

Supplementary notes to the biology
of *Cordyceps entomorrhiza* (Dicks. ex Fr.)
Link and the morphology
of its conidial stages

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Balazy S.: *Supplementary notes to the biology of Cordyceps entomorrhiza (Dicks. ex Fr.) Link and the morphology of its conidial stages*, Acta Mycol. 18(2):231-238, 1982(1986).

Cordyceps entomorrhiza develops on the larvae, pupae and adults on the ground beetles *Carabus* spp. (Coleoptera, Carabidae) and on the bugs (Heteroptera, Nabidae) of the genera *Himacerus* Wolff et *Nabis* Latr. There are two forms of the conidial sporulation of this fungus with an identical sporogenesis type (*Phialosporae*) but different as to the arrangement of sporogenous cells (*Tilachlidiopsis* and *Hymenostilbe*-like) The names *Tilachlidiopsis hippotrichoides* (Lindau) Keisler and *T. nigra* Yakushiji et Kumazawa should be treated as synonymous; they refer to the conidial stage of *C. entomorrhiza*. *Hirsutella eleutheratorum* (Nees ex Fr.) Petch — contrary to the hitherto existing notion — is not connected with the above mentioned fungus. It constitutes a separate species, close to or identical with *H. entomophila* Pat.

INTRODUCTION

Morphology of the perithecial stage of *Cordyceps entomorrhiza* (Dicks. ex Fr.) Link and its synonymy were comprehensively elaborated by Mains (1951a, 1958). However, despite extensive references in literature much controversy and vagueness is connected with conidial stages

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of this fungus. It is the purpose of this publication to set in order the state of knowledge of this problem and to present additional data on the biology of the species on the basis of the author's own observations.

MATERIALS AND METHODS

In the year 1977 there was discovered in the area of a reserve deciduous forest *Galio silvatici-Carpinetum* and *Calamagrostio-Quercetum* in the Wielkopolski National Park a locality with the abundant specimens of *C. entomorrhiza* appearing regularly in the following years. After a preliminary observations regular quantitative investigations on test surfaces 3×3 m and observations on the development of the fungus in natural conditions were begun. The morphology of the fungus was investigated with the application of the common microscopic techniques. For the comparative studies the material concerning the fungus genus *Tilachlidiopsis* Keissler (1924) was borrowed from the Naturhistorisches Museum in Vienna. The results were collated with the opinions and remarks of some authors (Petch 1933, 1937; Kobayasi 1937, 1941; Mains 1951; Koval 1974). At the same time attempts at isolation of the fungus as a pure culture were carried out. Standard organic and mineral media were initially applied but without success. Latter some special substrate used for the cultivation of the *Entomophthoraceae* or different rich mixtures were applied.

