

Contribution to the knowledge of the *Protomycetaceae* in Slovakia

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Abstract: The phytopathogenic fungi of the genera *Protomyces* and *Protomycopsis* (Archiascomycetes) are included in the present paper. They are biotrophic on leaves and stems, usually gall-forming. Mycelium is apparently diploid, grows intercellularly, forms thick-walled smooth or ornamented "resting-spores" - ascogenous cells. Detailed symptoms of descriptions on the hosts, anatomical-morphological and biometric characteristics of ascogenous cells, nomenclature, chorological data and notes on the ecology of the three taxa of *Protomyces* and two of *Protomycopsis*, associated with 10 taxa of vascular plants - *Protomyces macrosporus* (6); *Protomyces pachydermus* (1), *Protomyces kriegeianus* (1); *Protomycopsis leontodontis* (1), *Protomycopsis leucanthemi* (1) - are given as well. The new host-fungus combination and new locations of fungi specimens and their host plants in Slovakia are published.

Keywords: Archiascomycetes, *Protomyces*, *Protomycopsis*, biotrophic fungi, biology, ecology, chorology, *Apiaceae*, *Asteraceae*.

Introduction

Members of the *Protomycetaceae* are dimorphic parasites of flowering plants in *Apiaceae* and *Asteraceae*, and represent natural components of phytocenoses. The fungi cause morphological malformations - galls and lesions on stems, leaves and petioles, often with colour changes of infected host tissues. Since these species do not infect important agricultural or horticultural crop plants, the fungi are poorly known. Also the systematic and taxonomic position of fungi has been often discussed. More recent molecular studies confirmed that

Protomycetales are very closely related to *Taphrinales* (SJAMSURIDZAL et al. 1997, SUGIYAMA 1998, PRILLINGER et al. 1990). The authors used inferences from 18S rDNA sequence divergence to set up a new group of *Ascomycetes*, *Archiascomycetes*, containing both specimen of the order *Taphrinales*, genera *Taphrina*, *Protomyces* and *Protomycopsis* (NISHIDA & SUGIYAMA 1994, NISHIDA et al. 1995).

The family of *Protomycetaceae* includes genera: *Burenia*, *Protomyces*, *Protomycopsis*, *Taphridium* and *Volkartia* (BÜREN 1915, 1922, REDDY & KRAMER 1975) and *Mixia* (SALATA 1979, KRAMER 1987). *Taphridium* and *Volkartia* differ morphologically from *Burenia*, *Protomyces* and *Protomycopsis* in formation of their ascogenous cells in a layer just below the upper epidermis of the host leaves. The species of *Burenia*, *Protomyces* and *Protomycopsis* are form the ascogenous cells irregularly in the host tissue. In the case of the genus *Mixia* based on morphological and molecular phylogenetic analyses of *Mixia osmundae* on *Osmunda japonica* questions concerning the developement of endospores of *M. osmundae* and transfer of this species from Ascomycota to the Basidiomycota were answered (NISHIDA et al. 1995).

The species of *Protomyces* and *Protomycopsis* are very similar in forming its ascogenous cells irregularly in the host tissue as well as disorder symptoms on host plants. Mature ascogenous cells of *Protomyces* and *Protomycopsis* show no visible differences. Immature ascogenous cells of *Protomycopsis* are more or less pear-shaped and the walls are papillous. The walls of ascogenous cells of *Protomyces* are smooth.

In Slovakia *Protomycetaceae* is represented only by genera - *Protomyces* UNGER and *Protomycopsis* MAGNUS. The history and distribution of *Protomycetaceae* fungi in Slovak territory were briefly reported by BACIGÁLOVÁ (1991, 1995). The species *Taphridium algeriense* from Slovakia cited by BACIGÁLOVÁ (1995) was proved as *Protomyces macrosporus* on *Carum carvi*. The present paper summarizes new results of the mycofloristic research and reports on some new aspects of biology, ecology, infection symptoms and chorological observations of fungi in ecological conditions of the Slovak territory. The new specimens of *Protomycetaceae* and their host plants for Slovak mycoflora are also presented.

Material and methods

The results are based mainly on the study of materials obtained from own mycofloristic research in Slovakia during the last years (1980-2001), and on the examination of herbarium specimens deposited in the following institutes (acronyms according to HOLMGREN et al. 1990): BRA, TNP, PRM, PRC, BRNM, W.

All collecting site names cited ("specimens studied") are local names within the studied area. For details compare tourist maps of Slovakia 100 – 142, 1:50 000 (published by Vojenský kartografický ústav, Harmanec, Slovakia, 1992-1996).

For identification of the species of the order *Protomycetales* both visual symptoms of infected plants and anatomical-morphological characteristics of the

fungi were used. Taken cross and longitudinal sections from naturally infected leaves or stems were observed in drop of 50% lactic acid. An evaluation was made by means of Zeiss "Amplival" light microscope with microphotographic equipment. The specimens of *Protomyces* and *Protomycopsis* were identified according to BÜREN (1915, 1922), REDY & KRAMER (1975) and SALATA (1979); nomenclature of vascular plants followed MARHOLD & HINDÁK (1998). A list of fungi locations grouped according to their phytogeographical classification (FUTÁK 1966) was compiled. The maps of fungi extension in Slovakia were made followed according to JASIČOVÁ & ZAHRADNÍKOVÁ (1976). Voucher specimens are deposited in the mycological herbarium of the Institute of Botany, Bratislava, Slovakia (SAV).

PROTOMYCES

The species of the genus *Protomyces* are obligate parasites of plants within the *Apiaceae* and *Asteraceae*, causing galls on stems, leaves, flowers and fruits. On the leaves galls are restricted to petiole, midrib, veins and veinlets (Fig. 1, 8).

Spherical to subspherical ascogenous cells with thick and smooth walls are formed intercalarily in the intercellular mycelium throughout the infected tissue.

