

***OPHIOCORDYCEPS STYLOPHORA* (ASCOMYCOTA, HYPOCREALES), NEW SPECIES FOR POLAND**

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Abstract. The paper reports the first two records of *Ophiocordyceps stylophora* (Berk & Broome) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora in Poland, describes, illustrates and briefly discusses its macroscopic and microscopic characters, and briefly characterizes its habitats in Poland.

Key words: Fungi, *Ophiocordyceps stylophora*, distribution, Pieniny National Park, Poland, Europe

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INTRODUCTION

Ophiocordyceps stylophora (Berk & Broome) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora belongs to the family *Ophiocordycipitaceae* G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora (Sung *et al.* 2007). Until recently it was placed in the broadly conceived genus *Cordyceps* Fr. The anamorph of this species is *Hirsutella stylophora* Mains. Previously, 15 species of *Cordyceps* s.l. were reported from Poland (Krzemieniewski 1928; Bujakiewicz 2004; Nita & Bujakiewicz 2004; Van Vooren & Audibert 2005; Kautmanová & Kautman 2006; Kujawa & Gierczyk 2007; Chachula 2008; Mułenko *et al.* 2008).

According to Van Vooren and Audibert (2005) *O. ditmarii* is a European species, distinguished from *O. sphecocephala* which occurs in both Americas, Africa and Asia. The separation of these two species is also recognized by Sung *et al.* (2007), so presumably all the *Cordyceps sphecocephala* specimens reported from Poland actually belong to *Ophiocordyceps ditmarii*, the European vicariant of *C. sphecocephala*.

Ophiocordyceps stylophora is a fungus parasitizing larvae of the order Coleoptera (Kobayasi 1941). The specimens previously reported in the literature produced ochraceous, orange-brownish to dark cinnamon brown stromata. The stipes and aperithelial apices were concolorous with the fer-

tile part of the stroma. Total length of stromata varied from 1.5 and 4.5 cm, with thickness of 0.5–2.0 mm. The fertile part, cylindrical in shape and reaching 5.5–15.0 mm in length and 2–3 mm thickness, occurred below the aperithelial, pointed, 10–15 mm long apex. A distinctive feature of this species is the structure of the perithecial cortex, which constitutes a continuation of the outer hyphae of the stipe (Masse 1895; Mains 1958).

Ophiocordyceps stylophora is a rare species, known from only a few localities. It was first described from South Carolina by Berkeley (1857). The next locality was recorded in Minnesota at Minnehaha Falls near Minneapolis (MacMillan 1898). According to Kobayasi (1941) it was also found in North America in Michigan, New York and Tennessee, as well as in China (Tien-mu-shan in Chekiang Province) and Japan (Honsyū, Mino Province, Kawaue-mura). Mains (1958) provides further records from South Carolina, Nova Scotia and Illinois, and Glare (1992) provides the first finding of this species in New Zealand, where it was collected in the anamorph stage as *Hirsutella stylophora*.

So far the only published European locality of the *Ophiocordyceps stylophora* teleomorph is from Sweden (Vallda, Hördalens naturreservat, Halland County, Kungsbacka municipality) (Nordén *et al.* 2002). Moniz *et al.* (1999) reports finding

the anamorph, isolated from coleopteran larvae *Phoracantha semipunctata* in Portugal.

An interesting find was made in Japan (Settsu-kyo, Takatsuki, Osaka) where *O. stylophora* occurred as host for the probably hyperparasitic *Cordyceps cuboidea*, since a clump of stromata of this species was observed on its stipe (Ban *et al.* 2009).