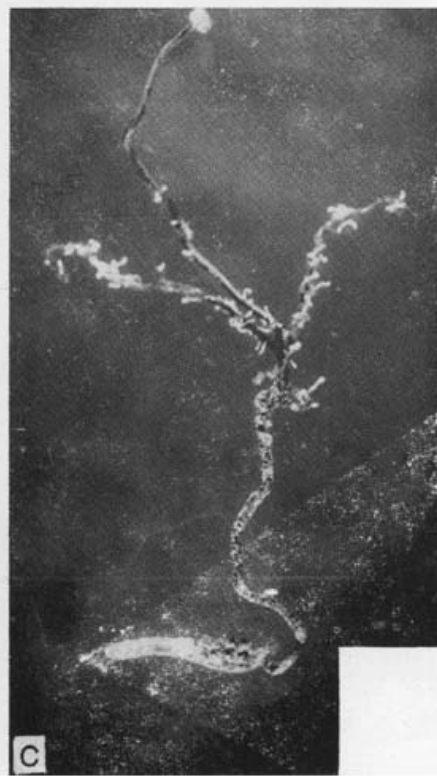
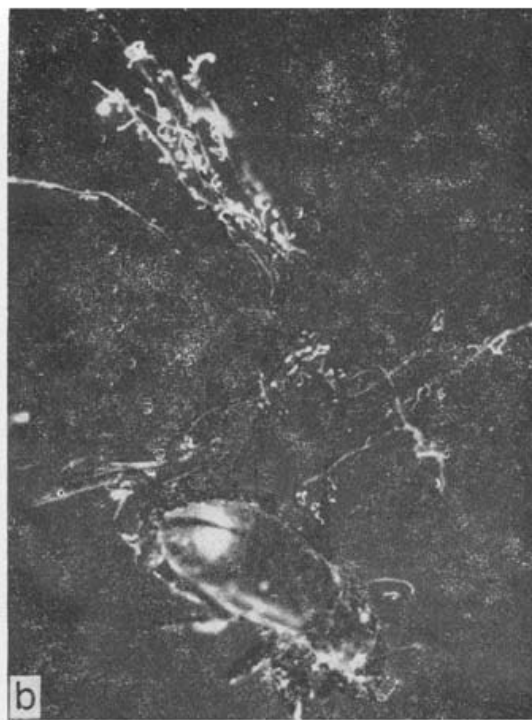
The majority of the materials collected in the Wielkopolski National Park have been deposited in the mycol. coll. of the Forest Res. Lab. in Poznań (Poland), but some selected well developed specimens representing a full spectrum of the morphological features were sent to the collections of the American Type Culture Collection, Rockville, Maryland, USA, British Mycological Society, Kew, Great Britain, Centraalbureau voor Schimmelcultures, Baarn, Netherlands, Naturhistorisches Museum in Vienna, Austria, and to some other institutes.

RESULTS AND DISCUSSION

Morphology and host-species. The stromata of the fungus grew singly, seldom in twos (Fig. 1), from the internal pseudo-

Fig. 1. *Cordyceps entomorrhiza*

a — a well developed specimen in the natural condition (on a ground beetle larva), *b* — the conidial stage of the *Tilachlidiopsis*-form on *Carabus nemoralis* (Müll.) from the forest litter (enlarged), *c* — the stromata with three types of sporulation on the *Carabus* larvae, (*a*, *c* — comprable size)



