120.0–175.0 × 15.0–23.0  $\mu$ m, with apex and a short stalk. Ascus apex obtused, inoperculate, 7.5–10.5  $\mu$ m thick, with thickened wall and strongly amyloid apical apparatus. Asci with eight spores in one row. Ascospores elliptical, finely smooth, blackish brown with a distinct longitudinal germ slit running near whole length on the flat side, 18.0–27.0 × 9.0–12.5  $\mu$ m, bounded by a hyaline gelatinous coat. Flesh pallid and tough. Odor and taste not distinctive (Tab. 1).

**List of localities in Poland.** All localities known until now are within four geographical macroregions of Poland: the Środkowomazowiecka Lowland (Warsaw, Wólka Węglowa, Otwock, Puławy), the Północnomazowiecka Lowland (Czartoria), the Północnopodlaska Lowland (Ogrodniczki), and the Południowopodlaska Lowland (Międzyrzec Podlaski). At present, two localities are within the area of the Północnomazowiecka Lowland (Czartoria – Fig. 2: number 2) and the Północnopodlaska Lowland (Ogrodniczki – Fig. 2: number 2) and the Północnopodlaska Lowland (Ogrodniczki – Fig. 2: number 7). All *P. punctata* localities, both historical and contemporary, occupy altogether one-fifth of the whole country's area.

**New localities/data.** 1. Warsaw, specimens found in the Herbarium of Botanical Garden at the University of Warsaw [Herbarium Szubertianum (1819–1845), WA0000051540 = WA 680]. 2. Czartoria, the Miastkowo Commune, the Łomżyński County, the Podlaskie Province, an extensive dry pasture at the Narew River, ca. 30 stromata on horse dung, Nov. 5, 2010, leg. et det. A. Szczepkowski. **Literature data.** 3.

	Features			
Source of data	disc of stromata (mm)	perithecia (mm)	asci (µm)	ascospores (µm)
Present studies (WAML 835)	3.0-9.5	0.3-0.6 × 0.2-0.4	120.0-170.0 × 15.5-23.0	18.0-24.0 × 9.0-11.0
Present studies (WA 0000051540)	2.5–12.0	0.3-0.6 × 0.3-0.5	145.0–175.0 × 15.0–20.0	19.0–27.0 × 9.0–12.5
[89]	1.0-6.0	0.5-0.6 × 0.32-0.55	135.0-160.0 × 14.5-17.0	16.6–21.7 × 8.3–9.9(11.0)
[23]	5.0–17.0	-	130.0-160 × 15.0-30.0	22.0–22.5 × 11.0–14.0 (16.5)
[15]	3.0–12.0 (5.0–20.0 in key)	0.55-0.62 × 0.35-0.50	140.0–150.0 × 15.0–17.0	(19)20.0–22.8 × 8.5–10.0 (17.0–26.0 × 8.0–14.0 in key)
[22]	2.8-8.5	$0.4-0.5 \times 0.3-0.4$	124.0-163.0 × 18.7-24.0	22.5-26.8 × 9.7-10.8
[90]	5.0-15.0	0.2–0.3	160.0-180.0 × 15.0-18.0	18.0–26.0 × 7.0–12.0
[12]	5.0-15.0	-	150.0-190.0 × 18.0-20.0	17.0-26.0 × 8.5-13.0
[11]	Up to 15	-	About 180.0 × 18.0	18.0-26.0 × 7.0-12.0
[9]	6.0–15.0	-	150.0-180.0 × 16.0-18.0	18.0-26.0 × 10.0-14.0

Tab. 1 Comparison of basic features of Poronia punctata (L.: Fr.) Rabenh. of examined specimens with selected descriptions.



**Fig. 2** Distribution of *Poronia punctata* (L.: Fr.) Rabenh. in Poland (black circles – new localities/data, white circles – localities known from the literature).

Wólka Węglowa near Warsaw [55]. 4. Otwock, ca. 20 km south of Warsaw [55]. 5. Surroundings of Międzyrzec Podlaski, on horse and cattle dung in a dry pasture behind the Żabiecki wood, spring [56]. 6. (?) Surroundings of Puławy, on horse dung [57,58]. 7. Circa 1 km south of Ogrodniczki, the Supraśl Commune, the Białostocki County, the Podlaskie Province, a pasture, on horse dung a few young stromata, Nov. 18, 2013 [59].

