CZECH MYCOL. 57(3–4): 221–237, 2005

Entomopathogenic fungi associated with insect hibernating in underground shelters

ALENA KUBÁTOVÁ[1] and LIBOR DVOŘÁK[2]

[1] Department of Botany, Faculty of Science, Charles University, Benátská 2, 128 01 Praha 2, Czech Republic
kubatova@natur.cuni.cz
[2] Šumava National Park Administration, Department of Science and Research, Sušická 399, 341 92 Kašperské Hory, Czech Republic
libor.dvorak@npsumava.cz

Kubátová A. and Dvořák L. (2005): Entomopathogenic fungi associated with insect hibernating in underground shelters. – Czech Mycol. 57(3–4): 221–237.

In the period 2001–2004, several hundreds of underground shelters (mainly abandoned galleries, caves, and cellars) in W and SW Bohemia (Czech Republic) were explored for insect cadavers with visible fungal growth. At 27 localities, 94 infected cadavers of six insect taxa were collected. The most frequent infected insects were *Triphosa dubitata*, *Scoliopteryx libatrix* (*Lepidoptera*; *Geometridae* and *Noctuidae*, resp.) and unidentifed mosquitoes (*Diptera*, *Culicidae*). On the collected cadavers, altogether 20 species of microfungi (including sterile mycelia) were recorded, most of them belonging to entomopathogens. The most frequent was *Paecilomyces farinosus* (36 % of all samples) and *Cordyceps* sp. (15 %) which had affinity to *C. tuberculata* and *C. riverae*. Close association with insects was shown by *Cordyceps* sp. (with *Triphosa dubitata*) and *Conidiobolus destruens* (with unidentified mosquitoes). On the contrary, *Paecilomyces farinosus* was recorded on five different insect species. Also several other interesting species were found (e.g. *Hirsutella guignardii*, *Engyodontium* cf. *parvisporum*), probably not yet recorded from the Czech Republic. Microphotographs of some microfungi studied are included.

Key words: entomopathogenic micromycetes, hypogean galleries, overwintering moths, butterflies and mosquitoes

Kubátová A. a Dvořák L. (2005): Entomopatogenní houby na hmyzu přezimujícím v podzemních prostorách. – Czech Mycol. 57(3–4): 221–237.

V letech 2001–2004 bylo prozkoumáváno několik set lokalit s podzemními prostorami (hlavně opuštěné štoly, jeskyně, sklepy) v západních a severozápadních Čechách. Na 27 lokalitách byl nalezen mrtvý hmyz s viditelným porostem mikroskopických hub. Celkem bylo sebráno 94 vzorků šesti taxonů hmyzu napadených houbami. Mezi napadeným hmyzem byly nejčastější zástupci řádu *Lepidoptera*: píďalka jeskynní (*Triphosa dubitata*, *Geometridae*) a můra sklepní (*Scoliopteryx libatrix*, *Noctuidae*) a *Diptera*: neurčení komáři (*Culicidae*). Na vzorcích hmyzu bylo zjištěno celkem 20 druhů mikromycetů (včetně sterilních mycelií), patřících většinou mezi entomopatogeny. Nejhojnější byl *Paecilomyces farinosus* (36 % vzorků) a *Cordyceps* sp. (15 %), blízký druhům *C. tuberculata* a *C. riverae*. Úzká vazba na hmyz byla zjištěna u *Cordyceps* sp. (na *T. dubitata*) a *Conidiobolus destruens* (na neurčené komáry). Naproti tomu *P. farinosus* byl zaznamenán na 5 různých druzích hmyzu. Nalezeny byly i další zajímavé druhy hub (např. *Hirsutella guignardii*, *Engyodontium* cf. *parvisporum*), které pravděpodobně nebyly dosud v České republice zaznamenány. Článek je doplněn mikrofotografiemi některých studovaných druhů.

INTRODUCTION

In underground habitats (galleries, caves, cellars etc.) live different types of organisms, hidden to the eyes of man. Many of them, so called troglobionts, are adapted to the somewhat unfavourable abiotic factors of hypogean shelters.

As regards insects, especially members of *Lepidoptera*, *Hymenoptera* and *Diptera* are found there in some phase of their life cycle. Several lepidopteran species of butterflies (family *Nymphalidae*), moths (family *Geometridae* and *Noctuidae*), and *Microlepidoptera* (family *Oecophoridae*) use underground shelters for hibernation. Those species live from summer to next spring and after hibernation they have to go outside where reproduction proceeds. In the Czech Republic, *Inachis io*, *Triphosa dubitata*, and *Scoliopteryx libatrix* are known as the most common hibernants in underground shelters in west Bohemia (Dvořák 2000). Concerning *Hymenoptera*, only fertilised females of several species of *Ichneumonidae* hibernate in underground shelters. Their life cycle is similar as in the mentioned *Lepidoptera* – ichneumonid females have to fly outside the hibernation shelter to lay eggs. The spectrum of hibernating species was studied in west Bohemia. *Diphyus quadripunctorius* is the most common species in underground shelters (Šedivý and Dvořák 2002). Members of *Diptera* (especially *Heleomyzidae*) occur in caves and galleries very regularly, more or less during the whole year and may reproduce there (Papp 1981). Fertilised females of mosquitoes (*Culicidae*) hibernate in underground shelters (e.g. Kjaerandsen 1993) and reproduce outside. According to Minář and Hájková (1966), *Culex pipiens* is the only common species in cellars and caves in south Moravia.

During the hibernation, insects can be infected by entomopathogenic fungi, other inhabitants of underground hollows. Fungi parasiting on insects in caves have drawn the attention of man for a long time. For example, Lagarde (1913, 1917, 1922) published extensive papers dealing with different groups of fungi (including entomopathogens) occurring in French, Algerian and Spanish caves. More recent papers are for example those by Rombach and Samson (1983), Samson et al. (1984), Pacioni (1978), Malloch (Internet), Gunde-Cimerman et al. (1998), and Matočec and Ozimec (2001), dealing with entomopathogenic fungi in Europaean caves.

In the Czech Republic only several authors have studied the mycobiota of caves or other underground habitats, e.g. Fassatiová (1970), Marvanová et al. (1992), Bosák et al. (2001), Kubátová et al. (2005), focusing on micromycetes predominantly in air, soil and on various surfaces of hypogean localities.

The study of entomopathogenic fungi has a long tradition in the Czech Republic which was formed e.g. by Rozsypal, Fassatiová, Samšiňáková, Krejzová, Weiser, and later by Landa, Oborník, etc. However, no papers (with exception of

Weiser and Batko 1966) have yet been published on entomopathogenic fungi on insects in underground shelters.

During recent excursions of the second author to underground galleries, caves and cellars mainly in western Bohemia (Czech Republic), numerous insects were found on which micromycetes parasitised. Thus, the main aim of the current paper is to extend our knowledge on the occurrence of entomopathogenic microfungi infecting insects in underground shelters.