Members of the *Protomyces* parasitizing on *Apiaceae* (*P. macrosporus*) are known from Europe, South Asia and North Africa. The other species (*P. pachydermus*, *P. kriegeanus*) parasitizing on *Asteraceae* (*Leontodon*, *Taraxacum*) occur only in Europe (KRAMER, 1975, SALATA 1979).

***Protomyces macrosporus* UNGER**

Syn.: *Physoderma gibbosum* WALLR., *Protomyces cari* B. BLYTT

Symptoms: The fungus causes white-green hart galls or round callosities within stem, leaves and fruits tissues (on leaves galls are usually restricted along petiole, veins and veinlets) of *Apiaceae* (*Aegopodium*, *Anthriscus*, *Carum*, *Chaerophyllum*, *Heracleum*, *Ligusticum*) (Fig. 1). In these galls, ascogenous cells are present. The ascogenous cells are formed intercalarily in the intercellular mycelium commonly concentrated along the vascular bundles of the host plant. The cells of surrounding host tissues shown hypertrophy and hyperplasia malformations.

The fact, that species *Protomyces macrosporus* caused infections of various host species of *Apiaceae* evoked numerous discussions about taxonomy of this specimen, (BÜREN 1915, 1922, GJAERUM 1964). Only genotypic identification of isolates from various host plants will prove taxonomical differences of these fungi.

The members of *Protomyces*, predominated by *Protomyces macrosporus* account the major Slovak records of the *Protomycetales* fungi. It occurs on 6 species of host plants (Map 1).

Host: *Aegopodium podagraria*

The fungus induced easily visible whitish galls on the very long green petioles and on the veins of the leaves. Spherical to roughly spherical ascogenous cells are affirmed intercalarily in the intercellular mycelium closely associated with the

conducting tissues within the veins stems and leaves. The size of the ascogenous cells varies between 42-77 µm in diameter (most frequently 64-67µm), with smooth wall, 3-6µm thick (Fig. 2).

The fungus locations and their ecological characteristics: *Aegopodium podagraria* is a common species of synantropic plant communities in various vegetation ranges in Slovakia. *Protomyces macrosporus* was collected on *Aegopodium podagraria* as early as in the last century by KMEŤ at Prenčov in 1978, 1886 (BRA), by HAZSLINSZKY at Prešov (Eperjes), (MOESZ 1939) and by BÄUMLER at Bratislava (Poszony) in 1890 (MOESZ 1939). Our mycofloristic observations confirmed the fungus occurrence mainly on the humid places along the brooks and streams during the whole vegetation season (spring, summer and autumn).

List of locations of studied species: (Map 1) **4.** Záhorská nížina lowlands: Bratislava – Devínska Nová Ves, 15. km. at the left riverside Morava (1993, SAV); **6.** Podunajská nížina lowlands: Hurbanovo (1983, SAV), Bratislava – Petržalka (1985, SAV), Mlyňany in the park (1988, SAV); **10.** Malé Karpaty Mts.: Bratislava – Železná studnička (1987, SAV), Píla (1989, SAV), Borinka at the margin of the forest (1992, SAV), Smolenice in the park (1999, SAV); **14e.** Štiavnické vrchy hills: Prenčov - Babí potok (KMEŤ 1886, BRA), Prenčov – Medzi vršky (KMEŤ 1878, BRA), Banská Štiavnica – surround of water reservoir (2002, SAV); **15.** Slovenské rudohorie Mts.: Kokava nad Rimavicou at the riverside Rimavica (1987, SAV), Revúca (1989, SAV); **21b.** Krivánska Malá Fatra Mts.: Štefanová – Zázrivá by the brook (1998, SAV); **21c.** Veľká Fatra Mts.: Vlkošinec (1989, SAV); **23a.** Západné Tatry Mts.: Roháčska dolina valley (1988, SAV); **23b.** Vysoké Tatry Mts.: Tri studničky along the path (1990, SAV), Hrebienok - along the old cableway (2000, SAV), Tatranská Lomnica, in the park (1988, 1999, 2000, 2001, omnia SAV), Starý Smokovec along the path (1988, SAV), Tatranská Polianka along the path (1990, 2000, 2001, SAV); **23c.** Belianske Tatry Mts.: Dolina Kežmarskej Bielej vody valley (1987, 1999, 2001, SAV), Kežmarské žľaby - blue tourist mark, (2001, SAV), Monkova dolina valley, (2001, SAV); **26a.** Liptovská kotlina hollow: Východná, in the village by the brook (1987, SAV); **28.** Západné Beskydy Mts.: Kysuce lazy Čierne (1987, 1989, (SAV), omnia (SAV) leg. et det. K. BACIGÁLOVÁ.

Host: ***Anthriscus sylvestris***

Galls of *Protomyces macrosporus* are very difficult to find out on flowering plants of *Anthriscus sylvestris*, but during the spring (in March), young new leaves emerge, often distorted by galls of *Protomyces macrosporus*. These symptoms can be seen before the young leaves turn green or greenish purple (Fig. 1).

Entire galls contain very thick walled ascogenous cells, closely associated with the vascular tissues of the host plant. Ascogenous cells are formed intercalarily in the intercellular mycelium. They are spherical to roughly spherical with size of 50-70µm (the most frequently 65 µm), with light yellow-brown smooth wall, 4-5 µm thick (Fig. 3). The ascogenous cells are known to produce vesicles and ascospores, but none have been seen to germinate on natural material of *Anthriscus sylvestris*.

The fungus locations and their ecological characteristics: *Anthriscus sylvestris* is a common species of synantropic plant communities in various vegetations ranges of the Slovak territory until now, but *Protomyces macrosporus* on *Anthriscus sylvestris* has not been collected in ecological conditions of Slovakia. The first one and new locations were found on *Anthriscus sylvestris* along the roadside Dúbravská cesta street (March 31, 2003), Rusovce (April 13, 2003) and along the path in Devínska Kobyla (April 20, 2003), omnia leg. et det. K. BACIGÁLOVÁ (SAV) (Map1).

