

Notes on the taxonomy of *Cordyceps longisegmentis* based on collections from the Czech Republic

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Fresh and herbarium material of the rare species *Cordyceps longisegmentis* (Ascomycetes, Clavicipitaceae) from the Czech Republic is thoroughly described and discussed. Fresh stromata were found in relict pine woods in the Bohemian Forest. Revision of the herbarium material from PRM showed that most collections labelled as *Cordyceps capitata* are in fact *Cordyceps longisegmentis*. In the collections studied the length of ascospore parts was constantly smaller than in the North American material. Other European mycologists have also observed this fact. Consequently, the European and American populations could be slightly different. Differences between the related species *C. longisegmentis* and *C. capitata* are discussed.

Key words: fungi, Ascomycetes, *Cordyceps*, taxonomy, ecology, distribution, Bohemian Forest

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V článku je podrobně popsán a diskutován vzácný druh *Cordyceps longisegmentis* (Ascomycetes, Clavicipitaceae). Popis je založen na čerstvém materiálu sbíraném v reliktních borech na Šumavě a je doplněn o některá pozorování získaná studiem sušených plodnic z herbáře mykologického oddělení Národního muzea. Revize herbářového materiálu ukázala, že téměř všechny starší položky původně určené jako *Cordyceps capitata* představují druh *C. longisegmentis*. U všech studovaných položek byla délka částí askospor menší než je udáváno v pracích založených na studiu materiálu ze Severní Ameriky. Totéž pozorovali i jiní evropští mykologové. Mohlo by to znamenat, že evropské a severoamerické populace tohoto druhu se poněkud liší. V článku jsou také probírány rozdíly mezi *C. longisegmentis* a příbuzným druhem *C. capitata*.

INTRODUCTION

Since 1992 the first author has carried out an intensive mycofloristic investigation of the Czech part of the Bohemian Forest (see e.g. Holec 1997a, 1997b, 1998, 1999, 2000, 2001a, 2001b; Holec & Pouzar 1999). This important mountain range is situated on the border between the Czech Republic on the one side and Germany and Austria on the other side. The local name of the Czech part is "Šumava". The most valuable area is protected as the "Šumava National Park"; on the German side as the "Nationalpark Bayerischer Wald". The mycoflora of

the German national park has been thoroughly described by Luschka (1993). Records from the Czech part are published in numerous smaller contributions of various authors (e.g. Hilitzer, Kavina, Velenovský, Pilát, Herink, Kubička, Svrček, Kotlaba, Pouzar, Holubová-Jechová, Réblová etc.). Short and incomplete reviews of these works were published by Svrček (1965), Váňa (1996) and Holec (1997a, 2000). Many rare, interesting or even new species of fungi are found in the Bohemian Forest every year. Finds of the rare species *Cordyceps longisegmentis* are discussed here. The results are completed by a study of herbarium specimens of *C. longisegmentis* from various regions of the Czech Republic. The ecology and distribution of *Cordyceps* species in the Bohemian Forest has been thoroughly described in a previous paper (Holec 2001b).

MATERIAL AND METHODS

Macrocharacters were observed on fresh stromata. Microcharacters were studied using hand-made sections of dried material. Sections were observed in various media: pure water, 5 % KOH solution, Melzer's reagent, and a 0.5 % solution of Toluidine Blue. Abbreviations: diam.: diameter, PRM: mycological herbarium of the National Museum, Prague.

RESULTS

Cordyceps longisegmentis Ginns, Mycologia 80(2): 219, 1988

Macrocharacters (according to fresh stromata found in the Bohemian Forest: PRM 897286, 897871): Stromata solitary or growing in a small group, 5–12 cm high, divided into a long stipitate part and an upper fertile part. Fertile part nearly globose, 0.7–1.0 cm diam., sharply separated from the stipe, viscid when fresh, lustrous, minutely verrucose by the protruding ostioles of the perithecia, upper part black-brown, lower part ochre-brown. Stipe 4–11 × 0.5–0.7 cm, cylindric, longitudinally striate, bright green-yellow, minutely grey-blackish granulose in upper part, basal part directly attached to fruitbody of *Elaphomyces* sp., no mycelial cords or fibrils observed.

