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# First localities in Poland of the recently described fungus – *Cordyceps bifusispora*

ANNA BUJAKIEWICZ[1], JOANNA NITA[1] and STANISŁAW BAŁAZY [2]

[1]Department of Plant Ecology and Environment Protection,
Adam Mickiewicz University Umultowska 89, PL-61-614 Poznań
[2]Research Centre for Agricultural and Forest Environment, Polish Academy of Sciences,
Bukowska 19, PL-60-809 Poznań

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Two localities of the entomopathogenic fungus *Cordyceps bifusispora,* hitherto not reported from Poland, are characterised by their site conditions and co-occurring macrofungi during the period of the appearance of its stromata. Description of this fungus culture is given and some remarks on the resemblance of its teleomorphs and anamorphs from different collections are discussed.

**Key words:** *Cordyceps bifusispora*, anamorphs, distinctive features, site conditions, macrofungi, riverside forests

## INTRODUCTION

In the genus *Cordyceps* (*Clavicipitaceae*: *Hypocreales*) almost 500 ascomycetous species of entomopathogenic and mycoparasitic fungi have been grupped. Its representatives are characterised by worldwide occurrence though their richest diversity is observed in subtropical and tropical regions. Apart from the characteristic clavate and usually colorful stromata with subapically and apically distributed perithecial clusters (teleomorphs) the majority of species are able to produce different forms of mitosporic (conidial) sporulation (anamorphs), usually of the propagative character. Numerous species hardly produce the typical ascogenous fructification especially in the temperate climatic zone, so they have been often known there as imperfect fungi (*Deuteromycetes*).

Although a relatively great number – about 250 species – of terrestial entomopathogenic fungi have been hitherto known in Poland, the ascomycetous teleomorphs of the order *Hypocreales* are among them very rare. According to Stec-Rouppertowa (1938) eleven species of *Cordyceps* were known from Poland just before the second World War, however she mentioned the names of only five including two non-entomopathogenic ones. In the historical review of Polish research on

insect diseases Lipa (1963) reported five entomopathogenic species belonging to this genus [*C. clavulata* (Schw.) Ell. et Everh., *C. ditmari* Quél., *C. militaris* (L.) Link, *C. myrmecophila* Cesati and *C. tuberculata* (Lebert) Maire. emend Petch], whereas in the Polish mycological bibliography until 1980s (Skirgiełło 1988) only seven papers were listed as containing data about the aforementioned species. In the last two decades two additional representatives of this genus [*C. entomorrhiza* (Dicks.) Fr. and *C. gracilis* (Grev.) Montagne et Durieau (Bałazy 1986; Miętkiewski and Bałazy (2003)] and unripe perithecia of the closely related ascomycete *Torrubiella* sp. (most probably *T. arachnophila* Johnston, *var. lepious* Mains) were found (Bałazy and Cysewski 2003). Surely, this does not bring the list of the Polish species to an end for several reasons, from which the following are the most important. The fungous group under discussion has never been a subject of thorough studies in our country or in Central European areas. So occasionally collected specimens can be found in many herbaria, as well as some reports can be hidden in hard-of-access contributions. Moreover, recent molecular studies based on PCR show genetical identity or very close affinity of particular species of *Hypocreales* with the most terrestrial entomopathogenic hyphomycetes (White et al. 2003; Stensrud, Hywel-Jones and Schumacher 2005). This allows to confirm species identity independently of sporulation type or other micromorphological features.

*Cordyceps bifusispora* was described twenty three years ago by Ove Eriksson (1982), basing on stromata that protrude from the pupa of a noctuid moth, found in the ravine of the rivulet Skravelsjöbäcken, Umeå region in Sweden. This entomogenous species is distinguished by a peculiar spore type. The basal and apical parts of its ascospores are narrowly fusiform, three-septate, and connected by a long filiform segment, without visible articulation. Until now clavicipitalean species of this feature have only been a few times found in Europe and Eastern Asia, (Eriksson 1982, 1988; Su and Wang 1986; Liu, Liang and Liu1996), so they seem to be very rare in natural habitats.

Because of the great potential of entomopathogenic fungi for arthropod population control and integrated pest management as well as their significance for pharmacy and medical biotechnologies, we consider it useful to publish information on the occurrence of the species *C. bifusispora* O. Eriksson in Poland and to describe its anamorphs on the basis of obtained cultures.