MATERIALS AND METHODS

Cadavers (= samples) of moths, mosquitoes and other insects with visible fungal growth were collected by the second author in several types of underground shelters (old abandoned mine galleries, caves and cellars) during three winters in the period December 2001 to January 2004. Most insect samples were collected in western and south-western Bohemia, one sample originated from the Moravian Karst (southern Moravia), Czech Republic. Although several hundreds of underground shelters were visited, insect cadavers were collected only at 27 localities. A great number of insect samples (13 cadavers) were collected in several galleries of nature reserve Amálino údolí near Kašperské Hory. Most localities were visited only once. Localities and collected insects are listed in Tab. 1. Altogether, 94 infected cadavers of 6 insect taxa were studied: 70 belonging to *Lepidoptera*, 21 to *Diptera*, and 3 to *Hymenoptera* (Tab. 2).

In the laboratory, both direct microscopic examination of infected insect cadavers and cultivation of fungi from insects were carried out. For the isolation of fungi, malt extract agar (MEA) after Pitt (1979) and potato carrot agar (PCA) after Fassatiová (1986) were used. Microscopic features were observed in mounts with lactic acid and cotton blue or in water. The microscopic fungi were identified according to Weiser and Batko (1966), Gams (1971), Weiser (1977), de Hoog (1978), Pacioni (1978), Gams et al. (1984), Domsch et al. (1993), Tzean et al. (1997), Matočec and Ozimec (2001), and Zare and Gams (2001). Photographs were taken on an Olympus BX–51 microscope using Nomarski (DIC) and phase contrast (Ph).

Herbarium specimens were deposited in PRC (Herbaria of Dept. of Botany, Faculty of Science, Charles University, Prague), six fungal isolates were deposited at CCF (Culture Collection of Fungi, Dept. of Botany, Faculty of Science, Charles University, Prague) – see note in Tab. 3.

Tab. 1. Localities of collected insect samples (all in the Czech Republic).
Note: PLA = Protected Landscape Area, NR = Nature Reserve, NM = Nature Monument, NP = Nature Park, x = lost.

PRC Herbarium specimens	Insect species, locality, date of sampling
	Diphyus quadripunctorius (*Hymenoptera*, *Ichneumonidae*)
64, 65	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „II“, c. 1.5 km SE of Kašperské Hory, 720 m a.s.l., 49°07'53" N, 13°34'29" E, I. 2002
x	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „I“, c. 1.5 km SE of Kašperské Hory, 710 m a.s.l., 49°07'51" N, 13°34'32" E, III. 2002
	***Heleomyza* sp.** (*Diptera*, *Heleomyzidae*)
57, 75, 88–91	SW Bohemia, NM Loreta, underground limestone mine, 1.5 km NE of Týnec, 495 m a.s.l., 49°21'26" N, 13°17'05" E, XII. 2001, IV. 2002, XII. 2002
95	W Bohemia, PLA Křivoklátsko, Modřejovice, gallery in valley of stream c. 2 km W of the village, 328 m a.s.l., 49°59'41" N, 13°40'11" E, I. 2003
	Inachis io (*Lepidoptera*, *Nymphalidae*)
80	SW Bohemia, Tuškov, SE periphery of the village, military bunker, 640 m a.s.l., 49°09'27" N, 13°32'43" E, VIII. 2002
132	W Bohemia, Luka, brewery cellars, near a pond N of the village, 630 m a.s.l., 50°09'43" N, 13°09'45" E, III. 2003
138	W Bohemia, PLA Slavkovský les, Kostelní Bříza, cellars in the castle park, SW of the village, 618 m a.s.l., 50°06'51'' N, 12°37'10'' E, I. 2004
	Scoliopteryx libatrix (*Lepidoptera*, *Noctuidae*)
63	S Bohemia, NM Kněží hora, between Katovice and Poříčí, graphite gallery, 430 m a.s.l., 49°16'50" N, 13°48'45" E, I. 2002
70	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „Myší díra“, c. 1.5 km SE of Kašperské Hory, 705 m a.s.l., 49°08'03" N, 13°34'14" E, III. 2002
71–73	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „Veřejné záchodky“, c. 1.5 km S of Kašperské Hory, 675 m a.s.l., 49°08'01" N, 13°33'45" E, III. 2002
74	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „A“, c. 1.5 km S of Kašperské Hory, 695 m a.s.l., 49°08'02" N, 13°33'46" E, III. 2002
76–78	SW Bohemia, NR Borek, gallery, SW of the village Velhartice, 610 m a.s.l., 49°15'44" N, 13°24'00" E, IV. 2002
81	SW Bohemia, NR Čepičná, gallery „Čepice“, 1 km W of the village Čepice, 500 m a.s.l., 49°16'03" N, 13°35'13" E, IX. 2002
92	W Bohemia, Stříbro, gallery „Urban III“, Úhlavka river valley, S periphery of the town, 380 m a.s.l., 49°44'17" N, 13°00'04" E, I. 2003
96	W Bohemia, Svinná, cellars of small castle in the village, 450 m a.s.l., 49°53'38" N, 13°37'19" E, I. 2003
97, 98	W Bohemia, NM Červený vrch, mine on Červený vrch hill, SE of the village Otov, 480 m a.s.l., 49°28'57" N, 12°51'14" E, I. 2003
100–102	W Bohemia, Černá Řeka, gallery in Mt. Jindřichova hora, c. 2 km WNW of the village, 575 m a.s.l., 49°25'50" N, 12°43'55" E, I. 2003
105, 106	W Bohemia, PLA Slavkovský les, Bečov nad Teplou, cellars of the castle, N periphery of the town, 520 m a.s.l., 50°05'07'' N, 12°50'21'' E, I. 2003
110, 136, 137	W Bohemia, Oloví, gallery in „Pplk. Sochora street“, 510 m a.s.l., 50°15'13'' N, 12°33'19'' E, I. 2003, II. 2004