MATERIAL AND METHODS

This study is based on examination of fresh material collected in April 2009 and 2011 in Pieniny National Park (Pieniny Mts, Western Carpathians, S Poland). Two specimens were identified according to the keys published by Kobayasi (1941) and Mains (1958). Microcharacters were examined with a Leica DM1000 microscope using 100 \times , 63 \times , 40 \times (phase contrast) and 20 \times objectives. Asci and ascospores were studied in Cotton Blue solution. All values are means of 15–20 measurements. The studied specimens are deposited in the fungal Herbarium of the Jagiellonian University in Kraków (KRA). Nomenclature of fungi follows Sung *et al.* (2007). Nomenclature of plants follows Mirek *et al.* (2002), Ochyra *et al.* (2003), and Szwejkowski (2006).

RESULTS

Ophiocordyceps stylophora (Berk & Broome)
G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora
Figs 1 & 2

Stud. Mycol. 57: 47. 2007. – *Cordyceps stylophora* Berk & Broome, J. Linn. Soc. Bot. 1(4): 158. 1857.

Stromata brown, 20–26 mm long. Stipes half-cylindrical and cylindrical in shape in cross section directly under the fertile parts of stromata. Fertile parts 5.5–7.0 cm long and 2 cm diam., cylindrical shape, with perithecia oriented at right angles to the surface. Stipes 0.5–1.0 mm thick. Aperithecial stromatal apices 7–8 mm long, pointed and slightly curved. Perithecia completely immersed in fertile parts of stromata. Cortex of hyphae in fertile parts constitutes a continuation of the outer hyphae of the stipes. Perithecia 270–417 \times 150–210 μ m, asci 8-spored, 154–220 \times 6–10 μ m with caps *ca* 3.0–5.2 \times 3.0–4.2 μ m. Ascospores 64.2–183.0 \times

2.0–3.5 μ m, multiseptate, with cylindrical cells 9–24 \times 1.8–3.0 μ m long, not breaking into segments.

STUDIED SPECIMENS AND HABITAT

The two localities of *Ophiocordyceps stylophora* are situated *ca* 1.4 km from each other in the Pieniny Właściwe Mts (Western Carpathians, South Poland) within Pieniny National Park in an area under strict protection.

1. Pieniński Potok stream (upper part), beech forest (*Dentario glandulosae-Fagetum abietetosum* var. *typicum*), GPS (WGS 84) 49°25'25"N; 20°25'00"E; 600 m a.s.l., 22 Apr. 2009, *leg. & det.* G. Vončina, P. Chachula & J. Kozik (KRA F-2009-5).

A single stroma of *Ophiocordyceps stylophora* was found on a coleopteran larva of the Staphylinidae family. The host was embedded in decaying wood of *Abies alba* overgrown with vascular plants: *Oxalis acetosella*, seedlings of *Abies alba* and *Picea abies*. In the bryophyte layer on the log were *Blepharostoma trichophyllum*, *Brachythecium rutabulum*, *Cephalozia bicuspidata*, *Dicranum scoparium*, *Herzogiella seligeri*, *Lepidozia reptans*, *Nowellia curvifolia*, *Orthodicranum montanum*, *Plagiochila asplenioides*, *Rhizomnium punctatum*, *Sanionia uncinata* and *Tetraphis pellucida*. The tree stand in the vicinity of the stream bed consisted of *Abies alba*, *Acer pseudoplatanus*, *Fagus sylvatica* and *Ulmus glabra*. The ground cover comprised *Aegopodium podagraria*, *Anemone ranunculoides*, *Angelica sylvestris*, *Asarum europaeum*, *Athyrium filix-femina*, *Caltha palustris*, *Cardamine amara*, *Chaerophyllum hirsutum*, *Circaea alpina*, *Dryopteris dilatata*, *D. filix-mas*, *Galeobdolon luteum*, *Geranium phaeum*, *G. robertianum*, *Glechoma hirsuta*, *Impatiens noli-tangere*, *Isophyrum thalictroides*, *Lamium maculatum*, *Mercurialis perennis*, *Milium effusum*, *Oxalis acetosella*, *Paris quadrifolia*, *Sorbus aucuparia*, *Stellaria nemorum*, *Symphytum cordatum*, *Thalictrum aquilegifolium*, *Valeriana sambucifolia* and *V. simplicifolia*. Species of bryophytes in the ground cover included *Conocephalum salebrosum*, *Ctenidium molluscum*, *Plagiochila porelloides* and *Plagiomnium undulatum*.