We assume, the fungus species occurs also in another part of the Slovak territory and has been overlooked investigated until now on *Anthriscus sylvestris*.

Host: *Ligusticum mutellina*

The fungus causes callosities and hard whitish galls on the stems, petioles and on the veins of the leaves. In these callosities ascogenous cells are present. Clumsy-globoid ascogenous cells are formed intercalarily in the intercellular mycelium throughout the infected host conducting tissues. The size of ascogenous cells is 44-72 µm (most frequently 51-56 µm) in diameter with smooth wall, 4-5 µm thick (Fig. 4).

The fungus locations and their ecological characteristics: The first locations of *Protomyces macrosporus* on *Ligusticum mutellina* in the Slovak territory were found during our mycofloristic observation in Vysoké Tatry and Nízke Tatry Mts. (BACIGÁLOVÁ 1991, 1995). The locations were situated along a tourist path, strictly only in the vegetation range at 1 500 m above sea level up to higher level. The fungus grew together with host plant and symptoms were visible within June, July and August. This host- fungus combination was recorded also in Polish part of the Tatra Mts. (SALATA 1979).

List of locations of studied specimen: (Map 1) **22.** Nízke Tatry Mts.: Chopok-Ďumbier Mt., along the tourist path (1985 SAV); **23a.** Západné Tatry Mts.: Tomanova dolina valley (1998 SAV), Tomanové sedlo saddle-back 1680 m (1999 SAV), Kôprová dolina valley (1987 SAV), Tichá dolina valley – Ľaliové sedlo saddle-back (1987 SAV), Tichá dolina valley - Kasprov vrch Mt. 1600 m (1999 SAV), Temnosmrečinová dolina valley 1674 m (1998 SAV), Kamenistá dolina valley 1440 m (1993, 1999, 2000, 2001 omnia SAV); **23b.** Vysoké Tatry Mts: Furkotská dolina valley 1800 m (1985, 1990 SAV), Mlynická dolina valley (1987, 1991 SAV), Dolina Zlomísk valley 1690 - 1830 m (1990, 1991, 1999, 2000, 2001 omnia SAV), Ostrva Mt. 1700 m (1991, 2000 SAV), Velická dolina valley above the Velické pleso lake 1700 m (1989, 1990, 1999, 2000 omnia SAV), Batizovské pleso lake (2001, SAV), Mengušovská dolina valley, Hincove plesá lakes - Kôprovské sedlo saddle-back (1990, 1999 SAV), Lomnické sedlo saddle-back 1850 m (1990, 2001, SAV), Dolina Kežmarskej Bielej vody valley 1500 m (1987, 1999, 2000, SAV); **23 c.** Belianske Tatry Mts.: Zelené pleso lake 1550 m (1989, 1990, 1999, 2000, omnia SAV). All herbarium items (SAV) were leg. et det. by K. BACIGÁLOVÁ.

Host: ***Heracleum sphondylium***

The fungus causes hard whitish galls on the long and bulky petioles and on the veins of the leaves. In these galls ascogenous cells are present.

Clumsy-round ascogenous cells are formed intercalary in the intercellular mycelium throughout the infected conducting host tissue. The ascogenous cells are 39-77 μm (the most frequently 61-64 μm) in diameter, with smooth wall 5-6 μm thick. Frequently, two ascogenous cells in pair are formed (Fig. 5).

The fungus locations and their ecological characteristics: The *Heracleum sphondylium* is a common species of plant communities in various vegetations ranges of Slovak territory, but the host-fungus combination occurred very rarely. The fungus was not found in the location detected by KMEŤ (1886, BRA), but new locations were found during mycofloristic observation situated only in mountain vegetation range at altitude of 1180 m. The fungus grew with the host thoroughly and visual symptoms were presented mainly during June and July. We assumed that ecological conditions of fungus location (humidity, temperature and altitude) were very important ecological factors for its live cycle on *Heracleum sphondylium*.

List of locations of studied species: (Map 1) **14e.** Štiavnické vrchy hills: Prenčov – Babí potok (KMEŤ 1886, BRA), **21b.** Malá Fatra (Krivánska Fatra) Mts.: Štefanová – Skalné mesto Mt., 1180 m (1998, SAV); **23a.** Západné Tatry Mts.: Javorový žľab valley 1300 m (1998, 1999 SAV); **23c.** Belianske Tatry Mts.: Dolina Siedmich prameňov valley (1999, SAV); omnia (SAV) leg. et det. K. BACIGÁLOVÁ.

Host: ***Chaerophyllum hirsutum***

Fungus causes white-green callosities on the stems, petioles and the veins of the leaves of *Chaerophyllum hirsutum*. In these callosities ascogenous cells are formed. The ascogenous cells are formed intercalarily in the intercellular mycelium restricted along conducting host tissue (veins and vessel). The ascogenous cells are globoid or broadly elliptical, with 45-71 μm (most frequently 58-64 μm) in diameter, with smooth hyaline wall, 5-6 μm thick (Fig. 6).

The fungus locations and their ecological characteristics: *Chaerophyllum hirsutum* is a very common species of plant communities near the roads, along the brooks, outside the woods and humid places in various vegetations ranges. The first locations of this host-fungus combination (*Protomyces macrosporus* on *Chaerophyllum hirsutum*) for Slovakia were recorded during our mycofloristic observations in the mountain vegetation ranges. The visual symptoms are presented during the June and July. Lately, the fungus causes dying away of the whole host plants. We assumed, that ecological conditions (humidity, temperature, altitude) were important for the live cycle of this fungus.