**Characteristics of a newly discovered locality.** The pasture in which *P. punctata* was collected is located on the flood terrace of the Narew River built of fluvioglacial sands transformed by aeolian erosion at the end of Pleistocene and fluvial erosion by the Narew in the early Holocene. At present, there occur slightly rolling plains built of sandy fen soils, locally small dunes with initial sandy soil and inactive oxbows filled with peat. In spring, oxbow depressions are partially flooded by the Narew's waters [60–62].

The pasture belongs to the inhabitants of the Czartoria village and it has the status of community land. Cattle and horses have been pastured there over the area of ca. 250 ha since as far back as the end of the nineteenth century. The herd had

numbered ca. 300 individuals till the 1990s. For the last decade, the herd's size has not exceeded 50 cows and a few horses. The pastured animals stay at night in outbuildings and are fed there. In the pasture they stay all day from early spring to late fall (the oral information given by the animals' owners and Aleksander Stepnowski – Chair of Board of the Commons Owners Association, 2015). A decrease in grazing intensity after 1990 caused development of shrubs in the pasture. Since 2008, the area has been protected as the Natura 2000 site – "Ostoja Narwiańska" (PLH 200024). Therefore, shrubs are now removed and extensive grazing is kept (Fig. 3).

In the pasture's area, a mosaic of psammophilous and acidic sedge fens has formed. The biggest area (ca. 50% of the land) in the plains of the proglacial valley is occupied by the compact mesophilous *Armeria* grassland *Diantho deltoidis-Armerietum* 



**Fig. 3** The physiognomy of the *Poronia punctata* (L.: Fr.) Rabenh. locality near the Czartoria village in the Narew valley, in Podlasie (photo by A. Szczepkowski, 2015).

elongatae Krausch 1959 (from the Koehlerio glauce-Corynephoretea canescentis Klika class in Klika et Novak 1941). The main building species are such grasses as: Agrostis capillaris L., Festuca ovina L., and Poa pratensis L. The contribution of Hieracium pilosella L., Achillea millefolium L., Leontodon autumnalis L., Galium verum L., Sedum acre L. is also important. The species Thymus serpyllum L. em. Fr., Armeria maritima (Mill.) Willd. subsp. elongata (Hoffm.) Bonnier, Dianthus deltoides L. and D. carthusianorum L., Potentilla argentea L., Hypochoeris radicata L., Cirsium vulgare (Savi) Ten. as well as a range of others are present constantly and in small density. In the shrub layer, there sporadically occur Rosa sp., Pyrus pyraster (L.) Burgsd., and Juniperus communis L.

The prominent sites on the tops of dunes (ca. 30% of the area) are occupied by the initial loose psammophilous *Corynephorus* grassland *Spergulo-Corynephoretum* (R. Tx. 1928) Libb. 1933 (from the *Koehlerio glauce-Corynephoretea canescentis* Klika class in Klika et Novak 1941). *Crynephorus canescens* (L.) P. Beauv., and *Festuca ovina* are the main building species here. The species *Chelichrysum arenarium* (L.) Moench, *Hieracium pilosella, Thymus serpyllum, Scleranthus annuus* L., *Sedum acre, Rumex acetosella* L., *Astragalus arenarius*, and *Veronica dillenii* Crantz have an important contribution. In the well developed lichen-moss layer, such lichens grow profusely as: *Cladonia mitis* Sandst., *C. foliacea* (Huds.) Willd., *C. rangiformis* Hoffm., *C. furcata* (Huds.) Schrad. et al., *Cetraria islandica* (L.) Ach., *C. aculeata* (Schreb.) Fr., *Peltigera canina* (L.) Willd., and *P. rufescens* (Weiss) Humb. as well as mosses *Polytrichum piliferum* Hedw., *Racomitrum canescens* (Hedw.) Brid., *Brachythecium albicans* (Hedw.) Schimp., *Abietinella abietina* (Hedw.) M. Fleisch. et al. In the shrub layer, there occur sparsely *Juniperus communis* and *Pinus sylvestris* L.