Fig. 1. *Ophiocordyceps stylophora* (Berk & Broome) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora. 1 – stroma, 2 – fertile part of stroma with perithecia, 3 – host – larva of Coleoptera, 4 – cross section of fertile part, 5 – perithecium, 6 – ascus, 7 – ascospore, 8 – component cells of ascospore.

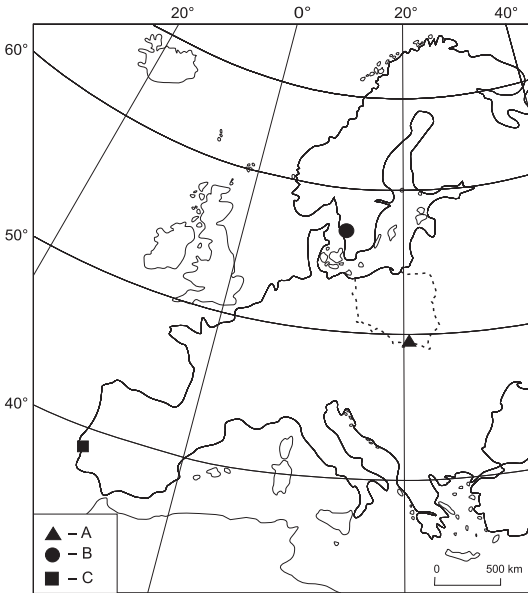


Fig. 2. Distribution of *Ophiocordyceps stylophora* (Berk & Broome) G. H. Sung *et al.* in Europe. A – new locality of *O. stylophora* (Poland), B & C – hitherto known localities in Sweden (B) and Portugal (C).

2. Skały Piecki rocks, sycamore forest (*Phil-litido-Aceretum*) with *Phyllitis scolopendrium*, GPS (WGS 84) 49°24'46"N/20°25'31"E; 617 m a.s.l., 6 Apr. 2011, *leg. P. Chachula* (KRA F-2011-1).

A single stroma of *Ophiocordyceps stylophora* grew on a coleopteran larva occurring in rotting

wood. It was embedded in a log of *Abies alba* overgrown with the bryophytes *Blepharostoma trichophyllum*, *Dicranum scoparium*, *Herzogiella seligeri*, *Hypnum cupressiforme*, *Lepidozia reptans*, *Lophocolea heterophylla* and *Pterigynandrum filiforme*. The tree stand in the vicinity consisted of *Acer pseudoplatanus* accompanied by *Fagus sylvatica*. In the ground cover were *Phyllitis scolopendrium*, *Polypodium vulgare* and *Urtica dioica*.

DISCUSSION

Previously, eight species of the genus *Cordyceps* s.l. were found in Pieniny National Park (Gumińska 1990; Kautmanová & Kautman 2006; Chachula 2008; unpublished information about *Elaphocordyceps rouxii* and *Ophiocordyceps forquignoni*) (Table 1). This is the first record of this species in Poland, and the second in Europe reporting the teleomorph stage (Nordén *et al.* 2002). When its anamorph (Moniz *et al.* 1999) is included, this is the third record of this species in Europe. All observations mentioned above were made in protected areas such as national parks or nature reserves. Such places provide protection for suitable habitats and are critical to maintaining the diversity of entomopathogenic fungi (Bałazy 2004).

According to Kobayasi (1941) *O. stylophora*

Table 1. Fungi of the genus *Cordyceps* s.l. occurring in Poland. * – species found in Pieniny National Park.