## MATERIALS AND METHODS

The species under discussion was found within the more extensive mycocoenological studies on macrofungi of Poland that have been carried on for several years on permanent research plots in different regions of the country. Particular plots of the size 200-400 sq.m each have been localized in alluvial forests of generally uniform site conditions and plant associations. Collections of the fungi and characteristics of their communities are carried out through many years (minimum three years), on permanent plots which are visited a number of times (in closer localities even 10-15 times) yearly with fortnight intervals in the growing season. Many of fungi form fruitbodies or stromata periodically in different seasons and some of them are only randomly found among the litter. For instance *C. bifusispora* found near Kiełpin (Fig. 1) was collected only once after 30 visits on the same plot. Occurrence

of fungous fruiting bodies has been estimated separatedly for particular plant storeys (tree stand, forest floor vegetation and epigeal moss layer including decayed wood of fallen branches and trunks). Collected materials have been preserved as dried herbarium specimens in Adam Mickiewicz University (POZM) in Poznań. A culture of *C. bifusispora* anamorph (POZM 3650) was isolated from the mycelium mass filling a microlepidopteran pupa after previous disinfection of its surface by 90% ethanol. The cultures on Sabouraud dextrose agar (SDA) and the same enriched by egg yolk (SDEYA) have been preserved in Research Centre for Agricultural and Forest Environment of the Polish Academy of Sciences in Poznań (Nr 3780).

## RESULTS AND DISCUSSION

Although the thorough studies on macromycetes in different regions of riverine forests have been carried on for many years, the specimens of *C. bifusispora* were found only twice, on distant localities in Poland (Fig. 1). With regards to morphology and sporulation elements of the stromata Polish specimens of *C. bifusispora* correspond entirely with Eriksson's description and Abrahamsson's (1988) photo (Figs 2, 3) with the exception of some asci which are often longer. The riverine forests throughout Poland shrink their extension and area very quickly as they are taken by agriculture or are victims of water reclamation. Those habitats are however very rich in various forms of fungi, which are rather weakly recognized.

The first post is located in the southernmost part of the country within Outer Western Carpathians (Kondracki 2000), on the left side of the Skawica river valley, near Białka village; the second one is situated in the northernmost part of the Wielkopolska region in the Gwda river valley near Kiełpin village. The habitat of

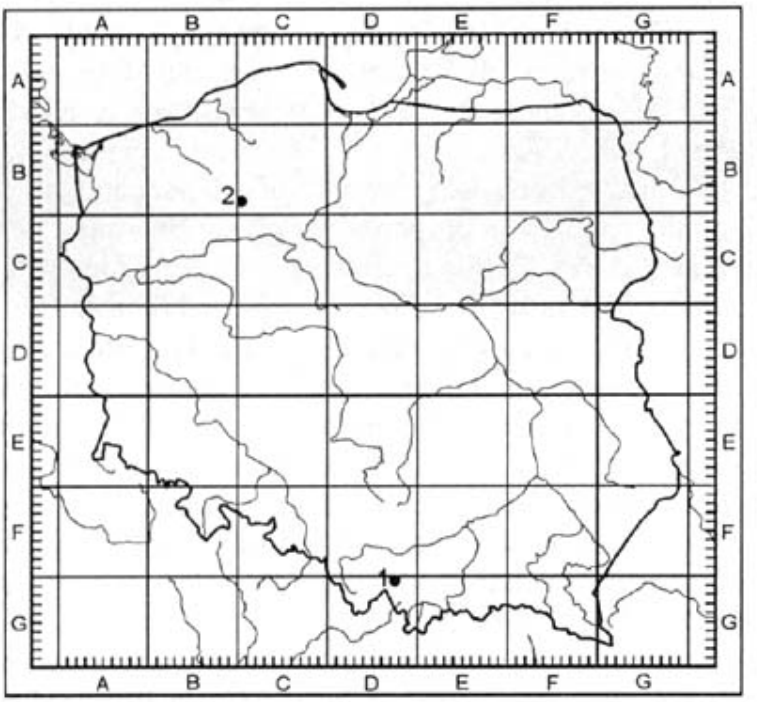


Fig.1. Distribution of *Cordyceps bifusispora* found in Poland. 1 – locality near Białka village; 2 – locality near Kiełpin village.