List of locations of studied specimens: (Map 1) **21a.** Malá Fatra (Krivánska Fatra) Mts.: Štefanová 625 m at brook in the village (1998, SAV); **23a.** Západné Tatry Mts.: Kamenistá dolina valley 1500 m (1999, SAV); **23b.** Vysoké Tatry Mts.: Malá studená dolina valley (1990, SAV), Tatranská Lomnica in the park, (1989, SAV); **23c.** Belianske Tatry Mts.: Dolina Siedmich prameňov valley (1999,

SAV), Monkova dolina valley (1998, 2000, SAV); **28.** Západné Beskydy Mts.: Kysuce, lazy Čierne (1995, SAV); omnia (SAV) leg. et det. K. BACIGÁLOVÁ.

Host: ***Carum carvi***

Protomyces macrosporus causes brown-orange round galls on the stems and leaves of *Carum carvi* (Fig. 1). In these galls ascogenous cells are present.

Ascogenous cells are formed intercalarily in the intercellular mycelium within the host tissue. They are globoid or broadly elliptical, 38-56 µm (the most frequently 40-50) µm in diameter, with smooth brown-orange coloured cells wall, 2-3 µm thick (Fig. 7).

The species *Protomyces macrosporus* on *Carum carvi* recorded by ZAVŘEL in Malá Fatra Mts. expected their occurrence in Slovakia as a new host plant-fungus combination. *Protomyces macrosporus* on *Carum carvi* has been identified only from Switzerland (BÜREN 1915, 1922) and from Norway (GJAERUM 1964) until now.

Location of studied specimen: (Map 1). **21b.** Malá Fatra (Krivánska Fatra) Mts.: Terchová, 570 m (leg. et det. ZAVŘEL, 1948, BRA).

Observations

Species identification of *Protomycetaceae* was carried out on the basis of morphological features, such as the size and shape of ascogenous cells, wall thickness of ascogenous cells, habit of mycelium, and pathological effect on host plant tissues (BÜREN 1915, 1922, GJAERUM 1964, SALATA 1964, KRAMER 1979). *Protomyces macrosporus* and its ability inoculated many species from family *Apiaceae* have been the research subject of mycologists for a long time. Based on inoculation experiments, BÜREN (1915, 1922) recognized 7 "formae speciales" of *P. macrosporus* (f. sp. *aegopodii*, f. sp. *chaerophyli*, f. sp. *carvi* and s.v.). Because no inoculation experiments have been carried out in any other countries and the diameters of ascogenous cells (Tab. 1.) do not support the creation of a new species, we accept created species *Protomyces macrosporus*, although BÜREN (1922), KRAMER (1975), SALATA (1979) in their monographs already described species of *Protomycetaceae* phenotypically. We suppose, the recent taxonomic studies (NISHIDA et al. 1993, SJAMURIDZAL et al. 1997) of the genus *Protomyces* using the methods of molecular biology help elucidate many discussed taxonomic and phylogenetic problems.

Tab. 1. *Protomyces macrosporus* and their host plants in some European countries.

Host	Slovakia	Switzerland BÜREN (1922)	Poland SALATA (1979)	Norway GJAERUM (1964)	Great Britain PREECE & HICK (2001)
Size of the ascogenous cells in µm					
<i>Aegopodium podagraria</i>	42-72 (64-67)	50-70	35-80 (50-60)	31-66	+
<i>Anthriscus sylvestris</i>	50-70 (65)	+	+	31-61	+
<i>Chaerophyllum hirsutum</i>	45-71 (58-64)	+	+	-	-
<i>Heracleum sphondylium</i>	37-77 (61-64)	+	+	-	-
<i>Ligusticum mutellina</i>	44-72 (51-56)	+	+	-	-
<i>Carum carvi</i>	38-56 (40-50)	+	-	31-66	-

+ = host / fungus combination is present

- = host / fungus combination was not found

***Protomyces pachydermus* THÜM.**

Host: ***Taraxacum officinale***

Symptoms: The fungus causes swellings and hard galls on peduncle and on the leaves along the main vein, often 2-15 mm long in diameter. The galls are limpid, white green to yellow or violet. Intensive fungus infections on the leaves are easily recognizable by the violet-brown or yellow coloured spots with a network of swollen veins and veinlets (Fig. 8).

The multinucleate mycelium with septa invades the host tissue intercellularly penetrating throughout all tissues and concentrates along the vascular bundles. The ascogenous cells are formed intercalarily in the intercellular mycelium. They are spherical to roughly spherical or elliptic 23-42 µm, (most frequently 25-35µm) in diameter, with smooth, yellow coloured wall, 2-6 µm thick (Fig. 9).

The fungus locations and their ecological characteristics: The host plant *Taraxacum officinale* is very often infected with *Protomyces pachydermus* mostly during humid vegetation season in various vegetation ranges. The first fungus locations founded by KMEŤ 1887 (BRA), were proved in the Štiavnické vrchy hills. Their occurrence in the Slovak territory is being confirmed during mycofloristic research every year (Map 2). The "white places" in the map of Slovakia (mainly the east part) here been poorly studied until now.

List of locations of studied species: **5.** Devínska Kobyla hill: Bratislava in the garden of Institute of Botany SAV (1990, 1991, 1992, 1993, 1999, 2000, 2001); **6.** Podunajská nížina lowland: Bratislava-Rusovce in the park (1991), Bratislava-Petržalka at the Danube riverside (1991), Veľký Grob in the garden (1991); **10.** Malé Karpaty Mts.: Dobrá voda (1989); **14e.** Štiavnické vrchy hills: Prenčov (KMEŤ 1887 BRA); **15.** Slovenské rudohorie: Revúca, at the brook (1989); **21a.** Malá

Fatra Mts.: Štefanová near the tourist path in Kreminná dolina valley (1998), Štefanová on the meadow 644 m (1998); **22.** Nízke Tatry Mts.: Krížna-Turecká Mt., along the tourist path (1987); **23b.** Štrbské pleso lake 1400 m (1990, 1991), Velická dolina valley (1989), along the tourist path to Popradské pleso lake (2000), Dolina Zlomísk valley (1991), Ostrva Mt. along the tourist path (1991), Starý Smokovec (1988); omnia (SAV) leg. et det. K. BACIGÁLOVÁ.