111, 112	W Bohemia, Zlatý Kopec, gallery „Ementál“, c. 1.2 km SW of the village, 852 m a.s.l., 50°25'53" N, 12°49'48" E, II. 2003
113–115	W Bohemia, Zlatý Kopec, gallery „Rezonanční“, c. 1.2 km SW of the village, 852 m a.s.l., 50°25'51" N, 12°49'48" E, II. 2003
133	W Bohemia, Andělská Hora, gallery under Stichlův Mlýn, c. 1.2 km NE of the village, 617 m a.s.l., 50°12'43" N, 12°58'50" E, III. 2003
135	W Bohemia, Vysoká Pec, gallery under the village, 688 m a.s.l., 50°20'46" N, 12°42'12" E,II. 2003
142	W Bohemia, Kamenec, gallery, c. 1.2 km E of the village, 390 m a.s.l., 49°52'49" N, 13°36'59" E, I. 2004
	Triphosa dubitata (*Lepidoptera*, *Geometridae*)
58–62	S Moravia, Křtiny, NR U Výpustku, Drátenická jeskyně cave, 395 m a.s.l., 49°17'26" N, 16°43'47" E, XII. 2001
66	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „Barbastelí“, c. 1.5 km SW of Kašperské Hory, 715 m a.s.l., 49°08'04" N, 13°33'46" E, II. 2002
67	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „Kristýna“, c. 1.5 km SE of Kašperské Hory, 700 m a.s.l., 49°07'49" N, 13°34'49" E, XII. 2001
68, 69	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „II“, c. 1.5 km SE of Kašperské Hory, 720 m a.s.l., 49°07'53" N, 13°34'29" E, III. 2002
103	W Bohemia, Černá Řeka, gallery in Mt. Jindřichova hora, c. 2 km WNW of the village, 575 m a.s.l., 49°25'50" N, 12°43'55" E, I. 2003
107–109	W Bohemia, PLA Slavkovský les, Bečov nad Teplou, cellars of the castle, N periphery of the town, 520 m a.s.l., 50°05'07'' N, 12°50'21'' E, I. 2003
116–118	W Bohemia, Horní Hrad, cellars of the castle, 470 m a.s.l., 55°20'44" N, 12°58'07" E, II. 2003
119, 143–145	W Bohemia, PLA Slavkovský les, Výškov, NR Lazurový vrch, gallery „Trampský převis“, c. 2 km NNE of the village, 600 m a.s.l., 49°54'47" N, 12°46'50" E, II. 2003, I. 2004
120–121	W Bohemia, PLA Slavkovský les, Výškov, NR Lazurový vrch, gallery „Pokojíček“, c. 2 km NNE of the village, 600 m a.s.l., 49°54'49" N, 12°46'50" E, II. 2003
122–131	W Bohemia, Valeč, cellars in the castle park, 550 m a.s.l., 50°10'37" N, 13°14'54" E, III. 2003
139	W Bohemia, Vítkov near Sokolov, mining magazine of former mine, 707 m a.s.l., 50°09'25" N, 12°41'21" E, I. 2004
146–149	W Bohemia, Valeč, cellars of farm building in the castle complex, 545 m a.s.l., 50°10'30" N, 13°15'01" E, I. 2004
	unidentified mosquito (*Diptera*, *Culicidae*)
79	SW Bohemia, Velhartice, NR Borek, gallery SW of the village, 610 m a.s.l., 49°15'44" N, 13°24'00" E, IV. 2002
82	SW Bohemia, NR Čepičná, gallery „Čepice“ 1 km W of the village Čepice, 500 m a.s.l., 49°16'03" N, 13°35'13" E, X. 2002
83–86	S Bohemia, NM Kněží hora, between Katovice and Poříčí, graphite gallery, 430 m a.s.l., 49°16'50" N, 13°48'45" E, XI. 2002
87	SW Bohemia, PLA Šumava, NR Amálino údolí, gallery „Kristýna“, c. 1.5 km SE of Kašperské Hory, 700 m a.s.l., 49°07'49" N, 13°34'43" E, XI. 2002
93	W Bohemia, Stříbro, gallery „Urban III“, Úhlavka river valley, S periphery of the town, 380 m a.s.l., 49°44'17" N, 13°00'04" E, I. 2003
94, 140, 141	W Bohemia, PLA Křivoklátsko, Písednice, Matčina hora hill, gallery, c. 0.5 km W of a village, 362 m a.s.l., 49°52'55" N, 13°44'20" E, I. 2003, I. 2004 (resp.)
99	W Bohemia, Černá Řeka, gallery in Mt. Jindřichova hora, c. 2 km WNW of the village, 575 m a.s.l., 49°25'50" N, 12°43'55" E, I. 2003
104	W Bohemia, Hostičkov, gallery in Trdlina hill, c. 2 km SSW of the village, 569 m a.s.l., 49°53'03" N, 12°48'57" E, I. 2003
134	SW Bohemia, NP Buděticko, Rabí, cellars of the castle, S periphery of the village, 500 m a.s.l., 49°16'46" N, 13°37'12" E, II. 2003

Tab. 2. Studied insect specimens collected in underground shelters in the Czech Republic.

Taxonomic group of insects (order, family)	Insect species (Latin, English and Czech name)	Number of cadavers
Lepidoptera, Geometridae	*Triphosa dubitata* – Tissue (píďalka jeskynní)	37
Lepidoptera, Noctuidae	*Scoliopteryx libatrix* – Herald (můra sklepní)	30
Lepidoptera, Nymphalidae	*Inachis io* – European Peacock (babočka paví oko)	3
Diptera, Culicidae	unidentified – mosquitoes (komárovití)	14
Diptera, Heleomyzidae	*Heleomyza* sp. – heleomyzid flies (lanýžovkovití)	7
Hymenoptera, Ichneumonidae	*Diphyus quadripunctorius* – ichneumonid (lumek)	3
Total number	**6**	**94**

RESULTS AND DISCUSSION

Overall results

On 94 cadavers of 6 insect species collected in underground shelters in the Czech Republic, altogether 20 species of microfungi (including four different types of sterile mycelium) were recorded (Tab. 3). The most frequent was *Paecilomyces farinosus* (36 % of all samples), *Cordyceps* sp. (15 %) and several types of sterile mycelia (32 %).

Most recorded fungi belong to entomopathogens: *Beauveria*, *Conidiobolus*, *Cordyceps*, *Engyodontium*, *Hirsutella*, *Lecanicillium*, *Paecilomyces*, and *Simplicillium*. Only *Acremonium bacillisporum*, *Aphanocladium album*, *Engyodontium rectidentatum*, *Mortierella* sp., and *Mucor* sp. are not generally considered to be entomopathogenic.

It is interesting that the majority of them belongs to anamorphic *Hypocreales* (Ascomycota), exception for three members of the Zygomycota: *Conidiobolus* (*Entomophthorales*), *Mortierella* (*Mortierellales*) and *Mucor* (*Mucorales*).

Insect – fungus associations

Most insect cadavers (85 %) were occupied by one fungus only. On 15 % of samples, two fungal species were recorded. More than two fungi on the same cadaver were not observed. Among the fungal couples on one insect body were *Conidiobolus destruens* and *Acremonium bacillisporum* (3 samples of unidentified *Culicidae*), *Paecilomyces farinosus* and *Lecanicillium muscarium* (3 samples of *Scoliopteryx libatrix*), *Cordyceps* sp. and *Simplicillium* cf. *lamellicola* (2 samples). Other couples occurred only once.

Tab. 3. Observed microscopic fungi and their association with insect species.
Notes: CCF = Culture Collection of Fungi, Prague, c = successful cultivation, e = empty perithecia, f = fertile perithecia, i = immature perithecia.