In land depressions that are former oxbows of the Narew River (ca. 20% of the land), there occurs an acidic low sedge fen with *Agrosis canina* L., *Carici canescentis-Agrostietum caninae* R. Tx. 1937 [from the *Scheuzerio-Caricetea nigrae* class (Nordh. 1937) R. Tx. 1937]. The main building species here are *Agrostis canina*, *Carex nigra* Reichard, *Juncus conglomeratus* L. em. Leers. Other species, such as *Alopecurus geniculatus* L., *Rumex palustris* Sm., *Ranunculus repens* L., *Rorippa palustris* (L.) Besser, *Mentha arvensis* L., *Myosotis palustris* (L.) L. em. Rchb., *Ranunculus flammula* L., *Eleocharis palustris* (L.) Roem. & Schult., and *Polygonum hydropiper* L., have an important contribution. Among the pasture species present, only occasionally are *Trifolium repens* L., *Potentilla anserina* L., *Deschampsia cespitosa* (L.) P. Beauv. and some others. In the shrub layer *Salix viminalis* L. occurs sporadically.

The locality of *P. punctata* was found in September 2010 on a dune, in a transitional zone between the sandy *Corynephorus* and mesophilous *Armeria* grasslands over-grown by perennials, pine, and juniper. The dung on which the stromata of *P. punctata* were found was much decomposed. It dated back probably to the beginning of the vegetation period or even to the previous year, thus it must have lain in the pasture for a few to several months. Searching through the pasture for other dung with the fungus was unsuccessful, both in November 2010 and again in September 2015.

In the horse dung collected in the area of the Czartoria pasture in 2015, mainly the remnants of narrow-leaved grasses mixed with few of those of dicotyledonous herbaceous plants were found. In addition, in one of three analyzed samples nearly 5% of oat husks and a bit of wooden shoots were found, whereas in another small amount of sedges were also noted.

# Discussion

#### Comparison of specimen dimensions

Until now, the dimensions of macro- and microtraits of *P. punctata* have not been given in the studies published by Polish authors. The characterization of traits shown in this study is the first one conducted on the grounds of specimens found in Poland. There were no differences in the trait dimensions between the specimens collected contemporarily by us and the collection by M. Szubert from the first half of the nine-teenth century. The measurements of stromata, perithecia, asci, and ascospores were within the range of values given by other authors (Tab. 1).

# Distribution of Poronia punctata in Poland

Until now, P. punctata has been known in Poland from at least four localities, though one of them (in the surroundings of Puławy) has an uncertain status. In the materials for recognition of higher fungi in Europe, Skirgiełło [58] reported erroneously that this fungus had been found in Poland only once. Subsequently, this information was repeated by other authors, e.g., Lohmeyer and Benkert [21]. Skirgiełło [58] based her report on the collection deposited in the Herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences (IB PAN) in Cracow ("Puławy (?), 4.15, rev. vel det. A. Wróblewski, KRAM-F 1898"). This collection comes from Feliks Berdau's collection located near Puławy, most probably from the second half of the nineteenth century, and it had not been published in his earlier works. The partially published and unpublished materials of Berdau were elaborated and published by Wróblewski [57], who did not indicate either the date or the locality of *P. punctata*, but described only the substratum - horse dung. It is known that Berdau collected fungi not only in the vicinity of Puławy, but also in the Świętokrzyskie Mountains and the surroundings of Ojców and Warsaw [63]. Therefore, it is not certain whether the collection cited comes from the vicinity of Puławy. It is not known either whether Berdau recognized this fungus on his own or it was done rather by Wróblewski, as according to Wróblewski "almost half" of the herbarium materials of Berdau "were well recognized". Skirgiełło [58] solves this question as follows: "rev. vel det. A. Wróblewski". It is worth mentioning that in the study cited the author has forecast the future status of this species - "one may expect a higher number of localities (on horse dung)". The specimens collected by Błoński [55] and Eichler [56] probably do not exist anymore. From the introductory part of the study by Błoński [55] one may suppose the author gathered his collection between 1885 and 1891. In the second case, Eichler collected his material in 1903-1904 [56].