***Protomyces kriegeerianus* BÜREN**

Host: *Leontodon hispidus*

Symptoms: The fungus causes swellings and galls along the main vein on the leaves, or easily recognizable yellow, red or violet brown coloured spots with a network of swollen vein or veinlets (Fig. 8).

The mycelium invades the host tissue intercellularly, penetrating throughout all tissues and concentrates along the vascular bundles. The ascogenous cells are formed intercalarily in the intercellular mycelium. They are spherical to roughly spherical or elliptic, 32-55 µm in diameter (most frequently 35-39 µm), with smooth yellow coloured wall, 4-6 µm thick (Fig. 10).

The fungus locations and their ecological characteristic: The first locations of *Protomyces kriegeerianus* on *Leontodon hispidus* in Slovakia were found during our mycofloristic studies (BACIGÁLOVÁ 1995). The further observations confirmed quite common occurrence of this host fungus combination in various vegetation ranges in Slovakia (Map 2).

List of locations of studied species: 5. Devínska Kobyla hill: Bratislava, in the garden of Institute of Botany SAV (1990, 1991, 1992, 1993, 1994, 1998, 1999, 2000, 2001); 10. Malé Karpaty Mts.: Dobrá voda valley (1989); 21a. Malá Fatra Mts.: Bystričianska dolina valley, Štefanová near the path in Kreminná dolina valley, Štefanová under Rozsutec Mt., Štefanová – Kopiská, Vrátna dolina valley on the north part of Stoh Mt., Štefanová near the path Šlahorka-Medziholie, Štefanová on the moist meadow near Biele skaly, Štefanová near the path to Podžiar (1998); 21c. Veľká Fatra Mts.: Selenec valley (1994) 23a. Západné Tatry Mts.: Tomanova dolina valley (1998), Kôprová dolina valley (1998), Tichá dolina valley –Kasprov vrch (1999); 23b. Vysoké Tatry Mts.: Mlynická dolina valley (1998), Dolina Bielej vody Kežmarskej valley (2001); omnia (SAV) leg. et det. K. BACIGÁLOVÁ.

Observations

BÜREN (1915, 1922) created species *P. pachydermus*, *P. kriegeerianus* and other species of *Protomycetaceae* on the basis of differences seen in pathogenicity studies and in variation in size of ascogenous cells and size of ascus –“vesicle”. On the other hand, the variations shapes and size of ascogenous cells in the species of *P. pachydermus*, *P. kriegeerianus*, *P. kreuthensis*, *P. picridis* and others species parasitizing on *Asteraceae* considered KRAMER (1975) as synonymous to *P. pachydermus*. Our observations were not in confirmation with KRAMER'S conclusions and we agree with conception of SAĽATA (1979). We suppose the taxonomic studies based on methods of molecular biology help elucidate discussed taxonomic problems of these fungi on *Asteraceae*.

Tab. 2. *Protomyces pachydermus* and *Protomyces kriegarianus* and their hosts in some European countries.

Fungus/host combination	Slovakia	Poland SAŁATA (1979)	Norway GJAERUM (1964)	Switzerland BÜREN (1922)
Size of the ascogenous cells in μm				
<i>P. pachydermus</i> /	23-42	23-44	22-46	30-45
<i>Taraxacum</i> sp.	(25-35)	(32-42)		
<i>P. kriegarianus</i> /	32-55	30-48	-	36
<i>Leontodon hispidus</i>	(35-39)	(30-40)		(26-52)

- = host / fungus combination was not found

PROTOMYCOPSIS

The specimens of the genus *Protomyopsis* are obligate parasites of plants in the *Asteraceae*, causing swellings and slightly raised coloured spots on the leaves (Fig. 8). Ascogenous cells are formed terminally in the intercellular mycelium throughout the infected tissues. They are spherical to subspherical, light yellowish-brown with characteristic rough-wall (echinaceous) at least in young stage (Figs. 11 and 12).

The specimens of *Protomyopsis* are very rare not only in Europe (GJAERUM 1964, SAŁATA 1979, REDY & KRAMER 1975, PREECE & HICK 2001). *Protomyopsis leucanthemi* was identified on *Leucanthemum vulgare* mainly (GJAERUM 1964, SAŁATA 1979, REDY & KRAMER 1975, PREECE & HICK 2001). The specimen *P. leucanthemi* on *Leucanthemum vulgare* from Slovakia cited by BACIGÁLOVÁ (1995) was proved as *P. leucanthemi* on *Pyrethrum clusii* (syn. *Leucanthemum corymbosum* ssp. *clusii*).

Protomyopsis leucanthemi MAGNUS

Syn. *Entyloma leucanthemi* SYD. et P. SYD.

Host: *Pyrethrum clusii* (syn. *Leucanthemum corymbosum* subsp. *clusii*, *Tanacetum corymbosum* subsp. *clusii*)

Symptoms: The fungus causes swellings and slightly raised, yellow to brown indistinctly limited spots, hypophyllously on the leaf (Fig. 8).

Thin mycelium growth in the intercellular spaces throughout the infected leaf parenchyma and along veins of leaf tissues. Spherical to subspherical, light yellow ascogenous cells are formed terminally on the mycelium. They are 35 – 74 μm in diameter (the most frequently 48-58 μm), with rough-wall, 6-8 μm thick (Fig. 11). The exosporium of the walls is echinaceous (verrucose) with small colourless lumps. In contrast to *Protomyopsis leontodontis* the wall of ascogenous cells of *Protomyopsis leucanthemi* are echinaceous also in matured stage, not only in the young one.