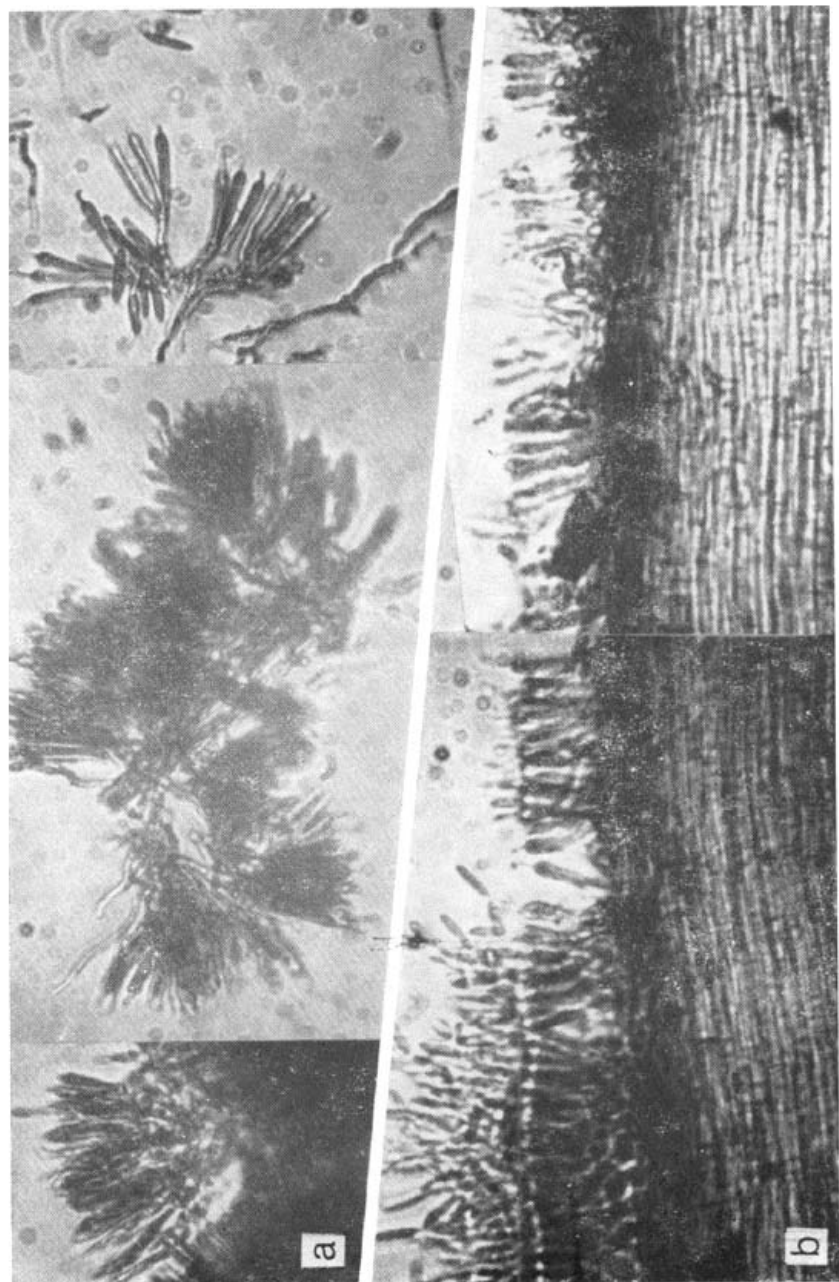


Fig. 2. Conidial stage of *C. entomorrhiza*:

a — brush-like arrangement of the phialides *Tilochlidiopsis hippotrichoides*, b — *Hymenostilbe*-like arrangement of the phialides

