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PC, PS: research designing, field works, writing the manuscript; GV: field works, writing the manuscript

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# ORIGINAL RESEARCH PAPER

# *Ophiocordyceps stylophora* (Ophiocordycipitaceae) in Poland: new localities and host species

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# Abstract

*Ophiocordyceps stylophora*, a rare fungus species in Europe, was first recorded in one locality in Poland in 2011. This paper concerns 33 specimens found between 2011 and 2015 in 23 new localities in five areas. Amongst the described specimens, one was found on the Tenebrionidae beetle, and others grew on Elateridae larvae. For the first time, Tenebrionidae larvae have been observed as hosts of *O. stylophora*. Five new positions have been located in the southern part of Poland in the area of Oszast and Reberce nature reserves, Babia Góra and Roztocze national parks, and one has been found in the northern part of Poland, in the Elblag Upland Landscape Park.

# Keywords

Hypocreales; *Cordyceps*; entomopathogenic fungi; fungal ecology; rare species; protected areas

# Introduction

*Ophiocordyceps stylophora* (Berk. & Broome) G. H. Sung et al. is a member of Hypocreales (Ascomycota). It was described in 1857, and classified within genus *Cordyceps* s. l. In 2007, it was transferred to the genus *Ophiocordyceps* [1,2]. This group of fungi exhibits parasitic tendencies [1]. In most cases, they parasitize Coleoptera larvae, but some species have been recorded on insects in the Lepidoptera, Heteroptera, Homoptera, Hymenoptera, and Araneae orders, as well as on fruit bodies of the hypogeous fungi from the genus *Elaphomyces* [1]. In Poland, 16 species of *Cordyceps* s. l. have already been recorded [3–5].

*Ophiocordyceps stylophora* is an entomopathogen that parasitizes beetle larvae from Cerambycidae, Elateridae, Scarabaeidae, and Chrysomelidae families [1]. The hosts are slightly recessed in wood (up to a few centimeters) when fungal structures are developed and visible on the wood surface. It is a rare species found in few countries in the world [3,6]. Recently, it has been observed in several places in Europe including Latvia [7], Norway [8], and Slovakia [9–11] (Fig. 1). Until now, recorded observations of this species in Poland have been limited to the Pieniny National Park [3]. Further fieldwork conducted between 2011 and 2015 revealed several other locations throughout Poland (Fig. 1).

The aim of the research is to present new localities of *O. stylophora* in Poland with reports on their hosts and description of its habitats. The ecology of the species is also briefly discussed.





### Material and methods

Between 2011 and 2015, researchers searched for O. stylophora in 15 protected areas: Babia Góra National Park, Magura National Park, Ojców National Park, Roztocze National Park, Little Beskids Landscape Park, Brzanka Range Landscape Park, Przemyskie Foothills Landscape Park, Elbląg Upland Landscape Park, Ciężkowice-Rożnów Landscape Park, Dłubnia Landscape Park, Poprad Landscape Park, Rudno Landscape Park, Tenczynek Landscape Park, Wiśnicz-Lipnica Landscape Park, and Żywiec Landscape Park. The collected samples were identified according to the keys developed by Kobayashi [12] and Mains [13]. Preparations of dry specimens mounted in water, Melzer's reagent, and Congo red were microscopically observed using the PZO 14 light microscope with Nomarski interference contrast [14]. Macro- and microfeatures of the specimens did not differ significantly from the specimens previously collected in the Pieniny National Park [3]. The Latin names of liverworts are derived from Szweykowski [15], those of mosses from Ochyra et al. [16], those of vascular plants from Mirek et al. [17], and those of plant communities from Matuszkiewicz [18,19]. Names and numbers of geographical regions were adopted from Kondracki [20]. Site localizations were marked using GPS Garmin III+. The localities were assigned to 10 × 10-km ATPOL squares calculated according to Snowarski's calculator [21]. Herbarium materials were deposited in P. Chachuła's private collection.

# Results

*Ophiocordyceps stylophora* (Berk. & Broome) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 47 (2007). Bas. & syn.: *Cordyceps stylophora* Berk. & Broome, in Berkeley 1857.