The fungus locations and their ecological characteristics: Host species *Pyrethrum clusii* grow only on locations from submontane slopes to higher altitudes on carbonate and silicate rocks as well as the limestone in the Vysoké and Západné Tatry Mts. *Protomyopsis leucanthemi* was found on *Pyrethrum clusii* near the tourist path in two valleys with cold and humid condition during the spring (June – early July).

List of locations of studied specimens: (Map 3) **23a.** Západné Tatry Mts.: Kamenistá dolina valley 1240 m (1988, 1993, 2003) (SAV); **23b.** Vysoké Tatry Mts.: Dolina Bielej vody Kežmarskej valley 1200 m (1995) (SAV), leg. et det. K. BACIGÁLOVÁ.

***Protomyopsis leontodontis* BÜREN**

Host. *Leontodon hispidus* subsp. *danubialis*

Symptoms: The fungus causes small galls on veins with yellowish-grey slightly raised spots on the leaves (Fig. 8).

Ascogenous cells are formed terminally on the intercellular mycelium occurring throughout the veins of the leaf tissue. They are round to oval 29-48µm in diameter, but most frequently 32-42 µm, and wall 3-5µm thick. On young ascospores the small papillae on the walls are formed (Fig. 12).

The fungus locations and their ecological characteristics: Host plant of this fungus (*Leontodon hispidus* subsp. *danubialis*) is one of the most widespread plants in various vegetation ranges (lowlands, hills, mountains) in Slovakia, but the host-fungus combination were found only on locations in subalpine or alpine mountain range. Similarly as for the species *Protomyopsis leucanthemi* and some other specimens of *Protomycetaceae*, ecological condition of the high mountains (extreme cold during the winter, cold and humid conditions during the spring, May, June), are favourable for growth and development of this fungus specimen (*Protomyopsis leontodontis*) on *Leontodon hispidus* subsp. *danubialis* only in this region.

List of locations of studied specimen: (Map 3) **23a.** Západné Tatry Mts.: Tichá dolina valley (1988), Kamenistá dolina valley, 1240 m along the tourist path (2003) (SAV); **23b.** Vysoké Tatry Mts.: Štrbské pleso, vodopád Skok (1990), Mlynická dolina valley (1991), Štrbské pleso, 1400 m (2000); omnia (SAV) leg. et det. K. BACIGÁLOVÁ.

This host-fungus combination is new not only for mycoflora of Slovakia, but also for the whole West Carpathian Mts. region. The fungus has been known only from Switzerland on *Leontodon montanus* until now (BÜREN 1922).

Tab. 3. Species of *Protomycopsis* and their host plants in some European countries.

Host/fungus combination	Slovakia	Poland SAŁATA (1979)	Norway GJAERUM (1964)	Switzerland BÜREN (1922)
Size of the ascogenous cells in μm				
<i>P. leucanthemi</i> / <i>Pyrethrum clusii</i>	35-74 (48-58)	-	-	-
<i>P. leucanthemi</i> / <i>Leucanthemum vulgare</i> / <i>Chrysanthemum leucanthemum</i>	-	24-61 (36-45)	27-51	35 (22-48)
<i>P. leontodontis</i> / <i>Leontodon hispidus</i> subsp. <i>danubialis</i>	29-48 (32-42)	-	-	+
<i>P. leontodontis</i> / <i>Leontodon autumnalis</i>	-	34 (30-41)	-	-

