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Competing interests

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ORIGINAL RESEARCH PAPER

The occurrence of *Microstoma protracta* (Fr.) Kanouse in Poland and assessment of its threat status

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Abstract

Microstoma protracta (Fr.) Kanouse is a strictly protected species in Poland, which is also present on the red list with the category R (rare). In Poland, there are five known localities for the species, including four historical sites discovered at the beginning of the twentieth century and one contemporary site discovered at the end of the twentieth century. In this paper, we present two new localities for *M. protracta*, discovered in April 2006 in Czeszewo (Wielkopolska Lowland, Jarocin Forest District) and in April 2017 in Krakow-Tyniec (Krakow-Częstochowa Upland, Myślenice Forest District). Sequences of the nuclear ribosomal DNA internal transcribed spacer regions of sporocarps from contemporary localities have been deposited in the GenBank database. This is the first record of the DNA barcode for this species. According to Criterion D of the IUCN recommendations, it has been suggested that *M. protracta* should be considered as a critically endangered species (CR: D) in Poland.

Keywords

Sarcoscyphaceae; Ascomycota; threatened fungi; Poland

Introduction

Microstoma protracta (Fr.) Kanouse (Sarcoscyphaceae, Pezizales, Ascomycota) is the only European representative of the genus *Microstoma* [1]. In Poland, it has been a protected species since 2004 [2,3], listed in all editions of the Polish red list of macromycetes with the Category R [4–6]. *Microstoma* ascomata appear at the end of winter or in early spring and usually grow on pieces of broadleaf tree wood buried in the ground or on the roots of trees. Apothecia are red, cup-shaped or urniform, deeply vase-shaped, and up to 2 cm in diameter. As they mature, they expand over the top and break, forming a distinctly serrated, white hair-covered edge (see Fig. 3, Fig. 4). The cups are mounted on a slender, 2–5 cm long, often branched, stipe embedded in the ground. The upper part of the stipe is colored similarly to the apothecium, but further down it brightens almost to white and finally, turns into a hard, dark pseudorhiza. *Microstoma* are easy to identify due to the characteristic morphology and color of their ascomata and their fruiting time. Different species of *Sarcoscypha* spp., with similar apothecia color, also fruit in similar habitats in spring. However, they have larger (1–6 cm in diameter) and shallower apothecia, with a uniform edge and single, sessile, or short (0–2 cm)

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stipitate. With regard to microscopic features, there is a clear difference in the size and shape of spores. *Microstoma protracta* spores are elliptic-fusoid, with a size of 25–45 × 10–14 µm [1] or larger (49–57 × 15–18 µm) [7]. *Sarcoscypha* spp. spores are elliptical to cylindrical, with a size of 27–43 × 11–15 µm [1]. Additionally, *Microstoma* asci mature simultaneously, while *Sarcoscypha* asci mature successively.

The aim of this article is to present the current distribution of *Microstoma protracta* in Poland and to assess the threat to the species, according to IUCN criteria.

Material and methods

The distribution of *Microstoma protracta* was determined based on the literature and the authors' own data. The threat status was assessed on the basis of IUCN criteria, taking into account the recommendations of Dahlberg and Mueller [8]. Ascomata were identified from two new localities, Czeszewo and Krakow-Tyniec. Identification was made based on macro- and micromorphological features, according to Hansen and Knudsen [1]. In addition, the specimens from new sites and one herbarium specimen (Herbarium Universitatis Lodziensis Fungi Poloniae, LOD 18203) were examined by molecular methods. For this purpose, dried fungal tissue fragments (ca. 5 mg) were lysed overnight at 56°C in ATL buffer containing 0.2 mg/mL Proteinase K (Qiagen GmbH, Hilden, Germany). Total genomic DNA was then extracted from the lysate using a DNeasy Blood and Tissue Kit (Qiagen), according to manufacturer's instructions. Nuclear ribosomal DNA internal transcribed spacer 1 (ITS1) was amplified with ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) primers developed by White et al. [9]. PCRs were performed in 10-µL reaction volumes containing 1× Type-it Microsatellite Kit (Qiagen), 0.25 µM of each primer, and 4 µL of DNA template, using a thermocycling profile of one cycle of 5 min at 96°C, followed by 35 cycles of 30 s at 95°C, 1 min at 50°C, and 1 min at 72°C, with a final step of 5 min at 72°C. After amplification, samples were purified with Exonuclease I and FastAP Alkaline Phosphatase (Fermentas; Thermo Fisher Scientific, Waltham, MA, USA) and sequenced using BigDye Terminator v3.1 chemistry on an ABI Prism 3130XL Analyzer (Applied Biosystems, Foster City, CA, USA). Herbarium samples were amplified using the same primers fused with indexed adapters and sequenced using Ion Torrent PGM system (Thermo Fisher Scientific) according to the manufacturer's protocols. Sequence reads were separated by barcodes, filtered, and assembled using Geneious R11 (Biomatters Ltd., Auckland, New Zealand).