Localities, habitats, and ecology

1. ATPOL: DB-06, Southern Baltic Coasts (313), Gdańsk Seashore (313.5), Elbląg Upland (313.55), Elbląg, Kumiela (Dzikuska) Stream region which is a tributary of the Elbląg Canal, Elbląg Upland Landscape Park, in the State Forests management area. GPS: 54°11′51″ N; 19°28′4″ E, 100-150 m above sea level (a.s.l.). The forest includes Alnus incana, Carpinus betulus, Fagus sylvatica, Fraxinus excelsior, and Populus tremula near streams (Fig. 2). An manage forest in which rotting wood is left in the ravine. Soil is usually naked with single herbaceous plants: Aegopodium podagraria, Impatiens sp., and Equisetum telmateia. Within this ATPOL square, 25 specimens including 22 teleomorphs and three sterile stromata were observed on 18 logs scattered in a 1-km<sup>2</sup> area. Stromata grew from Elateridae and Tenebrionidae larvae (Fig. 3A,D) recessed in the wood of the fallen log of a deciduous tree. Both mossy and uncovered logs were observed on the following dates: October 19 (Fig. 3C), October 20 and 22 (Fig. 3B), October 23 (Fig. 3E), October 29, November 23, 2013, February 15, 16, 20, 28, March 3, 4, 6, 18, June 22, July 18 (Fig. 3A), and September 25, 2014 (Fig. 3D); obs. and leg. P. Stanczak, det. P. Chachuła. Collection numbers: PC/1/23102013, PC/1/29102013, PC/1/06032014, PC/1/18032014, and PC/1/11042014 (Fig. 3F,G).

**2.** ATPOL: GE-91, Lublin-Lviv Upland (343), Roztocze (343.2), Central Roztocze (343.22), Roztocze National Park, and valley of the Świerszcz Stream, GPS: 50°35′27.0″ N; 22°59′04.0″ E, 230 m a.s.l., in lowland alder and ash-alder forest on periodically swamped ground-water soils *Fraxino-Alnetum* association. Recessed in surface layers



Fig. 2 The habitat of Ophiocordyceps stylophora in Kumiela (Dzikuska) Stream in Elbląg. February 28, 2014. Photo – P. Stanczak.



**Fig. 3** *Ophiocordyceps stylophora* – macro- and microfeatures. (**A**) Sterile stroma (anamorph) growing from Elateridae larva. (**B**) Stroma with perithecia (teleomorph) in the initial phase of growth. (**C**) Branched stroma in teleomorphic stage. (**D**) Stroma growing from Tenebrionidae larva. (**E**) Stroma in teleomorphic stage. (**F**) A group of asci. (**G**) Upper parts of asci with ascospores. Scales: (**A**–**E**) 1 cm; (**F**) 100  $\mu$ m; (**G**) 10  $\mu$ m. Photos: P. Stanczak (**A**–**E**); P. Chachuła (**F**,**G**). (**A**) July 18, 2014; (**B**) October 22, 2013; (**C**) October 19, 2013; (**D**) September 25, 2014; (**E**) October 23, 2013; (**F**,**G**) PC/1/11042014.

of fir logs in the neighborhood of bryophytes: *Cephalozia bicuspidata, Lepidozia reptans, Plagiothecium nemorale, Riccardia latifrons,* and *Tetraphis pellucida* and vascular plants: *Chrysosplenium alternifolium, Dryopteris carthusiana,* and *Oxalis acetosella.* A single stroma was observed with teleomorphic stage of *O. stylophora* growing from a larva from the Elateridae family. September 16, 2011, leg. G. Vončina, det. P. Chachuła. Collection number: PC/1/16082011.

**3.** ATPOL: FG-08, Outer Western Carpathians (513), Middle Beskids Foothills (513.6), Przemyskie Foothills (513.65), Reberce Nature Reserve, and Przemyskie Foothills Landscape Park, GPS: 49°39′01.8″ N; 22°30′45.3″ E, 400 m a.s.l., stream valley in submontane forb-rich Carpathian fir-beech forest; East Carpathian vicariant *Dentario* 

*glandulosae-Fagetum abietetosum* subassociation. One stroma was observed with teleomorphic stage *O. stylophora* growing from a larva from the Elateridae family, slightly recessed in a rotten fir log. September 12, 2015, leg. G. Vončina, det. P. Chachuła. Collection number: PC/1/12082015.

**4.** ATPOL: DG-17, Outer Western Carpathians (513), Western Beskids (513.5), Żywiec Beskids (513.51), Babia Góra Range (513.512), and Babia Góra National Park, in the area of the Górny Płaj trail, GPS: 49°35′28.2″ N; 19°33′34.5″ E, 1,020 m a.s.l. in montane forb-rich Carpathian fir-beech forest West Carpathian vicariant *Dentario glandulosae-Fagetum abietetosum* subassociation. One stroma was observed with teleomorphic stage *O. stylophora* growing from a larva from the Elateridae family, slightly recessed in the rotten wood of a fir log. May 19, 2012, leg. G. Vončina, det. P. Chachuła. Collection number: PC/1/19052012.