+ = host fungus combination was found

- = host fungus combination was not found

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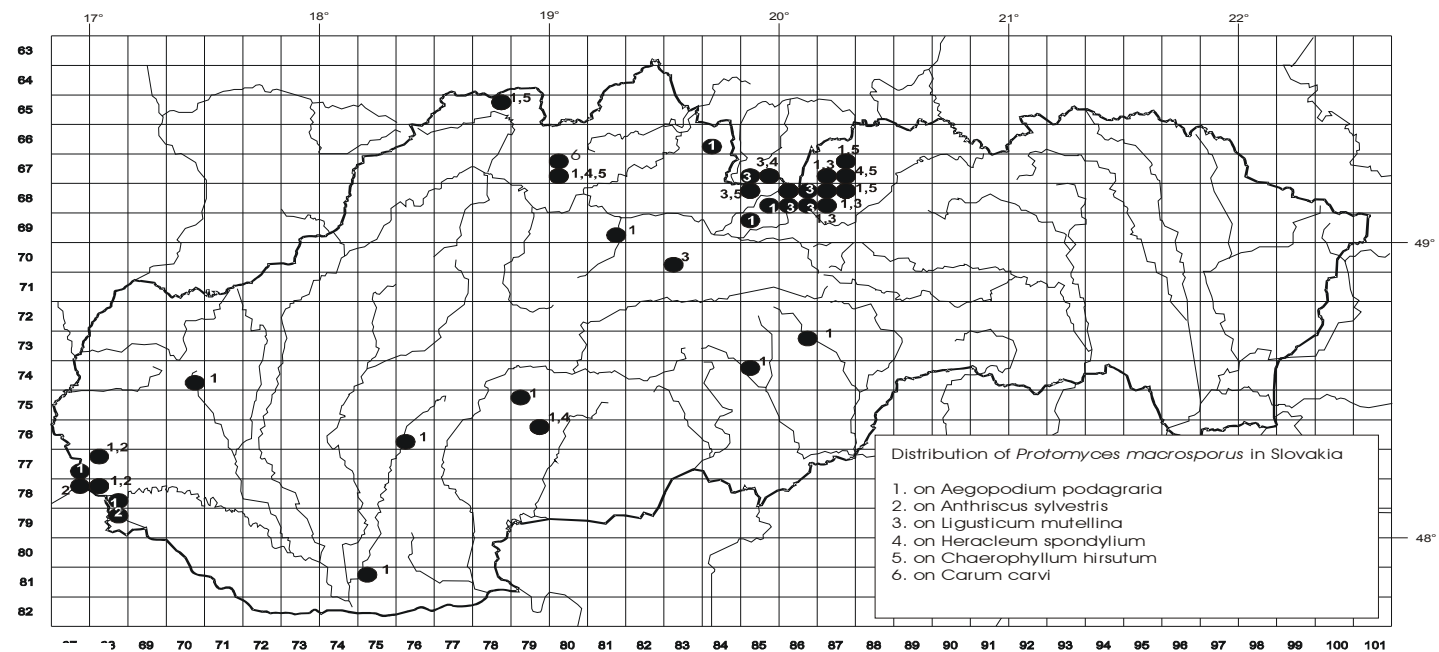
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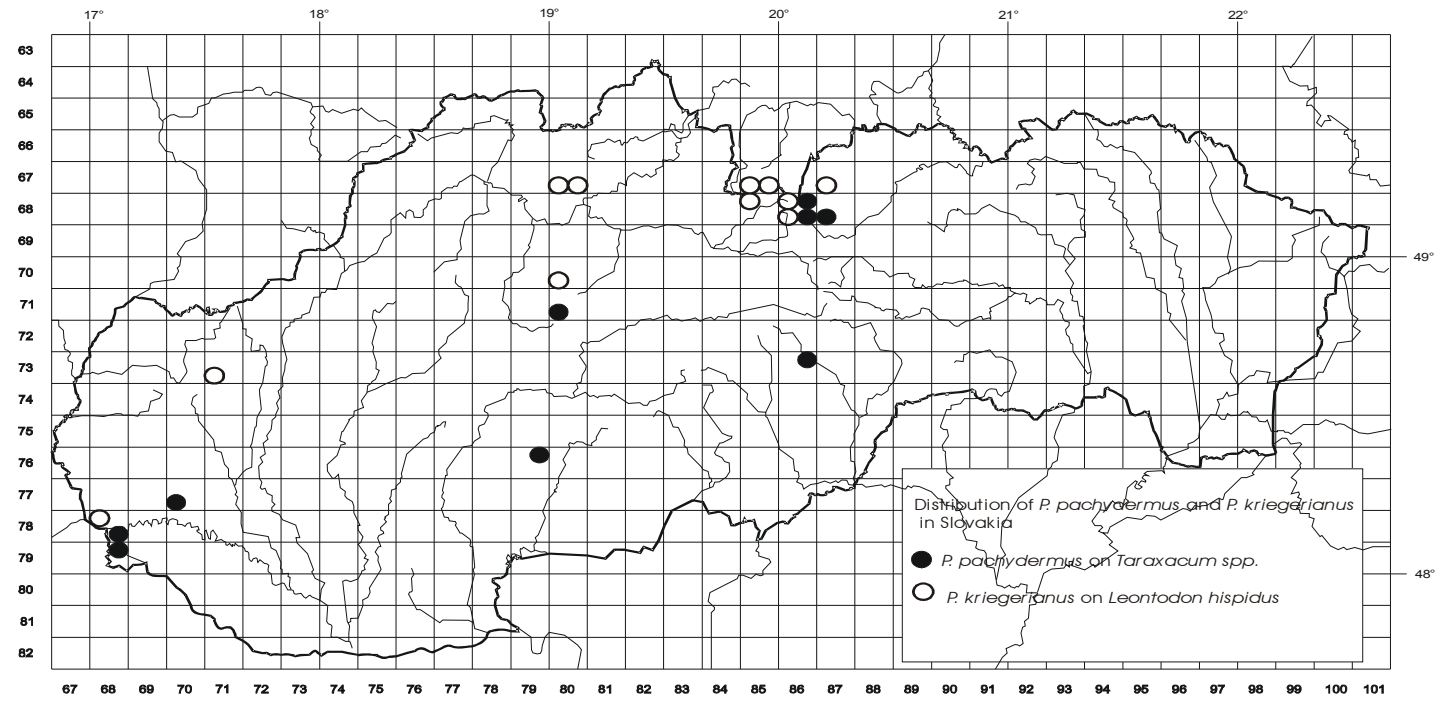
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Map 1. Distribution of *Protomyces macrosporus* in Slovakia.