Host	Species
<i>Elaphomyces</i> sp.	<i>Elaphocordyceps capitata</i> (Holmsk.) G. H. Sung, J. M. Sung & Spatafora* <i>E. longisegmentis</i> (Ginns) G. H. Sung, J. M. Sung & Spatafora* <i>E. ophioglossoides</i> (Ehrh.) G. H. Sung, J. M. Sung & Spatafora* <i>E. rouxii</i> (Cand.) G. H. Sung, J. M. Sung & Spatafora*
Lepidoptera	<i>Cordyceps bifusispora</i> O. E. Erikss. <i>C. militaris</i> (L.) Link <i>C. tuberculata</i> (Lebert) Maire
Coleoptera	<i>Ophiocordyceps entomorrhiza</i> (Dicks.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora <i>O. gracilis</i> (Grev.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora* <i>O. stylophora</i> Berk. & Broome G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora *
Homoptera	<i>Ophiocordyceps clavulata</i> (Schwein.) Petch
Hymenoptera	<i>Ophiocordyceps ditmarii</i> (Quél.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora* <i>O. forquignoni</i> (Quél.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora* <i>O. myrmecophila</i> (Ces.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora <i>O. unilateralis</i> (Tul. & C. Tul.) Petch.

Table 2. Comparison of macro- and microcharacters of *Ophiocordyceps stylophora* Berk. & Broome G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora from selected publications.

Publication Character	Massee 1895	MacMillan 1898	Mains 1958	This paper
Host	Larva of Hexapoda	Larva of Carabidae	Larva of Elateridae	Larva of Elateridae
Stipe	1.5–2.5 cm high 1.5–2.0 mm thick	–	1.5–4.5 cm high 0.5–1.0 mm thick	2.0–2.6 cm high 0.5–1.0 mm thick
Aperithecial apice	10–15 mm long	–	Long acuminate sterile apice	7–8 mm long
Fertile part of the stroma	10–15 mm long 2.5–3.0 mm thick	–	Up to 2 mm thick	5.5–7.0 mm long 2 mm thick
Perithecia	–	–	240–420 × 144–240 µm	270–417 × 150–210 µm
Asci	–	–	170–220 × 8–10 µm	154–220 × 6–10 µm
Ascospores	125–135 × 1 µm	150–180 × 2 µm	102–164 × 2–3 µm	64.2–180.3 × 2.0–3.5 µm
Component cells	3.5 × 1.0 µm	6–7 × 1 µm	12–29 × 2–3 µm	9–24 × 1.8–3.0 µm

occurs on larvae of beetles. Presumably the information from Massee (1895) also related to beetle larvae even though the hosts are given as from the subphylum Hexapoda, which included more taxa. The few descriptions of characters show considerable variation of the morphology and anatomy of this species (Table 2). Some stromata examined by Mains (1958) were almost twice the length of stroma as given by Massee (1895) and the stromata we collected. The specimens found in the Pieniny Mts had aperithecial stromatal apices 7–8 mm long, considerably shorter than the 10–15 mm length given by Massee (1895). The fertile parts of the stromata in the specimens we collected were 5.5–7.0 mm long, shorter than the 10–15 mm length given by Massee (1895). The perithecia and asci of our specimens resemble the description given by Mains (1958). These specimens also produced the longest ascospores (64.2–180.3 µm), similar to those described by MacMillan (1898) with length of 150–180 µm. Massee (1895) described the shortest ascospores, up to 135 µm long. Ascospore width in the specimens studied by MacMillan (1898) and Mains (1958) and in our specimens was between 2.0 and 3.5 µm, versus 1 µm given by Massee (1895). The biggest differences relate to cell segment length. The ascospores Massee (1895) observed by produced the shortest cells (3.5 µm long); in others they were 6–7 µm long (MacMillan 1898). The specimens collected in the Pieniny Mts had considerably longer cell

segments (9–24 µm), but the longest (12–29 µm) were studied by Mains (1958).

According to Mains (1941, 1958), *O. stylophora* stromata reach full maturity in May after overwintering. Most of the specimens housed in herbaria were collected in the summer, so only a few collections include fully mature stromata. The specimens we described were collected in spring and with mature ascospores, presumably having overwintered.

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