Results

Molecular analysis

The nucleotide sequences of the internal transcribed spacer region 1 (ITS1) region (227 bp) confirmed that all specimens belong to the same species. BLAST search showed that the obtained ITS1 sequence shared the highest similarity of 83% with sequences from species belonging to Sarcoscyphaceae. To date, only four ITS1 sequences have been published for the *Microstoma* genus and none of these are *M. protracta* sequences. Therefore, the ITS1 sequences obtained in this study (GenBank accession numbers MG920535–37) are the first records of the DNA barcode for this species.

Localities of *Microstoma protracta* in Poland (Fig. 1)

Material analyzed:

- Wrocław (Szczytniki, Swojszyce), Dolny Śląsk Province (KRA F-0-5268);
- Between Częstochowa and Mstów, Upper Silesia Province, Jurajski Landscape Park, planned reserve Gąszczyk, *Tilio-Carpinetum typicum*, 1998-03-26, leg. I. Walewska, det. M. Ławrynowicz, (LOD 18203) [10];

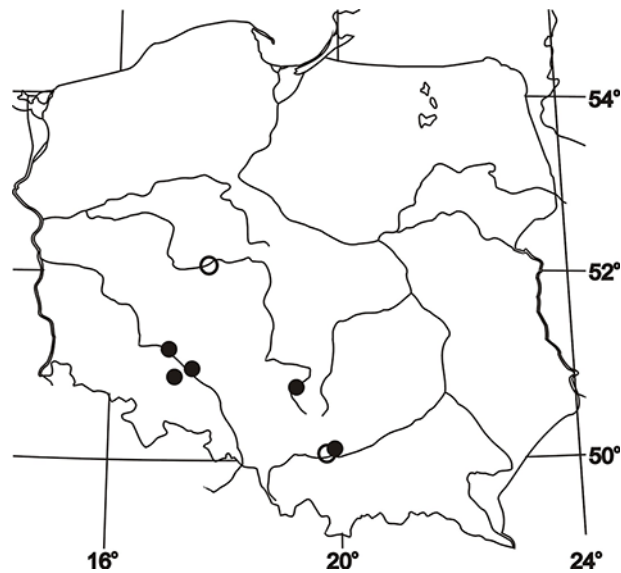


Fig. 1 Distribution of *Microstoma protracta* in Poland. Black circles – localities known from literature or herbarium specimens; white circles – new localities.

- Czeszewo, Wielkopolska Province, Żerkowsko-Czeszewski Landscape Park, 2006-04-21, leg. A. Winiecki & M. Hałas, det. A. Bujakiewicz & A. Kujawa (POZ, sine cat.) – new locality;
- Krakow-Tyniec, Małopolska Province, 2017-04-09, leg. & det. R. Kubiński (POZ, sine cat.) – new locality.

Localities known from the literature

- Oława, Dolny Śląsk Province, deciduous forest, before 1908 [11];
- Vicinity of Strzelin, Dolny Śląsk Province, deciduous forest, before 1908 [11];
- Wrocław (Osobowice, Popowice, Swojszyce), before 1908 [11];
- Krakow, Małopolska Province, slope of a mountain, Bobiak, 1889 [12,13].