In the Polish scientific literature from nineteenth century there is information on two extra localities of this fungus: in Grzybowice and Zniesienie (in both cases on cattle dung) in the vicinity of Lviv (Lwów) – nowadays in Ukraine [64,65].

Searching for specimens of this species in the Herbarium of the Faculty of Biology at the University of Warsaw we have found the unpublished collection including about

30 stromata of *P. punctata* (deposited as *Sphaeria poronia* Spreng) (WA0000051540, the old number on the envelope 680) of prof. Michał Szubert, the Polish botanist, founder and director of the Botanical Garden in Warsaw in 1818–1846. In the Herbarium envelope, there is a label with the following text: "Herbarium Horti Botanici Universtatis Varsaviensis; Herbarium Szubertianum; Plantae a clar. prof. M. Szuberto in Horto Botan. Varsaviensi annis 1819–1845 cultae". The residue of the substrate indicates the specimens were collected from horse dung. This is probably the oldest preserved Polish collection of this species. The discovery of this collection in the Herbarium at the University of Warsaw has also an additional historical value because until now there has not been any information in the literature on the preserved fungi collections made by Professor M. Szubert.

In the above-mentioned Herbarium, there are also three very poorly described collections of *P. punctata*. For the first one, apart from the name of the species hand-written on a sheet of paper, on the Herbarium envelope there is also a writing "coll. Heirich" (WA 5504). For the second one, apart from the species name there is also given a possible locality with an extra word "Pressburg Rolla" (?) (Pressburg is the German name of Bratislava, the capital of Slovakia) and the number 781 on the envelope. Lastly, the third one (WA 5505) has the species name, four synonyms, and the same numeral-character symbols as on the second label. It is highly probable that the three collections mentioned do not come from Poland.

In the Herbarium of the W. Szafer Institute of Botany of the Polish Academy of Sciences in Cracow, besides Berdau's collection mentioned there are also two others, dating back to the beginning of the twentieth century, from areas of pre-war Poland: in Połtawa (in present Ukraine – "leg.: an illegible surname, Połtawa 1902, in fimo equino", KRAM F 1896) and in Jukojnie (in present Lithuania – "leg. prof. dr Janczewski, Jukojnie, Żmudź, on dried horse dung, IX 1907, det. B. Namysłowski" KRAM F 1897).

In the Herbarium of the Institute of Botany at the Jagiellonian University in Cracow (KRA), there are deposited four envelopes with the *P. punctata* specimens. Two collections come from outside Poland, from the German collection of Ludwik Rabenhorst (KRA F-0-1560 and KRA F-0-1561), whereas the other two (KRA F-0-1929 and KRA F-0-1942) lack the date and place of their origin. In the other Polish herbaria (Lublin, Poznań, Toruń, Wrocław), that have answered our question whether they possess any collections of *P. punctata*, no specimens of this species have been found.

In 2010, after more than a hundred years of no records, the species was found again in Poland, in the pastures of the Czartoria village near Łomża (Fig. 1–Fig. 3, Tab. 1). In 2013, the stromata of *P. punctata* were found in Ogrodniczki near Białystok [59]. It means that within the present boundaries of Poland the species was found in five historical and two contemporary localities altogether. Of these, four findings date back to the nineteenth century, one – the beginning of twentieth century, and two – the beginning of the twenty-first century. Similar situation held place in Turingia (Germany), where this fungus was found after over a hundred years [23].

In some European countries (Belgium, Germany, Great Britain, the Netherlands, Spain) another species from the *Poronia* genus was noted as well. The species was described as *Poronia erici* Lohmeyer & Benkert (1988), which differs from *P. punctata* in bigger spores (longer and wider), smaller, perhaps more turbinate ascomata, and usually different substrates (hare, rabbit, goat, sheep, and marsupial dung), though it has sporadically been found as well on the horse and cattle dung preferred by *P. punctata* [13].

#### Habitat preferences of Poronia punctata in Poland

In Poland, *P. punctata* was found much more often on horse (4–5 records) than on cattle dung (one record). In two cases, the substrate was not given. If we consider data from Poland's area with its pre-war boundaries, then we have more records, two from horse and two from cattle dung. These observations confirm the preference indicated in the literature of this species for growing on horse dung (horses and ponies). According to Lundqvist [10], *P. punctata* is confined to horse dung in Nordic countries, but grows also on cattle dung in warmer regions. The species has also been collected

from donkey, mule, sheep, and elephant dung in other regions [11–13,19,21,22,24,66]. However it is possible that some other species of the genus *Poronia* may be involved in some of these reports, so the actual range may be smaller if a thorough worldwide revision would be done.