Microcharacters (studied in well-preserved dried material from the Bohemian Forest: PRM 897286, 897871; size of ascospore parts is based on measurements of all collections studied, see below): Cortical layer of the fertile part about 20–30 µm thick, pale olive-greenish, a distinct palisade of parallel to slightly flexuose septate hyphae 2.0–3.5 µm diam., in upper part embedded in a pale greenish gelatinous substance visible in Melzer's reagent but not in KOH, the substance stains slightly purplish in Toluidine Blue (metachromatic reaction). Tissue between this layer and

perithecia olive-brown, a textura epidermoidea, cells 2.5–8.0 μm diam., with wall thickened up to 0.5 μm , olive-brown. Perithecia embedded, ellipsoid with ostiolum, 450–550 \times 280–320 μm (without ostiolum). Perithecium wall 20–33 μm thick, outer layer pale beige-brown to brown, of elongated to nearly isodiametric cells with refractive wall up to 1 μm thick, size 3–4.5 \times 2.5–3 μm , inner layer pale ochre to pale beige-brown, of elongated thick-walled cells, size 6.5–16 \times 1.5–2.5 μm . Periphyses cylindrical, hyaline, smooth, apical part clavate, up to 3 μm broad, basal part septate. Asci cylindrical, slightly attenuated in basal and apical part, 350–430 \times 10–14.5 μm , thin-walled, not amyloid, with distinct ascoapical apparatus visible as two hyaline bodies separated by a narrow channel. Paraphyses not found. Ascospores not colouring in Melzer's reagent, filiform, narrow, multiseptate, breaking into parts when mature, parts (14.5–)25–40(–43) \times (2.5–)3.5–4.8(–5.2) μm (the smaller values relate to terminal parts, which are distinctly smaller than the medium ones), fusiform-cylindrical with slightly attenuated and truncate apices, wall 0.8–1.2 μm thick, thickened up to 2.5–5 μm at apices, content with many droplets when young, homogeneous when old. Stroma interior made up of cylindrical but flexuose and slightly interwoven hyphae 2.5–7.0 μm diam., hyaline, thin-walled. Cortical layer of the stipe about 300–400 μm thick, olive-brown, a textura porrecta of parallel cylindrical hyphae 3–7 μm diam., frequently septate, wall about 0.5 μm thick, green-brown, hyphae in the uppermost layer with olive-brown incrustations, with nests of interwoven hyphae forming a granulate covering on the stipe surface. Stipe interior a textura intricata of loosely arranged interwoven hyphae 1.5–5 μm broad, septate, hyaline, wall about 0.5 μm thick.

Collections studied: Czech Republic: Bohemian Forest, "Povydrí" protected area, W of Vydra river, stony NNE slope under "Mnich" rock (about 2.3 km SEE of Srní village), altitude 850 m, relict pine wood composed of *Pinus sylvestris* and *Picea abies*, sporadically with *Betula*, with undergrowth of *Sphagnum*, other mosses, and *Vaccinium myrtillus*; growing on *Elaphomyces* sp. among mosses, 19 Sept. 1998, leg. et det. J. Holec, PRM 897286 (JH 568/1998). – Bohemian Forest, "Povydrí" protected area, E of Vydra river, 1.9 km NNE of Srní village, relict pine wood composed of *Pinus sylvestris*, *Betula*, sporadically with *Picea abies*, on stony ground, stones covered with mosses, W slope; obviously on *Elaphomyces* sp. (but fruitbody not found) among mosses covering a big stone, 9 Oct. 2000, leg. et det. J. Holec, PRM 897871 (JH 203/2000). – Bohemia, Čáslav, 1854, leg. Veselsky, det. as *C. capitata* (PRM 169290). – Eastern Bohemia, Kačina, 1844, leg. Peyl, det. as *C. capitata* (PRM 169284, 169285). – Bohemia, Sušice ("Schuschitz", probably a village in eastern Bohemia near Chrudim), leg. Peyl, det. as *C. capitata* (PRM 169286). – Moravia, Žarošice, mixed broadleaved forest dominated by *Quercus*, on *Elaphomyces*, Sept. 1940, leg. V. Vacek, det. as *C. capitata* (PRM 169289).