Descriptions of new localities

Locality 1. Czeszewo, (52°08'06.00" N, 17°30'30.80" E), Miłosław Commune, Września District, Wielkopolska Province, Jarocin Forest District, Czeszewo Forest Range, compartment 180j, ATPOL CD 32, 2006-04-21, leg. Aleksander Winiecki, Michał Hałas, det. Anna Bujakiewicz, Anna Kujawa (Herbarium Instituti Botanici Universitatis Posnaniensis Fungi Poloniae, sine cat.)

This locality is situated west of the “Czeszewski Las” Reserve, in the Natura 2000 site “Żerkowsko-Czeszewski Forest” and Żerkowsko-Czeszewski Landscape Park, on the edge of the Warta River floodplain, on the slope of an oxbow lake bank (“Starucha”, Fig. 2), where brown alluvial soils occur. Altered and impoverished forest communities (*Carpinion betuli* Issler 1931 em. Oberda. 1957) occur here. The ascomata were observed in a patch of vegetation of a nitrophilic community of *Alliario-Chaerophylletum temuli* Lohmeyer 1949, with the following plant species: *Fallopia dumetorum* (L.) Holub, *Geranium robertianum* L., *Lapsana communis* L., *Geum urbanum* L., and *Cherophyllum temulum* L. The shrub layer consisted of *Crataegus monogyna* Jacq. and *Frangula alnus* Mill. and the tree stand was formed by *Acer campestre* L., *Quercus robur* L., *Ulmus laevis* Pall., and *Carpinus betulus* L. (*Pinus sylvestris* L., *Populus nigra* L., and *Robinia pseudoacacia* L. were growing nearby). Five apothecia, arising from the common stem, grew on a piece of wood buried in the ground (Fig. 3).

This locality has been monitored annually since 2006, but no fruiting has been found again.

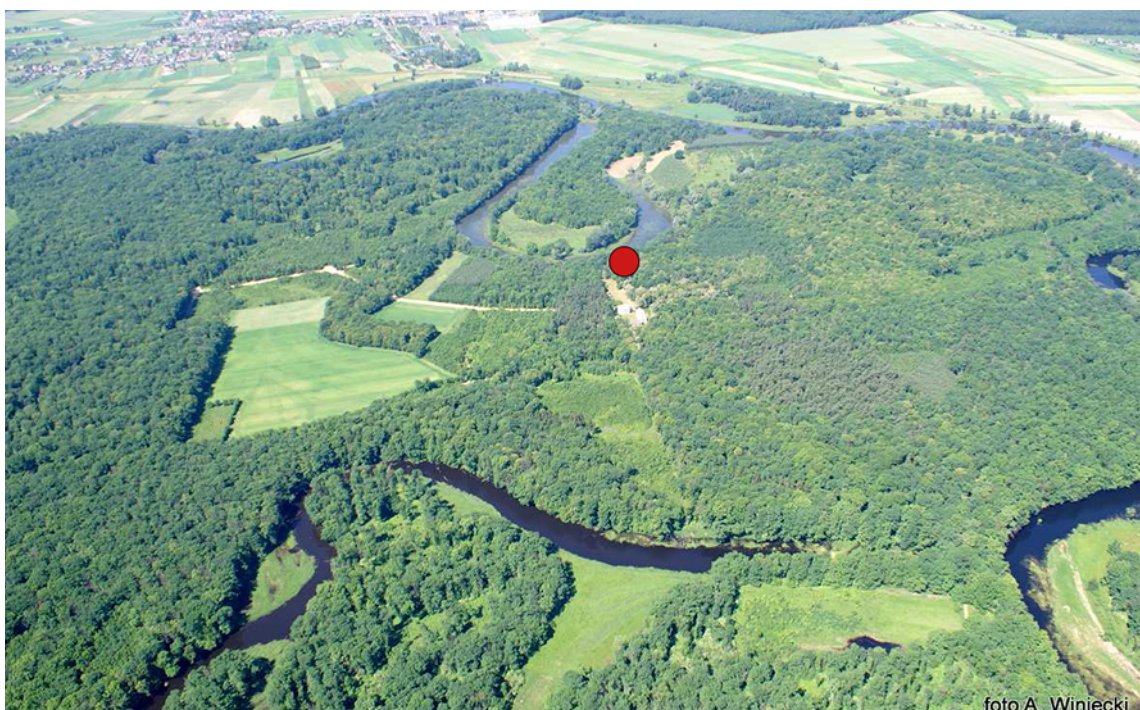


Fig. 2 Location of the locality in Czeszewo, fot. Aleksander Winiński.