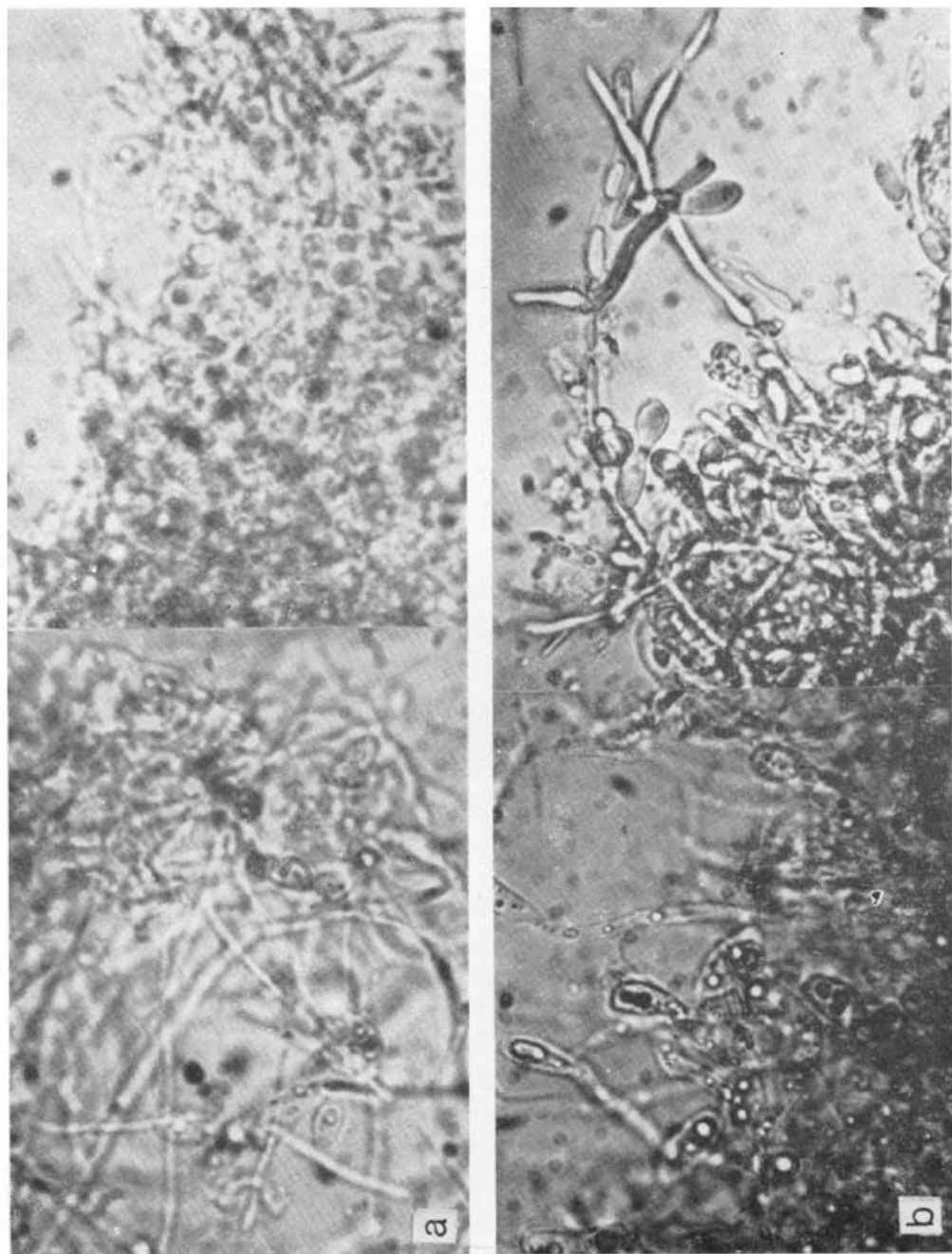


Fig. 3. A comparison of the internal mycelium taken from the hosts while the conidial sporulations were well developed (the sections soaked for 10 days in 5% KOH) — in the same magnification:
a — *Cordyceps entomorrhiza*, b — *Hirsutella* sp. from *Pterostichus vulgaris* L.

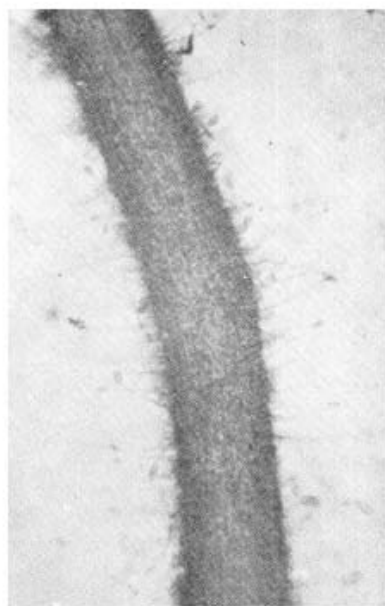


Fig. 4. A part of the synnema of *Hirsutella* sp. from *Pterostichus vulgaris* L.

sclerotia in dead larvae, pupae and imaginal ground beetles of the genus *Carabus* L. occurring in the superficial layer of the soil at the depth 3-15 cm. In the fresh state they were 5-18 cm long and 1-2 mm thick, dark brown sometimes with an indistinct violet shadow, along the upper part below the clava covered with white or light grey mealy coating consisting of the phialides and conidia of the *Hymenostilbe*-like (Mains 1951a) arrangement (Fig. 2). Independently, numerous white or cream, short coremia typical for the genus *Tilachlidiopsis* grew singly or in whorls along overground portions of the stromata. The clavae were verrucose, semispherical or irregularly flattened, of light grey lilac colour when fresh. The morphology of the ascosporic fructification was fully conformable to the descriptions by Mains (1951a, 1958). In cases when dead insects occurred in the forest litter on the surface of the soil the mycelium grew as numerous bundles (Fig. 1b), from among those the thickest resembled the typical stromata. However, they were strongly branched in the way characteristic for the species known under the name *Tilachlidiopsis nigra*. No ascosporic fructification was observed on such specimens.