Fungus – insect association	**PRC Herbarium specimens**	**Number of samples**
Acremonium bacillisporum (Onions et G. L. Barron) W. Gams		
unidentified *Culicidae*	83, 85, 93, 94	4
Aphanocladium album (Preuss) W. Gams		
Triphosa dubitata	139	1
Beauveria bassiana (Bals.–Criv.) Vuill.		
unidentified *Culicidae*	87	1
Beauveria brongniartii (Sacc.) Petch		
Heleomyza sp.	57^{c}	1
Conidiobolus destruens (Weiser et A. Batko) Ben–Ze´ev		
unidentified *Culicidae*	85, 93, 94, 141	4
Cordyceps sp.		
Triphosa dubitata	60^{e}, 67^{f}, 107^{f}, 108^{e}, 109^{i}, 119^{i}, 125^{i}, 128^{u}, 129^{u}, 130^{u}, 131^{e}, 139^{f}, 147^{u}, 148^{f}	14
Engyodontium cf. *parvisporum* (Petch) de Hoog		
Triphosa dubitata	145	1
Engyodontium rectidentatum (Matsush.) W. Gams, de Hoog, Samson et H. C. Evans		
Scoliopteryx libatrix	81^{c} = **CCF 3541**	1
Hirsutella guignardii (Maheu) Samson, Rombach et Seifert		
Heleomyza sp.	75	1
Lecanicillium muscarium (Petch) R. Zare et W. Gams		
Scoliopteryx libatrix	72^{c} = **CCF 3297**, 76^{c}, 78^{c}, 112^{c}, 113^{c}	5
Inachis io	138	1
Lecanicillium sp.		
unidentified *Culicidae*	79, 134, 140, 141	4
Mortierella sp.		
Heleomyza sp.	95	1
Mucor sp.		
Diphyus quadripunctorius	lost	1
Paecilomyces farinosus (Holmsk.) A. H. S. Br. et G. Sm.		
Scoliopteryx libatrix	63^{c}, 70^{c}, 71^{c}, 72^{c}, 73, 74, 76^{c}, 77^{c} = **CCF 3542**, 78^{c}, 97^{c}, 98^{c}, 100^{c}, 101^{c}, 102^{c}, 105^{c}, 106^{c}, 110^{c}, 111^{c}, 113, 114^{c}, 133^{c}, 135, 136^{c}, 137^{c}, 142^{c}	25
Diphyus quadripunctorius	64, 65^{c}	2
unidentified *Culicidae*	79^{c}, 84^{c}, 86^{c}, 99^{c}	4
Triphosa dubitata	103^{c}	1
Inachis io	80^{c}, 132^{c}	2
Paecilomyces fumosoroseus (Wize) A. H. S. Br. et G. Sm.		
Scoliopteryx libatrix	63^{c} = **CCF 3272**	1
Simplicillium cf. *lamellicola* (F. E. V. Sm.) R. Zare et W. Gams		
Triphosa dubitata	69, 108^{c} = **CCF 3524**, 131^{c} = **CCF 3525**	3
Scoliopteryx libatrix	71	1

Tab. 3. Observed microscopic fungi and their association with insect species – continued.
Notes: c = successful cultivation.

Fungus – insect association	PRC Herbarium specimens	Number of samples
sterile white mycelium with sterile synnemata		
Triphosa dubitata	66, 116, 117, 120, 121, 122, 123, 124, 126, 127, 143, 149	12
sterile white mycelium		
Heleomyza sp.	88, 89, 90, 91	4
Scoliopteryx libatrix	96, 115	2
Triphosa dubitata	58, 59, 61, 62, 68, 118, 144[c], 146, 147	9
unidentified *Culicidae*	104	1
sterile light sparse mycelium		
unidentified *Culicidae*	82	1
sterile non-septate light mycelium		
Scoliopteryx libatrix	92	1
total number of samples		**94**

In Tab. 4, associations of micromycetes with insect species are given. Each of the most frequent insect species (the moths *Triphosa dubitata* and *Scoliopteryx libatryx*, and an unidentified mosquito) hosted seven micromycetes. On three other less frequent insect species, only 2–4 microfungal species were recorded. These data correlate with the number of collected dead bodies. Thus, for more exact results on host – parasite specificity, much more collections should have had to be made.

Regarding entomopathogenic fungal species, the most frequent fungus *Paecilomyces farinosus* (Fig. 4, 6, and 8) was found as a parasite of a majority of studied insect taxa (five out of six). It occurred most frequently on *Scoliopteryx libatrix* cadavers. This corresponds with the overall knowledge about this species: a ubiquitous insect parasite, common both in temperate and tropical zones on a wide host range (Domsch et al. 1993). Tzean et al. (1997) mentioned in their comprehensive atlas that *Paecilomyces farinosus* belongs to the most often encountered *Paecilomyces* species in Taiwan. Samson et al. (1984) mentioned this species as a frequent parasite of insects in caves in the Netherlands. In the Czech Republic, Javůrková-Fassatiová (1956) found this species on members of several different insect groups; *P. farinosus* was also often recorded from soil in the Šumava Mts. (Kubátová et al. 1998, Nováková 2001) and from bark beetles (Landa et al. 2001).

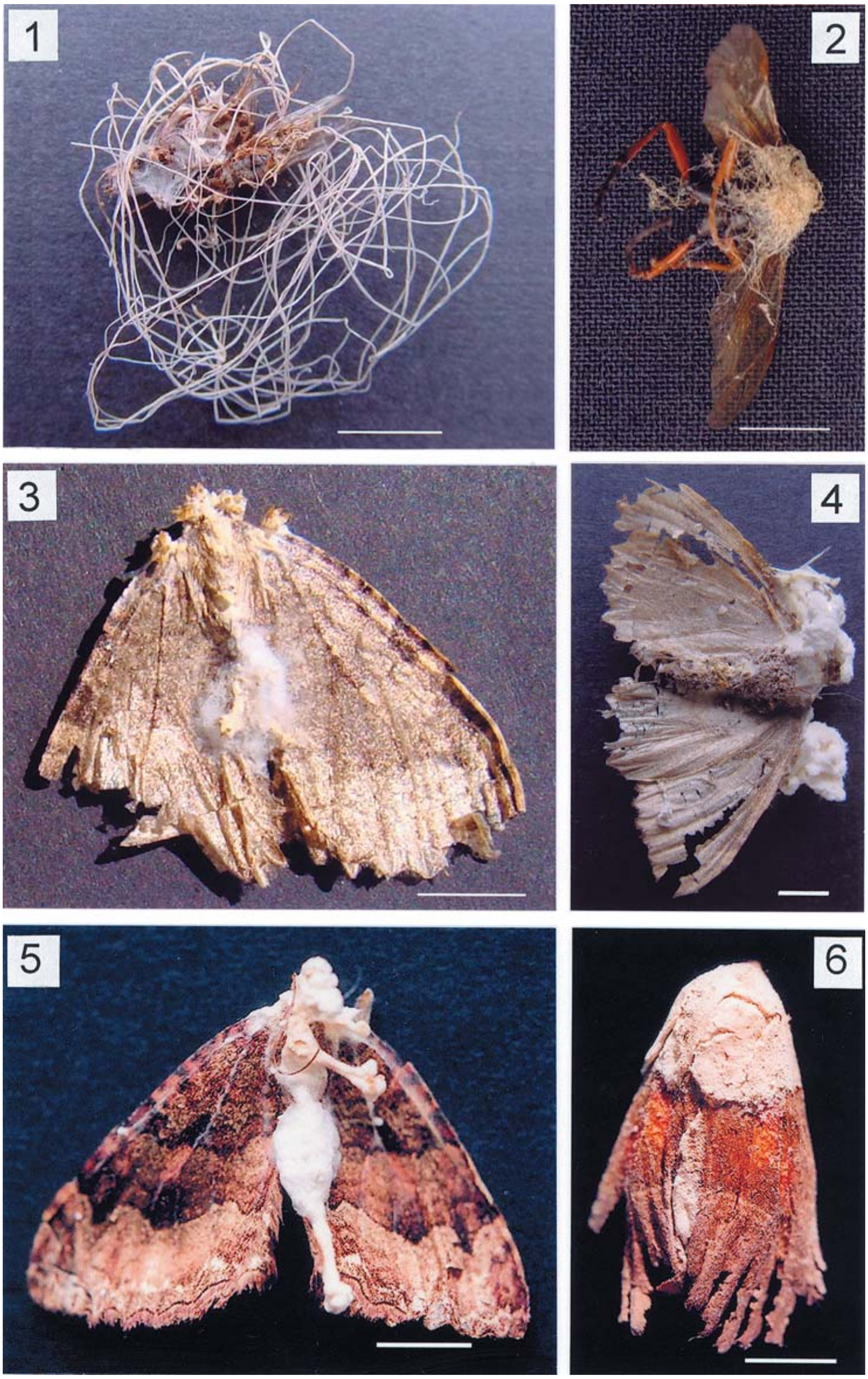
On the other hand, another frequent micromycete, unidentified *Cordyceps* sp. (Fig. 3, 5, and 7), was found associated with only one insect species, *Triphosa dubitata*. Pacioni (1978) described from this moth the morphologically similar species *Cordyceps riverae* Pacioni. Matočec and Ozimec (2001) observed *C. riverae* on *Triphosa dubitata*, too. Our collections however differ somewhat from this species (see below).