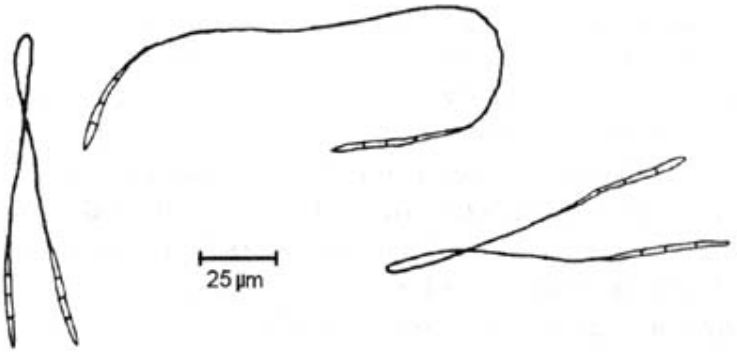


Fig. 3. Spores of *Cordyceps bifusispora* derived from the collection found near Kiełpin village on Sept.3.2005. (Drawn by J. Nita).

the first locality shows the features of *Alnetum incacae* plant community, overgrown by young trees of *Alnus incana, Fraxinus excelsior* and *Salix fragilis,* with *Petasites kablikianus, P. hybridus, Aegopodium podagraria, Chaerophyllum hirsutum, Poa trivialis, Lamium maculatum* and *Stellaria nemorum* in the herb layer and mainly with *Eurhynchium hians* in the moss layer. The stand shows some signs of occasional cutting and rather careless forest management.

Single specimens of *C. bifusispora* were found in this locality twice [on August 31, 1995 (POZM 286) and on September 6, 1995(POZM 320)] on pupae of *Microlepidoptera* in cocoons, as identified by Dr. Edward Baraniak most probably of *Yponomeuta evonymella* L. (*Lepidoptera*: *Yponomeutidae*). In the first term it occurred in association with the following macrofungi growing on the ground: *Cystolepiota seminuda* (Lasch) Bon, *Entoloma pleopodium* (Bull. ex DC.: Fr.) Noordel., *Laccaria tortilis* (Bolt.) Cooke, *Lepiota cristata* (Bolt.: Fr.) P. Kumm., *Melanophyllum haematospermum* (Bull.: Fr.) Kreisel), on litter *Calyptella capula* (Holmsk.: Fr.) Quél., *Cyathus striatus* (Huds.) Willd.: Pers., *Hymenoscyphus caudatus* (P. Karst.) Dennis, *Hemimycena cyphelloides* (P. D. Orton) Maas Geest., *Marasmius torquescens* Quél., *Mycena filopes* (Bull.: Fr.) P. Kumm., *Psathyrella candolleana* (Fr.: Fr.) Maire, *Psilocybe squamosa* (Pers.: Fr.) P. D. Orton v. *thrausta* (S. Schulz.) Guzmán and on wood *Mycena acicula* (Schaeff.) P. Kumm., *M. haematopus* (Pers.: Fr.) P. Kumm., *M. speirea* (Fr.: Fr.) Gillet, *Irpex ochraceus* (Pers.: Fr.) Kotiranta & Saarenoksa and *Trametes versicolor* (L.: Fr.) Pilát.

Within one week period between the first and the second collection of *C. bifusispora* considerable changes in carpophores set of macromycetes took place in the same habitat, as it shows the list mentioned below: on the ground *Agaricus silvaticus* Schaeff., *Agrocybe arvalis* (Fr.) Singer, *Coprinus atramentarius* (Bull.: Fr.) Fr., *C. comatus* (Müll.: Fr.) Pers, *Paxillus rubicundulus* P. D. Orton, *Phallus impudicus* L.: Pers., *Psilocybe rugosoannulata* (Farlow ex Murrill) Noordel.; on litter *Gymnopus confluens* (Pers.: Fr.) Antonín, Halling & Noordel., *Crucibulum laeve* (Huds.) Kambly, *Cyathus striatus* (Huds.) Willd.: Pers., *Marasmius rotula* (Scop.: Fr.) Fr.; on wood *Byssomerulius corium* (Pers.: Fr.) Parmasto, *Daedaleopsis confragosa* (Bolt.: Fr.) J. Schröt, *Psilocybe capnoides* (Fr.: Fr.) Noordel., *Mycena galericulata* (Scop.: Fr.) Gray, *Pluteus atricapillus* (Batsch) Fayod, *Schizophyllum commune* Fr.: Fr. and *Stereum hirsutum* (Willd.: Fr.) Gray.