Both conidial sporulation forms occurred commoly and constantly on sterile, ripening and ripe stromata of the fungus. The microscopic analysis of the mycelium did not confirm Petch's (1939) suggestion as to a parasitic character of the form-species *T. nigra* on the ascomycete. The mycelium forming pseudosclerotia was also identical in specimens with good development of both conidial and ascosporic stages and those of the conidial form only. Thus, there is no room for doubt that the conidial stage of *C. entomorrhiza* is *T. nigra*, which was very cautiously suggested by Kobayasi (1937). This author, however, in his later monograph (Kobayasi 1941) quoted after Petch (1933) *Stilbella setiformis* (Vahl) Petch and *Hirsutella eleutheratorum* (Nees ex Fr.) Petch as the conidial forms of this ascomycete.

As a matter of fact the second form of the conidial sporulation of *C. entomorrhiza* — mentioned by Mains (1951a) as resembling the genus *Hymenostilbe* — is identical with *T. nigra* as well in respect to the sporogenesis type as the morphology and dimensions of the phialides and conidia; the only difference is the arrangement of the phialides — in *Tilachlidiopsis* terminally brush-like and laterally perpendicular on the subterminal parts of the stromata (Fig. 2). None of these forms correspond to the species of the genus *Hirsutella* and the description of the species *H. eleutheratorum* quoted by Koval (1974) after the Mains' (1951a) data concerning the *Hymenostilbe*-like stage cannot be treated as correct. A more detailed analysis of the morphology and systematic position of the fungus reported as *Isaria eleuthe-*

ratorum Nees was given by Petch (1932). He stated that this name was applied for several undoubtedly different entomogenous species having synnematosus aerial mycelium and occurring on beetles. On the basis of two available collections he redescribed the species and transferred it to the genus *Hirsutella*. Moreover, he considered *Isaria guignardii* Macheu reported from the staphylinid beetle *Quedius mesomelinus* Marsh. to be conspecific. Pacioni (1980) confirmed an earlier Mains' (1951b) supposition about the identity of *H. eleutheratorum* and *H. entomophila*, however, he treated *I. guignardii* as a separate species and transferred in to the new monotypic genus *Troglobiomyces* Pacioni, close to *Hirsutella*. During the investigations in the Wielkopolski National Park in the same locality there were found two dead adult specimens of the ground beetle *Pterostichus vulgaris* L. on which the mycelium showing the characteristics typical for both mentioned above species of *Hirsutella* (Fig. 4) developed in damp chambers. The morphology of the mycelium filling body cavities of these beetles was distinctly different from that forming pseudosclerotia of *C. entomorrhiza* (Fig. 3). Although at present it lacks any good monograph or revisional treatment of the criteria making possible sure identification of all species described under the name *Hirsutella* there should not be any doubt that neither *H. eleutheratorum* nor any other species of this genus is connected in its development with the ascomycetous species *C. entomorrhiza*. There is also no basis for the treatment of the name *Stilbella setiformis* as a synonym of *H. eleutheratorum* — as it was done by Koval (1974).

In the four years of investigations in the same locality a number of dead specimens of *Himacerus apterus* (Fabr.) (*Heteroptera*, *Nabidae*) with the *Tilachlidiopsis*-like synnemata and conidial sporulation were simultaneously found. They were never observed in soil but only on the surface, in forest litter. The micromorphology of this fungus does not differ from that of the conidial *Tilachlidiopsis*-form of *C. entomorrhiza*. Keissler (1914) treated the fungus from *Nabis fesus* (L.) as a separate species *T. hippotrichoides* (Lindau) Keissler. Lindau (1908) was unaware of an entomogenous character of this fungus and described the species *Clavularia hippotrichoides* (basionym) on the assumption that it develops on plant roots. It seems to be more probable that his fungus occurred rather on a ground beetle larva in the soil than on a bug in the litter. Undoubtedly *T. hippotrichoides* and *T. nigra* are identical and thus the first name should be applied for the conidial stage of *C. entomorrhiza*. Dr. R. A. Samson (personal communication) who examined the type specimen of the genus *Tilachlidiopsis* (*T. racemosa* Keissler, 1924) stated, however, that it showed arthrosporic sporu-

lation resembling anamorphs of some *Basidiomycetes*. After his opinion the phialidic species described under this generic name should be rather transferred into *Polycephalomyces* Kobayasi, 1941.