***Cordyceps capitata* (Holmsk.: Fr.) Link, Handb. 3: 347, 1833**

= *Cordyceps canadensis* Ellis et Everh., Bull. Torrey Bot. Club 25: 501, 1898.

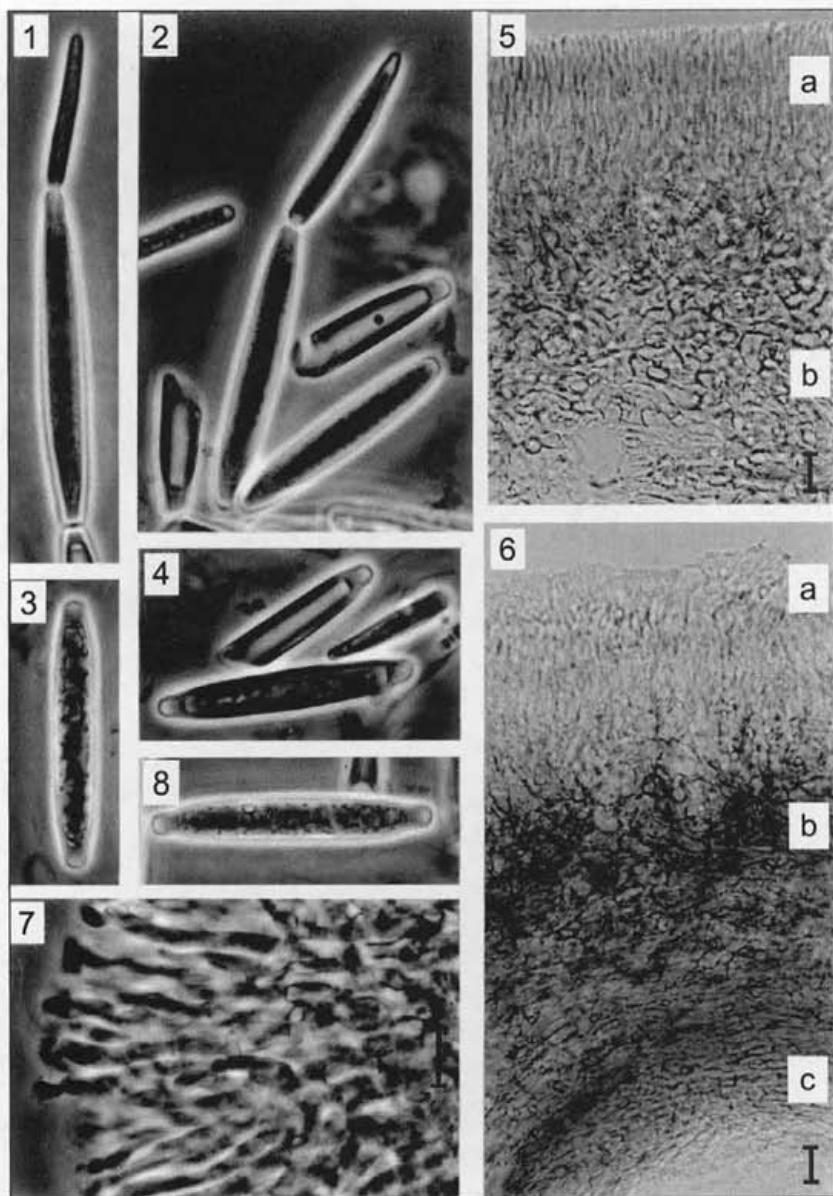
Short description (according to fresh stromata, see below): Stromata growing in a group but not fasciculate, divided into a long stipitate part and an upper fertile part. Fertile part nearly globose, about 1 cm broad, sharply separated from the stipe, finely verruculose from the protruding ostioles of the perithecia, dark brown with yellowish colour shining through. Stipe up to 18 cm long (in spite of this length no fruitbodies of *Elaphomyces* were observed), 0.3 cm thick, cylindrical, longitudinally striate, above-ground part 8 cm long, greyish-yellowish, floccose, underground part 10 cm long, white.