The second post of *C. bifusispora* was situated in the association *Querco-Ulmetum minoris chrysosplenietosum,* at the outskirts of the Gwda River valley, near Kiełpin village, after Kondracki (2000) Western Pomerania region, Krajeńskie Lake. Stromata of the fungus protrude from a similar microlepidopteran pupa, found on litter

Fig. 2. Stromata of *Cordyceps bifusispora* found in the *Querco-Ulmetum minoris* association near Kiełpin village on Sept.3.2005. (POZM 3650). Photo J. Nita.

Fig. 4. Ramose stromata of Chinese specimen of *Cordyceps bifusispora* (Courtesy of Prof. Dr. Zengzhi Li).

on September 3, 2005 (POZM 3650). The habitat was overgrown by *Alnus glutinosa, Padus avium, Corylus avellana* in the tree layer, by *Aegopodium podagraria, Ficaria verna, Galeobdolon luteum, Ranunculus lanuginosus* and *Stellaria nemorum* in the herb layer and by *Eurynchium hians* and *Mnium undulatum* in the moss layer. Stromata of *C. bifusispora* accompanied the following community of macrofungi: *Laccaria laccata* (Scop.: Fr.) Berk. & Broome, *Laccaria amethystina* (Bull.) Murrill, *Lactarius obscuratus* (Lasch.: Fr.) Fr., *Lactarius pyrogalus* (Bull.: Fr.) Fr. and *Mycena pura* (Pers.: Fr.) P. Kumm. on the ground, *Mycena tenella* (Fr.) Quél., *M. vitilis* (Fr.) Quél. on the litter and *Bjerkandera adusta* (Willd.: Fr.) P. Karst., *Calocera cornea* (Batsch.: Fr.) Fr., *Hymenoscyphus imberbis* (Bull.: Fr.) Dennis, *Hyphodontia paradoxa* (Schrad.: Fr.) E. Langer & Vesterholt ss. lato, *Inonotus radiatus* (Sowerby: Fr.) P. Karst., *Mycena acicula* (Schaeff.) P. Kumm., *Mycena galericulata* (Scop.: Fr.) Gray, *M. haematopus* (Pers.: Fr.) P. Kumm., *M. speirea* (Fr.: Fr.) Gillet, *Oligoporus alni* (Niemelä & Vampola) M. Piątek, *Oligoporus stypticus* (Pers.: Fr.) Gibertson & Ryvarden, *Orbilia delicatula* (P. Karst) P. Karst., *Pholiota mutabilis* (Scop.: Fr.) P. Kumm., *Stereum hirsutum* (Willd.: Fr.) Gray and *Stereum rugosum* (Pers.: Fr.) Fr. on the wood.

Hitherto accessible notes on the occurrence of *C. bifusispora* originate from Sweden (Eriksson 1982) and Quianlin Mts in the People Republic of China (Liu et at. 1996). Stromata of the fungus collected by the authors in the latter region were on some insects unbranched, but on the others – after illustrations greater – irregularly branched (Fig. 4). The cultures of the fungus obtained from the ascospores of unbranched stromata produced conidial sporulation characteristic for the form-genus *Septofusidium* W. Gams (1971), and described as the new form-species *S. bifusisporum* Liu et al. (1996). Similar arrangement of conidiogenous cells in cultures has been presented as *Acremonium*-anamorph by Su and Wang (1986) at the original description of very close species *Cordyceps ninchukispora* that, however, produced only unicellular or 1-septate conidia.