Tab. 4. Insect – fungus associations.

Insect species (No. of samples)	Associated fungus species	Number of infected samples
Triphosa dubitata	*Cordyceps* sp.	14
(37)	sterile white mycelium with sterile synnemata	12
	sterile white mycelium	9
	Simplicillium cf. *lamellicola*	3
	Aphanocladium album	1
	Engyodontium cf. *parvisporum*	1
	Paecilomyces farinosus	1
Scoliopteryx libatrix	*Paecilomyces farinosus*	25
(30)	*Lecanicillium muscarium*	4
	sterile white mycelium	2
	Engyodontium rectidentatum	1
	Paecilomyces farinosus	1
	Simplicillium cf. *lamellicola*	1
	sterile non-septate light mycelium	1
Inachis io	*Paecilomyces farinosus*	2
(3)	*Lecanicillium muscarium*	1
unidentified *Culicidae*	*Conidiobolus destruens*	4
(14)	*Acremonium bacillisporum*	4
	Lecanicillium sp.	4
	Paecilomyces farinosus	4
	Beauveria bassiana	1
	sterile white mycelium	1
	sparse sterile light mycelium	1
Heleomyza sp.	sterile white mycelium	4
(7)	*Hirsutella guignardii*	1
	Mortierella sp.	1
	sterile white mycelium	1
Diphyus quadripunctorius	*Paecilomyces farinosus*	2
(3)	*Mucor* sp.	1

Another fungal species, *Conidiobolus destruens* (Fig. 14), was also observed on only one insect species, an unidentified mosquito (*Culicidae*). According to Weiser and Batko (1966) who described this species (as *Entomophthora destruens* Weiser et A. Batko), it is a typical pathogen of mosquitoes in underground shelters. They found it in caves and cellars in Moravia and Bohemia (Czech Republic), Slovakia, England and France on *Culex pipiens* as the only host.

Lecanicillium muscarium (known formerly as *Verticillium lecanii* (Zimm.) Viégas) (Fig. 12) was found on two members of *Lepidoptera* (Table 3). It is a very frequent polyphagous species occurring mainly in temperate regions whereas the


1
2
3
4
5
6

closely related species *L. lecanii* (Zimm.) R. Zare et W. Gams prefers tropical regions (Zare and Gams 2001). The pathogenic abilities of *L. muscarium* are also utilised commercially (Zare and Gams 2001). In the Czech Republic it was reported infrequently from soil (Kubátová et al. 1998, Nováková 2001) and more frequently from bark beetles (Landa et al. 2001).

Hirsutella guignardii (Figs. 1 and 9) is known in Europe as an entomogenous fungus parasiting troglobiotic insects (dipteran and coleopteran) (Samson et al. 1984, Malloch – Internet). We found it only once on *Heleomyza* sp., a dipteran member. The find in the Czech Republic is probably the first.

Engyodontium parvisporum (Fig. 10) is known to be associated with insects from warm regions. De Hoog (1978) cites several records from Sri Lanka. Our specimen differs somewhat from the description by de Hoog (see below).

It is noteworthy that well–known entomopathogenic fungi like *Beauveria bassiana*, *B. brongniartii*, and *Paecilomyces fumosoroseus* were recorded only once. The main niche of these fungi is probably elsewhere, although Samson et al. (1984) found *Beauveria bassiana* frequently on troglobiotic insects. Another well–known entomopathogen, *Metarhizium anisopliae* (Metschn.) Sorokin, was not observed on our insect material. This species occurs only on coleopteran members (Domsch et al. 1993).

On the other hand, we found several fungi which are not typical parasites of insects. For example, *Mucor* species (Fig. 2) are generally coprophilous and "sugar" fungi. Sporadic data about its associations with insects however exist. Lagarde (1922) found *Mucor* sp. on debris of insects. Heitor (1962) experimentally demonstrated that *M. hiemalis* Wehmer can cause infection of wounded insects; the fungus penetrates through the wound. Recently, Zalar et al. (1997) described a new species, *M. troglophilus* Zalar, infecting *Troglophilus neglectus* (*Orthoptera*).

Among other microfungi with unknown association with insects are *Mortierella* sp., *Acremonium bacillisporum*, *Aphanocladium album* (Fig. 11), and *Engyodontium rectidentatum*. They are known predominantly from soil (Gams 1971, Gams et al. 1984, Domsch et al. 1993). *Acremonium bacillisporum* was found on insects by O. Constantinescu in Romania (CBS Filamentous Fungi Database 2005). *Aphanocladium album* was often recorded from myxomycetes and fungi, too. In the Czech Republic this fungus is found rarely; it was reported

---

Fig. 1. Synnemata of *Hirsutella guignardii* on *Heleomyza* sp. (PRC 75).
Fig. 2. *Mucor* sp. on *Diphyus quadripunctorius*.
Fig. 3. Perithecia of *Cordyceps* sp. in the head part of *Triphosa dubitata* (PRC 139).
Fig. 4. Synnemata of *Paecilomyces farinosus* on *Triphosa dubitata* (PRC 103).
Fig. 5. Sterile mycelium and stalks of *Cordyceps* sp. on *Triphosa dubitata* (PRC 67).
Fig. 6. *Paecilomyces farinosus* on *Scoliopteryx libatrix* (PRC 74).

Scale bars: 5 mm. Photo A. Kubátová

e.g. from the air of caves (Bosák et al. 2001). *Engyodontium rectidentatum* is known predominantly from soil (Gams et al. 1984). In the Czech Republic this species is rare; it was isolated from air in caves (Marvanová et al. 1992). It is possible that these fungi contamined dead bodies of insect and grew there as saprotrophs.