Despite the rich and differentiated fauna of other beetles mostly of the families *Curculionidae*, *Staphylinidae*, *Silphidae* and *Elateridae* as well as the ground beetles of the subfamily *Harpalinae*, no other infected species were found. In the light of the above considerations it does not seem probable that the precinct of the hosts of *C. entomorrhiza* exceeds the genera *Carabus* and perhaps some others closely related among the ground beetles, and *Nabis* and *Himacerus* among the bugs. The selectivity in relation of these insects could be due to a comparable composition of their constitutional substances resulting from the similarity of behaviour and food (predatory insectivorous forms). This supposition requires, however, a confirmation by detailed physiological investigations.

Occurrence and significance. The presence of the fungus *C. entomorrhiza* was stated only in one locality in the Wielkopolski National Park on a surface of about 11 ha, where, however, it was occurring in great numbers, evenly during four years of the observations. The flora of entomogenous fungi occurring in the place where *C. entomorrhiza* was observed at the time of its sporulation and their quantity is shown in Table 1. The investigations were carried out also in many other areas of the plant communities such as *Galio silvatici-Carpinetum*, *Fraxino-Ulmetum*, *Carici elongatae-Alnetum*, *Salicetum albo-fragilis* in the Wielkopolski National Park and *Pino-Quercetum* and *Leucobryo-Pinetum* in some managed forests. They did not give any positive results. The circumstances concerning collections reported in the references cited above seem also to show that the fungus was comparatively abundant but in dispersed localities. In the letter of March the 10, 1931 included to the Keissler's collection Professor Humazawa wrote „The fungus is found very often in our botanic garden, and one of us found and collected within an hour 17 individuals of the infected insects, however the fungus has not yet been found in other places". Thus, the fungus is able to cause considerable wastes in the local populations of the useful ground beetles.

C. entomorrhiza is easy to notice owing to the characteristic snow-white heads of the conidial sporulation (Fig. 1a) appearing from the second decade of June till August when they steadily disappear. The ascosporic fructification ripened only on a part of specimens in July and August. In September and October the fungus was not found. The stromata in living state, however, may hibernate, because on May the 29, 1980 a specimen of *Carabus nemoralis* Müll. with very old stromata

Table 1

Results of the quantitative investigations on the mortality of insects in the forest litter during the summer periods of 1977-1980 in the locality of *C. entomorrhiza*

Subject		Data
General	The surface investigated (total)	194 m ²
	The period considered	June 10-Aug. 15
Population of Carabidae	<i>Carabidae</i> — alive (larvae and adults)	68 individuals
	— — in that <i>Carabus</i> spp.	6 „
	<i>Carabidae</i> — dead (due to undefined) causes	6 „
	Mycoses of <i>Carabidae</i>	88 „
Mycoses of insects	<i>Cordyceps entomorrhiza</i> (Dicks. ex Fr.) Link.	85 cases
	— — in this on <i>Carabus</i> spp. (larvae, pupae and adults)	80 „
	— — on <i>Himacerus apterus</i> (Fabr.)	5 „
	<i>Beauveria bassiana</i> (Bals.) Vuill.	111 „
	<i>Hirsutella gigantea</i> Petch	1 „
	<i>Hirsutella</i> sp. near (<i>eleutheratorum</i>)	1 „
	<i>Mucor</i> sp.	1 „
	<i>Paecilomyces fumoso-roseus</i> (Wize) Brown et Smith	32 „
	<i>P. farinosus</i> (Dicks. ex Fr.) Brown et Smith	186 „
	<i>Sesquicillium</i> sp.	1 „
	Unidentified species of fungi	2 „
Unidentified group of pathogens	22 „	

was collected and after 5 days in laboratory conditions of moist chamber the well developed sporulation of the *Tilachlidiopsis*-type appeared. It is conformable with Klevenhusen's (1926) note.