Diagnostic microcharacters: Cortical layer of the fertile part made up of densely interwoven hyphae showing a pseudoparenchymatous structure in section. Ascospores filiform, at maturity breaking into several parts, size of the parts (15-)17-20(-23) × 2.5-3.0(-3.5) μm, shape cylindrical or slightly fusiform-cylindrical, thin-walled, with truncate apices when mature.

Collection studied: Czech Republic: Bohemian Forest, 3 km N of site called Březník near Modrava village (distr. Klatovy), "Cikánská slat" peat bog, eastern part between the Modravský potok stream and the central part of the peat bog, altitude 1070 m, humid to waterlogged spruce forest with undergrowth of *Vaccinium myrtillus* and *Sphagnum* (forest with a natural character); obviously on *Elaphomyces* (its fruitbodies not found) among *Sphagnum*, 9 Oct. 1998, leg. et det. J. Holec, PRM 897594 (JH 926/1998).

Habitats of *C. longisegmentis* with special emphasis on the Bohemian Forest

Most collections of *Cordyceps longisegmentis* held at PRM are from the 19th century. No data on the habitats are written on their labels. The only collection provided with these data is from Moravia (Žarošice, leg. V. Vacek, PRM 169289). The species was found in a mixed broadleaved forest dominated by *Quercus* at an altitude of about 400 m. The two recent finds of *Cordyceps longisegmentis* from the Bohemian Forest are from relict pine woods of the association *Betulo carpaticae-Pinetum* Mikyška 1970. This forest type is typical by its occurrence on rocks, boulder slopes or screes. The tree layer is composed of *Pinus sylvestris*, *Picea abies* and *Betula* (mainly *B. carpatica*). The soil surface is densely covered with mosses (often *Sphagnum*), lichens and *Vaccinium myrtillus*. The geological background is made by granite. The vegetation is natural, minimally influenced by man and included in the first (strictly protected) zone of the "Šumava National Park". This fact also refers to the habitat of *Cordyceps capitata* in the "Cikánská slat" peat bog. The species was found in a humid to waterlogged montane spruce forest with undergrowth of *Vaccinium myrtillus* and *Sphagnum*. The only find



Figs. 1, 2, 3, 4, 8: Ascospore parts observed in phase contrast. Terminal parts are short and narrow. The wall of mature parts is distinctly thickened at apices. Fig. 5: Longitudinal section of the fertile part of the stroma mounted in KOH, a: cortical layer (palisade of narrow hyphae), b: tissue between cortical layer and perithecia (textura epidermoidea). Fig. 6: Longitudinal section of the fertile part of the stroma mounted in Melzer's reagent, a: cortical layer, hyphal ends are embedded in a gelatinous substance, b: see above, c: perithecial wall. Fig. 7: Cortical layer of the fertile part of the stroma; the hyphae form a palisade-like structure. Scale bar = 10 μ m.



Fig. 9. Fresh stromata of *Cordyceps longisegmentis* in the field (Bohemian Forest, "Povydří" protected area, PRM 897286, for details see collections studied).



Fig. 10. Fresh stromata of *Cordyceps longisegmentis* with their substrate - *Elaphomyces* sp. (for explanations see Fig. 9).

of *C. capitata* from the German part of the Bohemian Forest (Luschka 1993) is from an almost identical habitat (waterlogged spruce forest of the association *Soldanello-Piceetum bazzanietosum* at an altitude of 755 m). In the Bohemian Forest, both *Cordyceps* species seem to be confined to relics of natural vegetation.