Notes to the identification of some recorded fungi

Cordyceps sp. (Fig. 3, 5, and 7). We found 14 specimens with a fungus identified as *Cordyceps* sp. It formed whitish stalks varying in length (max. 5 mm long), at the end often club-shaped. Perithecia grew terminally on the stalks, sometimes also laterally; in some specimens on the surface of the stalk, in others partially embedded in white felted mycelium. Mature perithecia with ascospores were yellow. However, only four specimens were fertile (see Table 3) and formed long cylindrical asci with filiform hyaline ascospores, which fragment to 1–celled ascospores, c. 10 x 1 µm. All other specimens formed immature yellow perithecia or old brown, empty perithecia. Because of the same general habitus we assigned them also to *Cordyceps* sp. In species identification of *Cordyceps*, the host is of great importance. Our specimens are very close to *Cordyceps riverae* which was described by Pacioni (1978) on *Triphosa dubitata* from caves in Italy and Belgium and later found also by Matočec and Ozimec (2001) on *T. dubitata* and *T. sabaudiata* in Croatian caves. However, *Cordyceps riverae* differs by smaller part spores (c. 4–6.5 x 0.6–0.7 µm). Another similar species often found on lepitopteran members is *C. tuberculata* (Lebert) Maire. This species is known from many parts of the world, and was recorded also in Slovakia (Kautmanová 2002). It differs from our specimens by smaller part spores, too (2–6 x 0.5–1 µm after Mains 1958). In the Czech Republic, at least 8 species of *Cordyceps* have been recorded, six of them belonging to parasites of insects (Javůrková-Fassatiová 1955), and two of *Elaphomyces* (Holec 2001).

Sterile mycelia. This type of fungus could not mostly be identified with morphological methods. However, some morphological similarities were observed between „sterile white mycelium with sterile synnemata“ and *Cordyceps* sp., both found on *Triphosa dubitata* (Table 3). The sterile synnemata could be initials of stalks of *Cordyceps*. In this case, molecular methods could yield some results. However, for this „floristic case study“ it would be too expensive.

Hirsutella guignardii (Fig. 9). It has prominent thin long synnemata, lageniform phialides and ellipsoidal-fusiform conidia with mucus. This mucus was not observed in lactic acid mounts (Fig. 9c), only in water mounts (Fig. 9d).

Lecanicillium sp. (Fig. 13). This species was found only on cadavers of unidentified mosquitoes. It forms verticillate conidiophores with hyaline conidia of highly variable size, c. 4.5–15 x 1.2–2 µm. The longer conidia (macroconidia) are

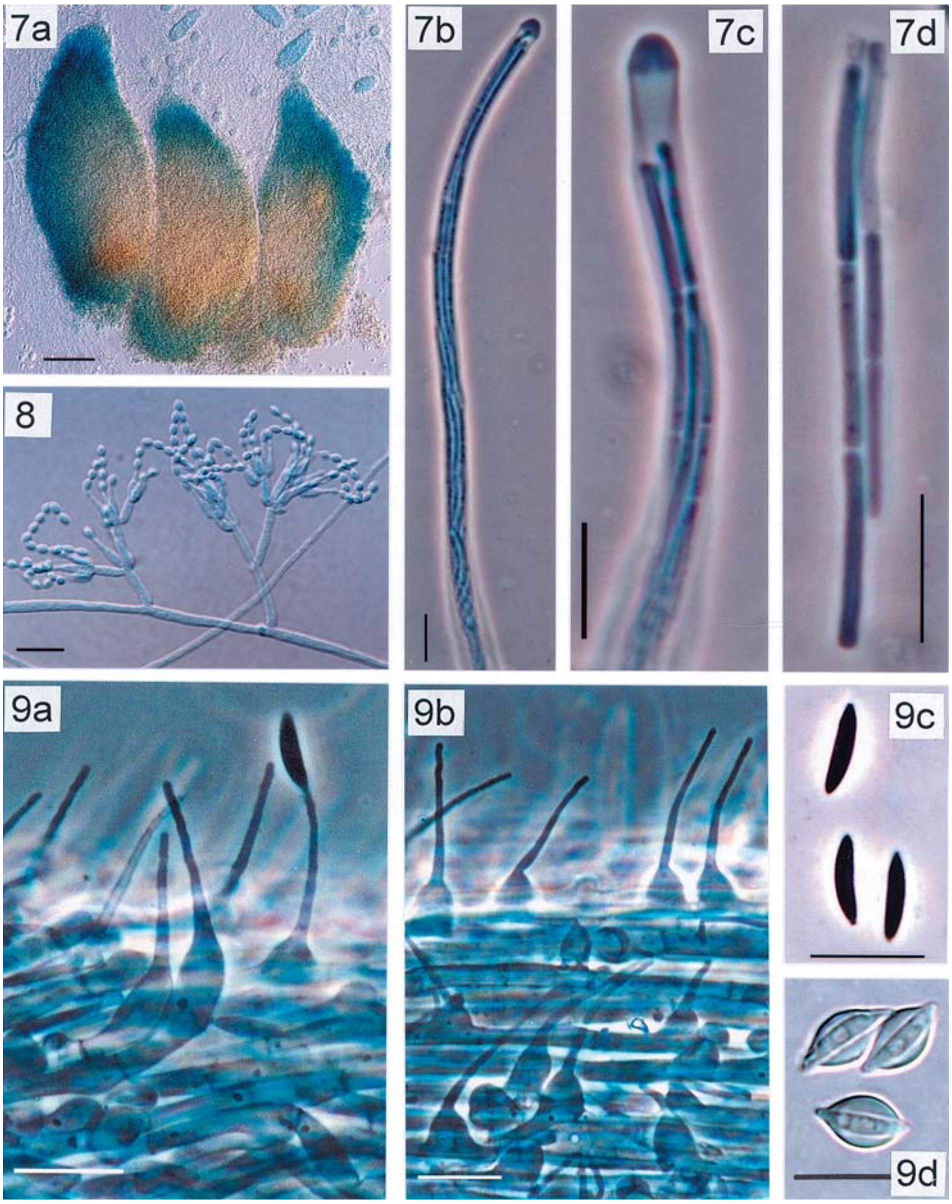


Fig. 7. *Cordyceps* sp.: a – young perithecia (PRC 144), DIC; b, c – upper part of a long cylindrical ascus with ascospores (PRC 139), Ph; d – ascospore fragments (PRC 139), Ph.
Fig. 8. *Paecilomyces farinosus* (PRC 80), conidiophores with conidia, DIC.
Fig. 9. *Hirsutella guignardii* (PRC 75): a, b – phialides with long necks, Ph; c – conidia in lactic acid mount, Ph; d – conidia in water mount, DIC.