Other observations. The attempts at isolation of the fungus on media did not give any encouraging results. On the standard organic-and-mineral media the mycelium did not develop either from the spores or after inoculation with pieces of pseudosclerotia. On the dextrose agar enriched with the coagulated egg-yolk very scarce mycelium — at first milky white, later darkening — developed slowly, not giving any sporulation and dying after several weeks. The attempts at the artificial infection of the larvae and adults of different species of *Carabidae* in a terrarium, by applying the sporulating specimens as a source of the infective material failed, too. The narrow precinct of the host species and peculiar requirements as to nutritive substances and environmental conditions are undoubtedly the causes limiting the distribution and frequency of the fungus in nature.

CONCLUSIONS

There is a need of a critical analysis of the available materials concerning the hyphomycetous species *Hirsutella eleutheratorum* and *Stilbella setiformis*, because — contrary to the earlier statements — they cannot be the conidial forms of the ascomycete *Cordyceps entomorrhiza*. It is probable that under the names *H. eleutheratorum* and *H. entomophila* some other species of the fungi pathogenic to beetles can also be found.

In the face of a possibility of epizootics caused by *Cordyceps entomorrhiza* in the populations of useful ground beetles it would be advisable to carry out wider investigations on its distribution and significance in our country. This fungus should be also considered in the studies of the population dynamics of the ground beetles by entomologists.

The author express his sincere thanks to Dr. Uwe Passauer (Naturhistorisches Museum, Bot. Abt., Wien, Austria) for sending the materials from Keissler's collection and to Dr. Alicja Cmóluchowa (M. Curie-Skłodowska University, Dept. of Zool., Lublin, Poland) for identification of the infected specimens of *Himacerus apterus* (Fabr.). The author is also indebted to Ing. Wanda Hoffman (Wielkopolski National Park, Puszczykowo, Poland) for the assistance and considerable help at the quantitative researches in the area.

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**Uwagi uzupełniające do biologii
Cordyceps entomorrhiza (Dicks. ex Fr.) Link
i morfologii jego stadiów konidialnych**

Streszczenie

Cordyceps entomorrhiza (Dicks. ex Fr.) Link rozwija się na larwach, poczwarkach i dorosłych osobnikach chrząszczy z rodzaju *Carabus* L. oraz na pluskwiakach z gatunku *Himacerus apterus* (Fabr.). Typowe podkładki i owocniki pojawiają się tylko na osobnikach chrząszczy znajdujących się w powierzchniowej warstwie gleby na głębokości od 3 do około 15 cm. Na osobnikach obumierających w ściółce leśnej — w tym na wszystkich okazach zainfekowanych pluskwiaków — spotykano tylko na osobnikach chrząszczy znajdujących się w powierzchniowej warstwie gleby forma-gatunek *Tilachlidiopsis hippotrichoides* (Lindau) Keissler z synonimem *T. nigra* Yakushiji et Kumazawa. Gatunki *Hirsutella eleutheratorum* (Nees ex Fr.) Petch i *Stilbella setiformis* (Vahl) Petch, podawane dotychczas w piśmiennictwie jako formy konidialne *C. entomorrhiza*, stanowią odrębne taksony. *Cordyceps entomorrhiza* występuje niekiedy licznie, jednakże na rozproszonych stanowiskach — jak się wydaje — grzyb ten związany jest z bogatymi zbiorowiskami roślinności drzewiastej na żyznych siedliskach. Lokalnie może powodować epizootcje w populacjach drapieżnych gatunków biegaczowatych ustawowo uznawanych w naszym kraju za gatunki chronione. W Wielkopolskim Parku Narodowym, gdzie prowadzono czteroletnie obserwacje nad rozwojem i wahaniami liczebności na jedynym znalezionym tu stanowisku, stwierdzono, że podkładki i owocowania konidialne pojawiały się regularnie od połowy czerwca do sierpnia, natomiast owocowania workowe — dojrzewające tylko na części okazów — spotykano w lipcu i sierpniu.