**5.** ATPOL: DG-16, Outer Western Carpathians (513), Western Beskids (513.5), Żywiec Beskids (513.51), Babia Góra Range (513.512), Babia Góra National Park, Czatoża forest, GPS: 49°35′49.6″ N; 19°28′59.4″ E, 876 m a.s.l. in montane forb-rich Carpathian fir-beech forest West Carpathian vicariant *Dentario glandulosae-Fagetum* association. A single stroma was observed with teleomorphic stage *O. stylophora* growing from a larva from Elateridae family, recessed in the surface layers of a strong fir log in the vicinity of *Herzogiella seligeri* and *Dicranum scoparium*. April 9, 2013, leg. G. Vončina, det. P. Chachuła. Collection number: PC/1/09062013.

**6.** ATPOL: DG-34, Outer Western Carpathians (513), Western Beskids (513.5), Żywiec Beskids (513.51), Żywiec-Orawa Beskid (513.511), Oszast Nature Reserve, Żywiec Landscape Park, GPS: 49°24′37.7″ N; 19°11′18.6″ E, 1,120 m a.s.l., in beech-fir forest with sycamore. Three stromata were observed with teleomorphic stage *O. stylophora* recessed in the surface layers of rotten wood of a fir log. March 9, 2014, obs. R. Rutkowski, det. V. Kautman (by photos).

# Discussion

The localities presented in this paper are upland, foothill, and mountain regions with elevations ranging from 100 to 1,120 m a.s.l. It should be emphasized that out of the 15 studied regions, O. stylophora was found in five. Of these, localities (Fig. 1) are located in areas under strict protection, excluded from forest management activities. They are in the Reberce and Oszast nature reserves (Fig. 1) and the Babia Góra National Park (Fig. 1). One site in the Roztocze National Park is located in an area of active protection. The site located in Elblag Upland Landscape Park (Fig. 1) is managed by the State Forests National Forest Holding. In this area, suitable conditions for the development of O. stylophora were created by leaving dead wood in inaccessible places, such as the deep valleys of the streams. Among six areas in 23 localities, 33 specimens of O. stylophora were observed, including 30 in the teleomorphic stage and three in the anamorphic stage. This fungus is most commonly found on click beetle larvae from the Cerambycidae, Elateridae, Scarabaeidae, and Chrysomelidae families [1-3,7,8,10-13]. A similar pattern was observed in this study of the 33 specimens, the hosts for the fungus in 29 cases were larvae from the Elateridae family (in the case of three specimens from Oszast the hosts were not identified). The exception was the fungi specimen observed in the Kumiel (Dzikuska) Stream in Elbląg (Fig. 1), where the stroma of O. stylophora emerged from a larva of the Tenebrionidae family. The insects from this family were never mentioned in literature as hosts for O. stylophora and our finding extends the list of potential hosts for this species [1].

In reports on *O. stylophora*, the authors rarely described the habitat of this species precisely [3,7]. Generally, it can be said that vegetation in which this species is found is diverse. In Elblag Upland and Roztocze National Park, specimens were found in the riparian forest in valleys of streams, similar to regions in Latvia and Norway [7,8]. In higher-elevation areas of the Reberce nature reserve and Babia Góra National Park, this fungus grew in old beech and fir, such as those in the Pieniny National Park [3]. In

Slovakia, they were found in beech forests [10,11] while in Mexico, they were found in oak and pine forests [6]. Although most observations from Europe [7-11,22] have been done in October and one in April [3], the observations described in this paper indicate that the anamorphic and teleomorphic stages of *Ophiocordyceps stylophora* can be found year-round. Moreover, unlike Mains' conclusions [13,23], the results demonstrate that the mature stage of *O. stylophora* can also be observed at nearly anytime in the year (February–June, September–November) (Tab. 1).

 Tab. 1
 Dates and development stages of the observed specimens of Ophiocordyceps stylophora.

	Month								
Form	II	III	IV	V	VI	VII	IX	X	XI
Anamorph	+	-	-	-	+	+	+	+	-
Teleomorph	+	+	+	+	+	-	+	+	+

The substrates in which beetle larvae were observed were coniferous and deciduous wood. The following tree species are mentioned in previous studies: *Alnus glutinosa* [7,8], *Fagus sylvatica* [10,11], and *Abies alba* [3]. In this respect, the following correlation was observed in Poland: in the natural ranges of *Abies alba*, *O. stylophora* was found in fir logs, while beyond the area of the fir range, larvae infected by this fungus were found in deciduous wood.

The observation of *O. stylophora*, a notably rare taxon, and the recent discovery of new *Cordyceps* s. l. species from Poland [4] prove that the fungal species in Poland have not been comprehensively investigated.

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