Scale bar in Fig. 7a: 100 µm, in all other figs. 10 µm. Photo A. Kubátová

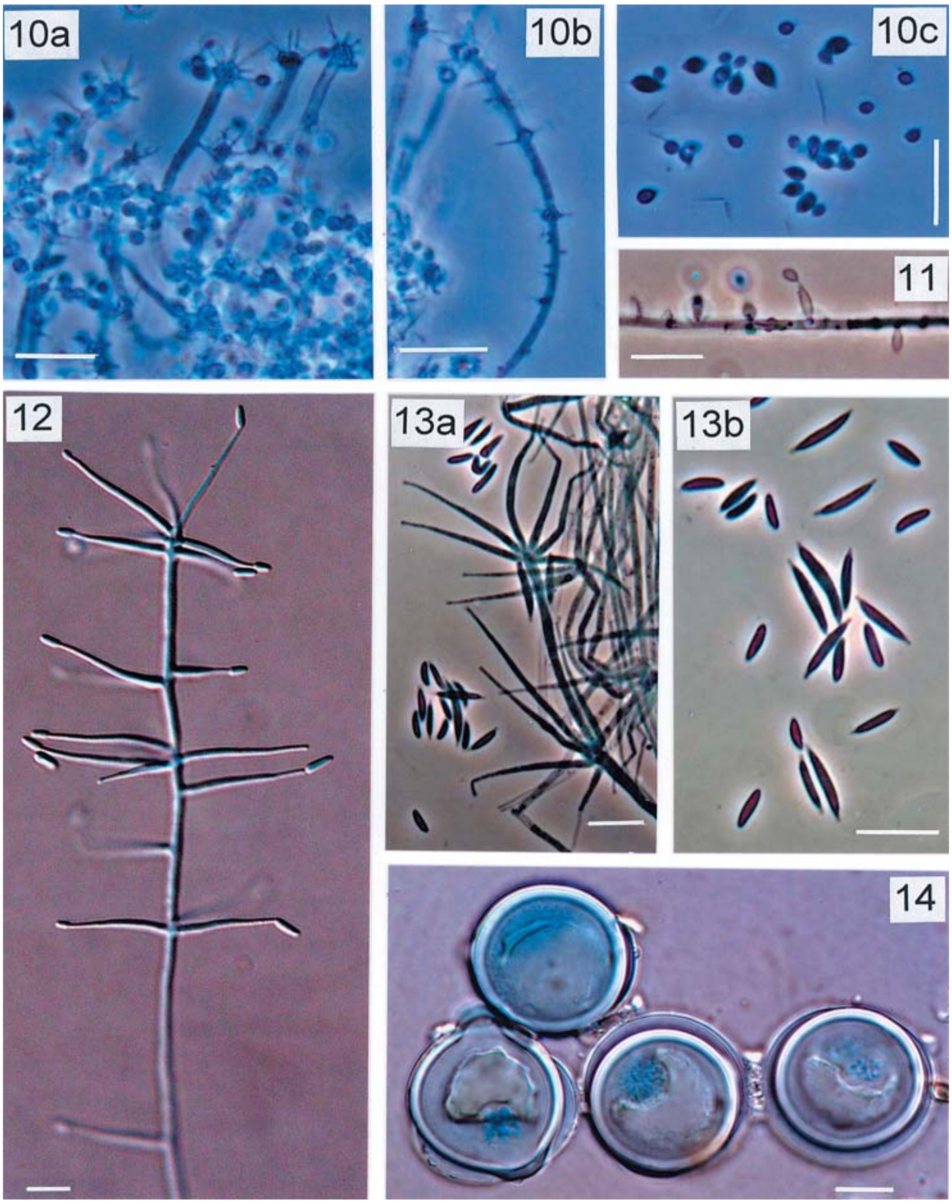


Fig. 10. *Engyodontium* cf. *parvisporum* (PRC 145): a, b – conidiophores with thin denticles and conidia, Ph; c – conidia, Ph.
Fig. 11. *Aphanocladium album* (PRC 139), conidiogenous hypha with phialides and conidia, Ph.
Fig. 12. *Lecanicillium muscarium* (PRC 138), conidiophore with conidia, DIC.
Fig. 13. *Lecanicillium* sp. (PRC 140): a – conidiophore with conidia, Ph; b – macro- and microconidia, Ph.
Fig. 14. *Conidiobolus destruens* (PRC 94), resting spores, DIC.

Scale bars: 10 µm. Photo A. Kubátová

spindle-shaped, straight or slightly curved, with acute ends, sometimes two-celled. The smaller conidia (microconidia) have rounded ends. The division between macroconidia and microconidia is not sharp. On account of the conidium sizes this species is intermediate between *Lecanicillium psalliotae* (Treschew) R. Zare et W. Gams (having shorter macroconidia) and *Lecanicillium acerosum* R. Zare et W. Gams (longer macroconidia). Whilst *L. psalliotae* is often found worldwide and in the Czech Republic predominantly in soil, *L. acerosum* was described only in 2001 from insects in Brazil and its distribution is not yet known. According to data by Zare and Gams (2001), our isolates are similar to strain CBS 171.97 isolated from insects in Spain, which has been provisionally assigned to *L. psalliotae*.

Simplicillium cf. *lamellicola*. This species is morphologically very similar to *Lecanicillium psalliotae*. According to Zare and Gams (2001) it differs by more straight conidia (in *L. psalliotae* they are more curved) and by a brown colony reverse (in *L. psalliotae* it is red). Our two isolates have more or less straight conidia but failed in pigmentation of the reverse. Thus some uncertainty remains about their identification. Both strains are maintained in the CCF, Prague.

Engyodontium cf. *parvisporum* (Fig. 10). Our specimen differs somewhat from the description by de Hoog (1978). The size of conidia in our fungus is 2–4 x 1.5–2 µm, whereas de Hoog reported 1.2–3 x 1.2–1.5 µm. Another difference was found in the shape of conidiogenous cells. They are slightly swollen at the end in our specimen, whereas de Hoog reported that they are often very slightly tapering towards the tip. The identification of our specimen thus remains open. Morphologically similar is *Aphanocladium aranearum* (Petch) W. Gams which differs from our fungus by somewhat larger conidia (2.8–4.6 x 2–3.4 µm according to Gams 1971).

Concluding, *Paecilomyces farinosus* was found to be the most frequent parasite of different insects in the explored underground shelters and thus plays probably the important role in this habitat. Two fungi were recorded in association with one insect species: *Cordyceps* sp. is the major fungal pathogen of *Triphosa dubitata* and *Conidiobolus destruens* the pathogen of an unidentified mosquito. The identification of several fungal entomopathogens found during this “case study” (especially *Cordyceps* sp., *Lecanicillium* sp., sterile mycelia) requires a more detailed (preferably molecular) study.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Institutional Project MSM 0021620828).

REFERENCES

BOSÁK P., VAŠÁTKO J., CÍLEK V., DUMNICKA E., HANULÁKOVÁ D., HORÁČEK I., JENÍK J., KOPECKÝ J., MARVANOVÁ L., MLEJNEK R., RŮŽIČKA V. and ZACHARDA M. (2001): Czech Republic. – In: Juberthie C. and Decu V. (eds.), Encyclopaedia Biospeleologica, Tom III, p. 1405–1426, Moulis and Bucarest.

CBS Filamentous Fungi Database (2005): http://www.cbs.knaw.nl/

DOMSCH K. H., GAMS W. and ANDERSON T.–H. (1993): Compendium of soil fungi. Vol. I [reprint]. – 860 p. Eching.