The scarce descriptions of the localities given in the domestic literature and the newly discovered locality (Czartoria), may indicate that *P. punctata* prefers dry and poor pastures. From such a site it was reported by Eichler [56]. The localities from Otwock and Wólka Węglowa given by Błoński [55] are also sandy, dry areas.

The Czartoria site was localized on a dune, in the transitional zone between the sandy grassland *Spergulo-Corynephoretum* (R. Tx. 1928) Libb. 1933 and the mesophilous *Armeria* grassland *Diantho deltoidis-Armerietum elongatae* Krausch 1959, overgrown by perennials, pine, and juniper (both communities from the class *Koehlerio glauce-Corynephoretea canescentis* Klika in Klika et Novak 1941).

It is not clear where the vouchers of *P. punctata* from the historical Szubert collection were found. It is highly probable that the specimens originate from the Botanical Garden in Warsaw and their occurrence was related with the presence of manure of horses used in the gardening management system of those times. *Poronia punctata* specimens found in the nineteenth century in hot-beds in northern Scotland might have had similar determinants of occurrence [67].

In other localities in Europe, *P. punctata* was reported from mesotrophic grazed grasslands, often dry pastures; e.g., alvar meadows (Estonia), dune areas (Finland), karstic rockfield grasslands (Croatia), chalk grassland *Mesobrometum*, subpannonic steppic grasslands (Germany), steppes (Hungary, Ukraine), but also from heaths, low acidic grasslands, and open woodlands (UK), xerothermic grasslands *Festucetum sulcatae* and dry pastures *Festuceto rubrae-Agrostidetum* (Romania) as well as pasturages within settlements (Ukraine) [22–24,68,69].

As this is a coprophilous species, the soil features seem not to be especially important for its occurrence. The colonized excrements were found on brown soil (Germany) [24] as well as regosol, rendzina (Romania), grey forest soil and leached cernoziomic soil (Bulgaria). In northern countries, the fungus prefers rather dry and warm soils (Iceland, Sweden) [66]. It seems the fungus is limited to grasslands on poor soils not artificially fertilized and not polluted by pesticides and emissions [69].

In Poland, *P. punctata* was found in November (two contemporary records) and spring (one historical record). The collection date of other specimens is unknown. For localities outside the present, but within the pre-war, boundaries of Poland, information on the collection date of two specimens was given: in March and September. The literature data suggest that in Europe stromata may be found all year round, but more often in fall (October to December) and spring (March to May) [66,67]. As the species colonizes rather large-sized dung storing much water, stromata are made from spring to late fall. In France, Macedonia, the Netherlands, and Great Britain they were also found in winter [69]. Structure of the dung pile does not seem to have an effect on the presence of *Poronia*, but the moisture content of the bolus is important, with most fungi being found on those in "normal" condition [68].

# Threat assessment

*Poronia punctata* within the present boundaries of Poland was found in only five historical localities – from at least the year 1845 (discovered in M. Szubert's collection from 1819–1845, cited for the first time in the present paper and concurrently the oldest record of this species from Poland) to 1903–1904 [56]. For the next hundred years (1905–2009), *P. punctata* was not recorded, which may be surprising because the substrate (horse or cow dung) occurred commonly until the first half of the twentieth century, and in some regions of Poland even longer. In the year 1937 there were ca. 4 million horses in Poland, in 1970 – ca. 2.4 million, and in 2010 – ca. 0.25 million [70].

There were not any records of *P. punctata* almost for the whole twentieth century, probably because stromata of this fungus might have been overlooked by the few Polish mycologists who did not devote much purposive sampling effort to coprophilous fungi at the time. Until the year 1945, only a few studies had been published

concerning solely or primarily this particular group of fungi; moreover the papers described mainly laboratory cultivations [71–76]. The historical data on this species was published when mycobiota of selected larger areas was elaborated [55–57]. After 1945, a few more studies dedicated to coprophilous fungi were published, and they concerned mainly forest communities but not open areas, meadows, and pastures, where *P. punctata* was more likely to occur. These communities seem to be less attractive to mycologists and much less often studied systematically by them [77–82]. Moreover, *Poronia stromata* appear chiefly in fall and spring, thus in many cases before or after the period of standard field studies, which may be the reason for rare records.