The micro-morphology of the Polish cultures, isolated from the internal mycelium filling a pupa with three single and one furcate but underdeveloped stromata (Fig. 2) is in no essential detail comparable with the Chinese culture characteristics. The fungus develops well on SDA and SDEYA media, producing moderately intensive conidial sporulation of *Paecilomyces* type. Two-week-old cultures can be briefly characterized as follows. Mycelium on SDA abundant, cottony, fast growing, about 40 mm of diameter and up to 8 mm high in the center, of intensively orange color apart from about 2 mm white marginal zone. Reverse intensively orange in central part, steadily more and more diluted towards the margin. Aerial hyphae filamentous (0.8) 1-2.5 (-3.0) μm thick, scarcely branched, light golden-yellow to light orange, without differentiated conidiophores. Sporogenous structures appear mostly in the terminal parts of hyphae and their branches as one- two- or three levels of losely arranged prophialides 5-7 (10) μm long and 2-2.5 (-3) μm thick, each terminated with more compact bundle of 3-6 phialides 5-12 μm long and 2-2.5 μm thick; the longer of them (10-12 μm) are steadily attenuated upwards whereas the shorter are rather abruptly constricted into short necks. Single prophialides protrude often laterally from hyphae and they are usually longer - up to about 20 μm, giving divaricate terminal clusters of more plump phialides 5-6 x 3-3.3 μm. Sparse longer fusiform phialides 12-16 x 2.5-3 μm also protrude individually in some points of aerial hyphae. Phialospores spherical, subglobose or short-ovoid, 2-3 (-3.5) x 1.8-2.5 μm, most frequently of the diameter 2.2 μm, in catenulate arrangement.

The microscopical picture of the sporulation type is very close to that of *Paecilomyces farinosus* (Holm.: S. F. Gray) Brown et Smith. The essential differences are: generally globose and subglobose conidia, intensive orange color of aerial mycelium and much less regularly penicillate aggregations of conidiogenous structures.

As comparing published descriptions of the Swedish and Chinese specimens (Eriksson 1982, Liu et al. 1996) the latter have perithecia up to about 100 $\mu$m longer and both asci and ascospores greater. According to presented drawings of ascospores the ends of their thickened fusiform parts are sharply apiculate in Chinese material, whereas steadily attenuating towards subacute tip in the Swedish. Moreover, the colors of stromata in both collection differ very significantly. In Chinese specimen of richly branched stromata they are of red-vinaceous color (color file sent kindly by Professor Zengzhi Li), whereas in the Swedish (Abrahamsson 1988) as well as in Polish ones here discussed - whitish-cream to light yellow-brownish in basal unfertile part to dilute yellow-orange clava with deep orange perithecial necks. Moreover, the perithecia in Chinese specimens are much more densely arranged on the fertile part of stromata and not so deeply submerged than in the Swedish and Polish ones. These differences – although some of them rather fine – should be taken into account at species identification, because there have been numerous cases of uncertainty, vagueness or errors in *Cordyceps* and allied species taxonomy resulting from incomplete descriptions.

On the basis of the above, rather scarce, collections and published data the following conclusions can be expressed:

Three "bifusisporous" forms of *Cordyceps* can be considered for the present, i.e. Swedish and Polish ones as the typical *C. bifusispora*, Chinese *Phytocordyceps ninchukispora* Su et Wang (1986) from Taiwan - growing in seeds of the tree *Beilschmiedia erythroploia* Hay, and entomopathogenic of red, branched stromata from Quianlin Mts. identified as *C. bifusispora*. More detailed characteristics of their ascogenous and mitosporic sporulations could be helpful for confirmation of their separate positions.

Peculiar form of ascospores justify inclusion of these fungi into the separate subgenus *Bolacordyceps* – according to Eriksson and Hawksworth (1986) proposal.

Species of this group seem to be very rare. Polish collections of *C. bifusispora* suggest its preference to fertile sites of wet floodplain forests, where it was found as accessory element in late summer communities of macrofungous fruit-bodies. Though such habitats favour rich biodiversity and often unique associations of organisms, they are highly vulnerable to side effects of land use changes, hydrotechnological reclamations and pollution. For these reasons they require special care within the national and international systems of nature protection in order to preserve rare and ecologically sensitive biota.

It would be desirable to more precisely elucidate trophic relations within this group of pathogens, in particular their linkage with plant-seeds consumers. In the latter cases particularly important is to check if there are no residues of arthropod sclerites within seeds overgrown by mycelia, because the cases of entomopathogenic hyphomycetes development on internal seed pests are pretty common in nature.

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## Pierwsze stanowiska w Polsce nowo opisanego maczużnika – *Cordyceps bifusispora*

Streszczenie

W artykule scharakteryzowano dwa stanowiska entomopatogenicznego workowca *Cordyceps bifusispora*, dotychczas nie notowanego w Polsce, opisano warunki siedliskowe fitocenoz oraz współwystępujące gatunki macromycetes. Zamieszczono również opis kultur omawianego gatunku oraz uwagi i dyskusję dotyczącą podobieństw jego teleomorf i anamorf pochodzących z różnych kolekcji.