DVOŘÁK L. (2000): Poznámky k přezimování motýlů v podzemních úkrytech v oblasti Šumavy a v západních Čechách [Notes on hibernation of *Lepidoptera* species in underground shelters of the Bohemian Forest and of West Bohemia]. – Silva Gabreta 5: 167–178 [in Czech with English summary].

FASSATIOVÁ O. (1970): Micromycetes inhabiting the mines of Příbram (Czechoslovakia). – Čes. Mykol. 24: 162–165.

FASSATIOVÁ O. (1986): Moulds and filamentous fungi in technical microbiology. – 233 p. Amsterdam etc.

GAMS W. (1971): *Cephalosporium*–artige Schimmelpilze (Hyphomycetes). – 262 p. Stuttgart.

GAMS W., de Hoog G. S. and SAMSON R. A. (1984): The hyphomycete genus *Engyodontium* – a link between *Verticillium* and *Aphanocladium*. – Persoonia 12(2): 135–147.

GUNDE–CIMERMAN N., ZALAR P. and JERAM S. (1998): Mycoflora of cave cricket *Troglophilus neglectus* cadavers. – Mycopathologia 141(2): 111–114.

HEITOR F. (1962): Parasitisme de blessure par le champignon *Mucor hiemalis* Wehmer chez les insectes. – Ann. Epiphyties 13(3): 179–203.

HOLEC J. (2001): Vzácné houby z rodů *Cordyceps* a *Phaeocollybia* na Šumavě – rozbor taxonomie a ekologie nových nálezů z Povydří a celkové shrnutí výskytu [Rare fungi of genera *Cordyceps* and *Phaeocollybia* in the Bohemian Forest – taxonomy and ecology of new records from Povydří area and general distribution]. – Silva Gabreta 6: 87–96 [in Czech with English summary].

HOOG G. S. de (1978): Notes on some fungicolous hyphomycetes and their relatives. – Persoonia 10(1): 33–81.

JAVŮRKOVÁ–FASSATIOVÁ O. (1956): O našich entomofágních houbách I. [On our entomophagous fungi I.]. – Univ. Carol., Biol. 2(2): 233–252 [in Czech with English summary].

KAUTMANOVÁ I. (2002): *Cordyceps entomorrhiza* and *Cordyceps tuberculata* (Ascomycetes, *Clavicipitales*) new for Slovakia. – Acta Rer. Natur. Mus. Nat. Slov. Bratislava 48: 40–43.

KJAERANDSEN J. (1993): *Diptera* in mines and other cave systems in southern Norway. – Entomol. Fennica 4: 151–160.

KUBÁTOVÁ A., VÁŇOVÁ A. and PRÁŠIL K. (1998): Contribution to the biodiversity of soil microfungi of the Šumava Mts., Czech Republic. – Silva Gabreta 2: 23–34.

KUBÁTOVÁ A., PRÁŠIL K. and VÁŇOVÁ M. (2005): Půdní mikromycety v prostředí podzemního tunelu – předběžné výsledky [Soil micromycetes in the environment of underground tunnel – preliminary results]. – In: Voříšek K. et al. (eds.), Život v pôde, p. 91–98, Praha and Bratislava [in Czech with English summary].

LAGARDE J. (1913): Biospeleologica. Champignons (1re série). – Arch. Zool. Exp. Gen. 53: 277–307.

LAGARDE J. (1917): Biospeleologica. Champignons (2me série). – Arch. Zool. Exp. Gen. 56: 279–314.

LAGARDE J. (1922): Biospeleologica. Champignons (3me série). – Arch. Zool. Exp. Gen. 60: 593–625.

LANDA Z., HORŇÁK P., OSBORNE L. S., NOVÁKOVÁ A. and BURSOVÁ E. (2001): Entomogenous fungi associated with spruce bark beetle *Ips typographus* L. (*Coleoptera*, *Scolytidae*) in the Bohemian Forest. – Silva Gabreta 6: 259–272.

MAINS E. B. (1958): North American entomogenous species of *Cordyceps*. – Mycologia 50(2): 169–222.

MALLOCH D.: Fungi in the Ramioul cave: http://www.botany.utoronto.ca/ResearchLabs/MallochLab/Malloch/Caves/Ramioul.html

MARVANOVÁ L., KALOUSKOVÁ V., HANULÁKOVÁ D. and SCHÁNĚL L. (1992): Microscopic fungi in the Zbrašov aragonite caves. – Čes. Mykol. 46(4–4): 243–247.

MATOČEC N. and OZIMEC R. (2001): Observation on *Cordyceps riverae* (*Hypocreales*, Ascomycota) in Croatian caves. – Nat. Croat. 10(3): 197–206.

MINÁŘ J. and HÁJKOVÁ Z. (1966): Knowledge on the hibernation of mosquitoes in our territory, especially in South Moravia. – Folia Parasitol. 13: 237–247.

NOVÁKOVÁ A. (2001): Soil saprotrophic micromycetes in Norway spruce forests in the Šumava National Park. – Silva Gabreta 7: 177–184.

PACIONI G. (1978): Un nuovo ascomicete entomogeno rinvenuto in grotta: *Cordyceps riverae*. – Giorn. Bot. Ital. 112: 395–398.

PAPP L. (1981): 54. család: *Heleomyzidae* – Tüskésszárnyú legyek [54th family: *Heleomyzidae*]. – In: Papp L. and Soós Á., Tüskésszárnyú legyek – Szikilegyek. *Heleomyzidae – Tethinidae*, p. 1–77, Budapest [in Hungarian with English summary].

PITT J. I. (1979): The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. – 634 p. London etc.

ROMBACH M. C. and SAMSON R. A. (1983): Insektenschimmels uit Limburgse kalkgrotten. – Natuurhist. Maandblad 72(3): 45–49.

SAMSON R. A., ROMBACH M. C. and SEIFERT K. A. (1984): *Hirsutella guignardii* and *Stilbella kervillei*, two troglobiotic entomogenous hyphomycetes. – Persoonia 12(2): 123–134.

ŠEDIVÝ J. and DVOŘÁK L. (2002): Přezimování lumků ve štolách, jeskyních a sklepech (*Ichneumonidae*, *Hymenoptera*) [Hibernation of ichneumonids in mine galleries, caves and cellars (*Ichneumonidae*, *Hymenoptera*)]. – Sborn. Přírodověd. Klubu v Uher. Hradišti 7: 182–184 [in Czech with English summary].

TZEAN S. S., HSIEH L. S. and WU W. J. (1997): Atlas of entomopathogenic fungi from Taiwan. – 214 p. Taipei.

WEISER J. (1977): An atlas of insect diseases. – 81 + 240 p. Praha.

WEISER J. and BATKO A. (1966): A new parasite of *Culex pipiens* L., *Entomophthora destruens* sp. nov. (Phycomycetes, *Entomophthoraceae*). – Folia Parasitol. 13: 144–149.

ZALAR P., HENNEBERT G. L., GUNDE–CIMERMAN N. and CIMERMAN A. (1997): *Mucor troglophilus*, a new species from cave crickets. – Mycotaxon 65: 507–516.

ZARE R. and GAMS W. (2001): A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. – Nova Hedwigia 73(1–2): 1–50.