Fig. 3 Ascomata of *Microstoma protracta* from Czeszewo, fot. Aleksander Winiński.

Locality 2. Krakow-Tyniec, Bielańsko-Tyniecki Landscape Park, in private forests at the eastern border of forest compartment 277b (Myślenice Forest District, Radziszów Forest Range), ATPOL DF-78, 2017-04-09, leg. & det. Radosław Kubiński.

The ascomata were found in a deciduous forest, with a small amount of litter and a poorly developed herb layer. In the shrub layer, there was *Sambucus nigra*, and in the layer of trees: *Betula pendula* Roth, *Quercus robur* L. and *Carpinus betulus* L. Four *M. protracta* apothecia grew individually on distinct stems, most likely from one piece of wood (Fig. 4). *Caloscypha fulgens* (Pers.) Boud. ascomata were also found near the *M. protracta* ascomata.

Microstoma protracta ascomata were again observed on 2018-03-28 at this locality, with seven apothecia arising from one piece of wood.

A distribution map of the known localities of *M. protracta* in Poland is shown in Fig. 1.



Fig. 4 Ascoma of *Microstoma protracta* from Krakow-Tyniec, fot. Radosław Kubiński.

Threat assessment

The threat to the species was assessed based on the following information:

- Based on knowledge of the new localities and the availability of potentially suitable habitats for this species, it can be concluded that the national population is not decreasing, nor is it expected to do so in the future (Criterion A).
- The current extent of occurrence is estimated at approximately 35,000 km², but the actual area of occupancy (assuming that the locality is a square with an area of 10 km²) is 30 km². The extent of occurrence, the area of occupancy, the number of habitats suitable for this species, the number of localities, and the number of mature ascomata (Criterion B) are not expected to decrease. It is not possible to assess fluctuations in the number of ascomata at the localities, due to a lack of monitoring data. Long-term observations have only been performed in the Wielkopolska region and they suggest the ephemeral production of ascomata every few years or extinction of mycelium.
- The number of ascomata is extremely small, with only single ascomata observed at each of the localities. The size of the national population is estimated at 30 mature individuals (Criterion C, D). This estimate assumes the maximum number of mature individuals for each inhabited piece of wood, as proposed by Dalberg and Mueller [8].

After analyzing Criteria A–D, *Microstoma protracta* should be considered as critically endangered, due to meeting Criterion D – the population size is estimated at less than 50 mature individuals (CR: D).

Discussion

Microstoma protracta is a very rarely recorded species in Poland. This may be influenced by its fruiting in early spring, when the activity of observers is usually low. However, *Sarcoscypha austriaca*, a species similar to *M. protracta* in terms of ascomata color, time of appearance, and habitat (fertile deciduous forests), is known to occur in at least 170 localities [14]. Therefore, it should be recognized that *M. protracta* is extremely rare and is threatened by extinction. All known sites of this species should be monitored in order to identify the dynamics of the subpopulations, assess the threats, and develop methods of conservation. When the red list is updated, the existing R category should be replaced with the CR category.

Similar to Poland, *Microstoma protracta* is placed on red lists in several other European countries, including Austria (with the EN category) [15], Bulgaria (EN) [16], the Czech Republic (EN) [17], Slovakia (LR) [18], Germany (G – species of undetermined threat) [19], and France (in the Franche-Comté region, CR) [20]. The records presented in this paper suggest that the population of *M. protracta* in Poland is an important component of the Central European population of this rare species.

Acknowledgments

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