DISCUSSION

Cordyceps longisegmentis is readily recognised by its long and broad ascospore parts (longer than 30 μm , 4–5 μm broad, see Ginns 1988) with truncate, thick-walled apices and narrowly fusiform shape. In the related and similarly looking species *Cordyceps capitata*, the ascospore parts are thin-walled, cylindrical and usually measure 12–27 \times 2–3.5 μm (Maas Geesteranus 1963, Ginns 1988). Moreover, the cortical layer of the stroma has a palisade-like structure in *C. longisegmentis*, whereas in *C. capitata* the layer consists of interwoven hyphae pseudoparenchymatous in section (Mains 1957, Maas Geesteranus 1963, Ginns 1988).

Until 2000, *Cordyceps longisegmentis* has not been reported from the Czech Republic. However, the species was collected here in the past (as *C. capitata*, see collections studied). The recent finds described here are the first ones for the Bohemian Forest (see also Holec 2001b).

In Europe, *Cordyceps longisegmentis* was recognised by Maas Geesteranus (1963), but the finds were published under the name *Cordyceps canadensis* Ellis et Everh. Maas Geesteranus applied the conclusions of Mains (1957), who clearly described differences between the two taxa in the *C. capitata* group using material from North America. Mains used the name *Cordyceps canadensis* for the species with greater ascospore parts. However, a review of the original descriptions of *C. capitata* and *C. canadensis* and a detailed type study of *C. canadensis* showed (Ginns 1988) that the two taxa are conspecific. Thus, the name *C. canadensis* is a synonym of *Cordyceps capitata* (Holmsk.: Fr.) Link. As no older name was available for the species with greater ascospore parts, Ginns (1988: 219) described it as a new species under the name *Cordyceps longisegmentis*. Consequently, older European finds published as *Cordyceps canadensis* (Maas Geesteranus 1963, Dennis 1981, Phillips 1981) should be reported as *Cordyceps longisegmentis*. The correct name was used e.g. by Arnolds et al. (1995) and Hansen & Knudsen (2000).

It is worth mentioning that in collections of *Cordyceps longisegmentis* from the Czech Republic the length of ascospore parts is only (14.5-)25–40(-43) μm . Similarly, Maas Geesteranus (1963) reports (19.7-)22.5–49.5(-62.0) μm for collections from the Netherlands (given as *C. canadensis*) and Hansen & Knudsen (2000) 30–47 μm for material from the Nordic countries. On the other hand, Ginns (1988) gives (12-)40–65 μm for material from Canada and the United States. The comparison shows that the length of ascospore parts is somewhat different in

European and North American collections. Except for the length, other characters remain identical. Consequently, the European and North American populations seem to be conspecific at the present state of knowledge. It is possible, however, that the European populations distinguished by shorter ascospore parts slightly differ from the American ones. An isoenzyme or DNA study would be desirable to evaluate the difference.

The related species *Cordyceps capitata* has also been found in the Czech part of the Bohemian Forest (see results). Its find (PRM 897594) perfectly fits the descriptions in the literature (Mains 1957, Maas Geesteranus 1963, Dennis 1981, Breitenbach & Kränzlin 1981, Kobler 1984). The species is macroscopically indistinguishable from *C. longisegmentis*. However, microcharacters summarised in the first paragraph of the discussion enable its reliable identification.

Kobayasi & Shimizu (1960) observed that the fertile parts of the stromata of *Cordyceps longisegmentis* (named *C. capitata* in their paper) were "glossy", whereas those of *C. capitata* lacked the glossiness. Ginns (1988) confirmed this observation stating "the specimens of *C. longisegmentis* I examined at 10 × were glossy, whereas those of *C. capitata* were dull". We also observed a lustrous surface on fresh fertile parts of the stromata of *C. longisegmentis*. Unfortunately, the character of the fresh surface was not noticed in our find of *C. capitata*. Under a stereomicroscope at a magnification of 10–20 times, no difference in character of the surface was seen on dried material of both taxa from the Bohemian Forest.

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