It is unknown whether and how weather conditions influence the development and emergence of the fungus stromata. In the area where both contemporary localities have been found, the years of the finding were unusually wet and as regards temperature, the year 2010 was described as normal and the year 2013 as warm with an extremely warm fall [83,84]. Such a setting of weather conditions might have positively influenced the emergence of stromata found in the localities in Czartoria and near Ogrodniczki.

The recent discoveries in the period 2010–2013 of two localities at the distance of about 100 km from each other, independently by two researchers, may indicate much better condition of the species population in Poland than one may conclude from the foregoing literature data and the threat category assigned to the species. The recent records of *P. punctata* in Poland prompt us to expect finding further localities of the species, especially in areas east of the Vistula River. These areas are characterized by a less transformed environment, especially in regions with poorer soil where grazing is kept on extensive pastures. Pastures, especially the ones with a continuous grazing tradition, are potential refuges of *P. punctata*, as they are rich in the proper substrate (dung) for growth of this species.

However, it seems less probable to find the fungus in many other parts of Poland, i.e., western regions (Silesia, Great Poland, Kujawy, Pomerania), where it has never been noted. The possible reasons for this is that much more fertile soil occurs there and, as a result, long-time intensive farming might have developed. Nevertheless, while searching for localities of the fungus one cannot neglect also this area. The agrotouristic and ecological farms that are nowadays very popular keep horses for recreational purposes, which may aid the appearance of *P. punctata*.

Looking for the right habitats and studying the occurrence of the fungus species there seems appropriate to verify the thesis that the number of the *P. punctata* localities in Poland is higher and the population condition is better that might have been imagined. The cause of the species decline has never been ascertained. It is supposed that a drastic decline in the *P. punctata* records in Europe in the twentieth century was caused by a decrease in populations of horses, ponies, and donkeys, replacing traditional free grazing with intensive closed animal husbandry, loss of primitive low-production dry grasslands, possible use of farming herbicides, plant pesticides and synthetic chemicals for "worming" horses [12,21,23,66].

Dry sandy grasslands are characterized by rich flora and entomofauna, often with the contribution of rare and threatened species. For this reason, they have been registered in the list of protected natural habitats of the European Union [85]. The case of *P. punctata* described by us indicates as well an important role of sandy grasslands for preserving specific threatened fungi. This is an additional argument for protection of these communities. The main threat to the grasslands is vegetation succession due to their initial character. Therefore, the best way to preserve these communities may be grazing which inhibits succession [86].

For the reason mentioned above, a basic manner to secure *P. punctata* seems to be keeping forms of traditional grazing at least in chosen areas. This solution may be ensured by agricultural/environmental programs, especially the variant that concerns keeping extensive agricultural land. It compensates farmers for the costs of keeping traditional extensive forms of farming [86–88]. The finding of the Czartoria locality is probably an indirect effect of agricultural/environmental programs, because the farmers who breed animals there use support payments aimed at preserving natural values of the Nature 2000 area – "Ostoja Narwiańska" (PLH 200024). Presumably, grazing would not be kept in this area any longer without this financial support.

After the latest discovery of the *P. punctata* localities in Poland, according to the IUCN [5,6] and Dahlberg and Mueller [8], while assessing a threat to this species in Poland, even if we exclude the probability of occurrence of other localities, it seems advisable to change the endangered (E) category, that had been assigned to it before the current criteria of IUCN became valid, to the vulnerable (VU) one. The main reason for this is a tendency to the increased instead of decreased number of localities and individuals. It should not be suggested, however, the species be removed from the list of the threatened ones due to "a very restricted number of locations (typically five or fewer)" such that it is prone to the effects of human activities or stochastic events within a very short time period in an uncertain future, and is thus capable of becoming critically endangered or even extinct in a very short time period, in accordance with the criterion D2 of the VU category.

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