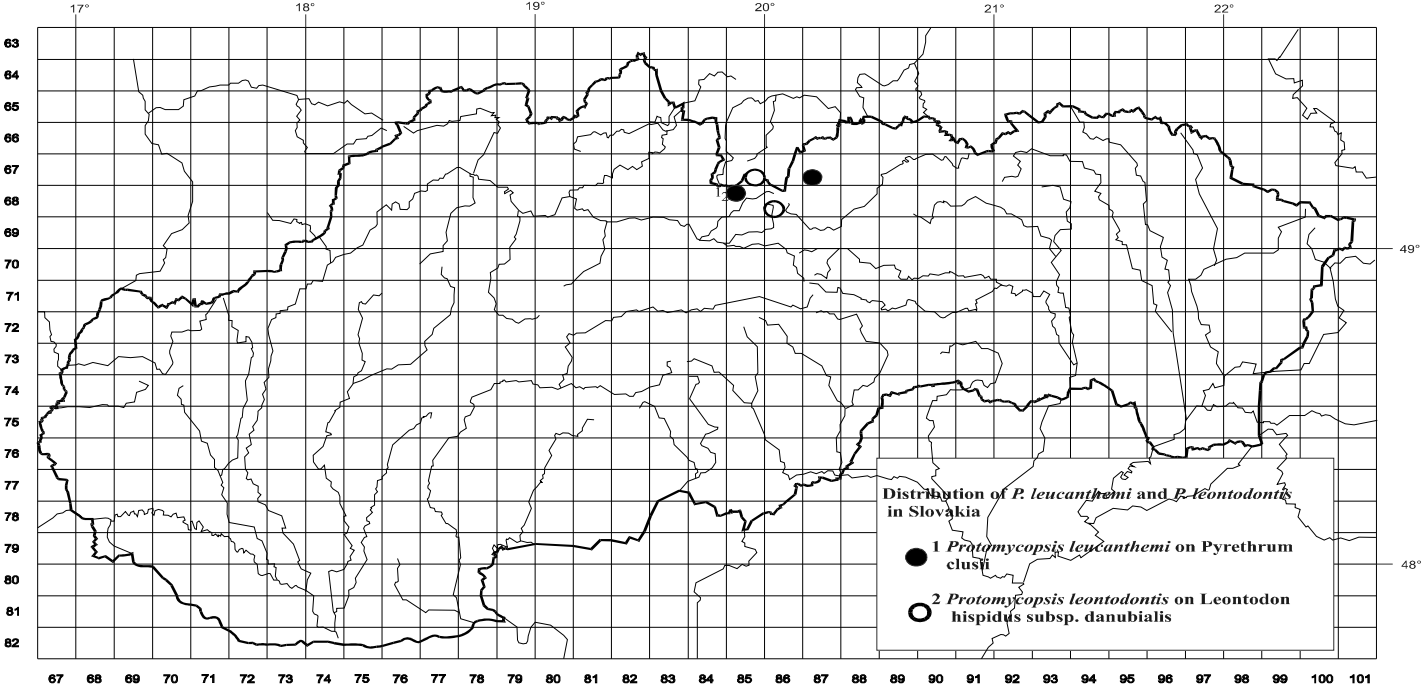


Map 1.

Map 2. Distribution of *Protomyces pachydermus* and *Protomyces krieglerianus* in Slovakia.



Map 3. Distribution of *Protomyropsis leucanthemi* and *Protomyropsis leontodontis* in Slovakia.



map 3.

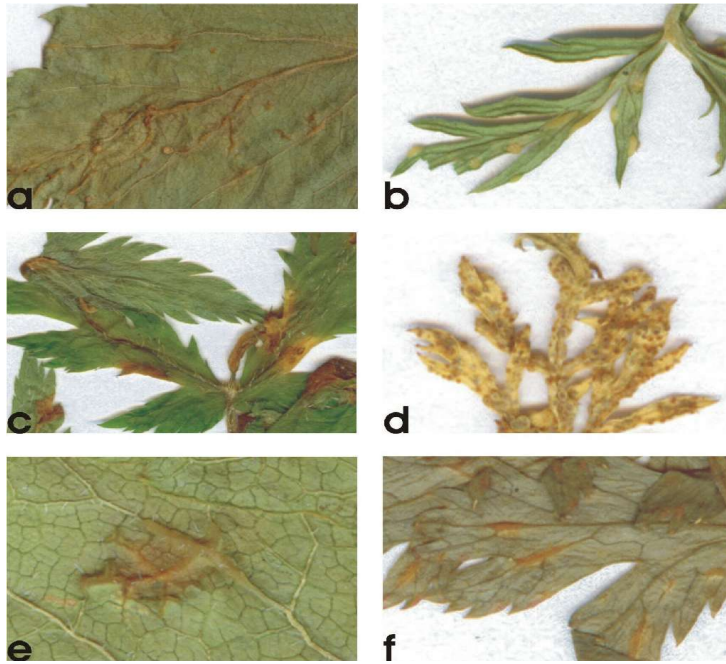


Fig. 1. *Protomyces macrosporus*: galls on leaf veins of: a) *Aegopodium podagraria* (Smolenice, 1999, SAV), b) *Ligusticum mutellina* (Kamenistá dolina, 2000, SAV), c) *Chaerophyllum hirsutum* (Monkova dolina, 1998, SAV), d) *Carum carvi* (Terchová, 1948, BRA), e) *Heracleum sphondylium* (Štefanová-Skalné mesto, 1998, SAV), f) *Anthriscus sylvestris* (Devínska Kobyla, 2003, SAV).

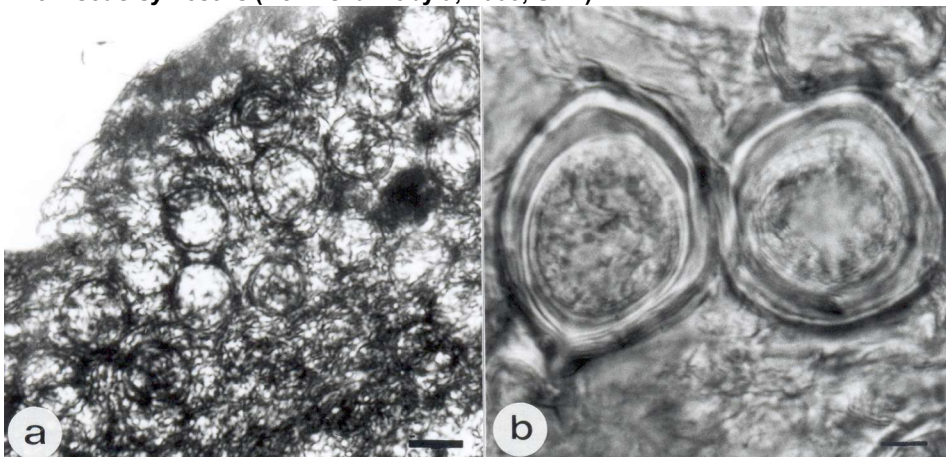


Fig. 2. *Protomyces macrosporus* within the leaves of *Aegopodium podagraria*
a) relationship of the entire gall with ascogenous cells near the vascular tissue. Bar = 100 µm
b) ascogenous cells *in situ*. Remnants of hyphae can be seen, showing their intercalary position. Bar = 10 µm

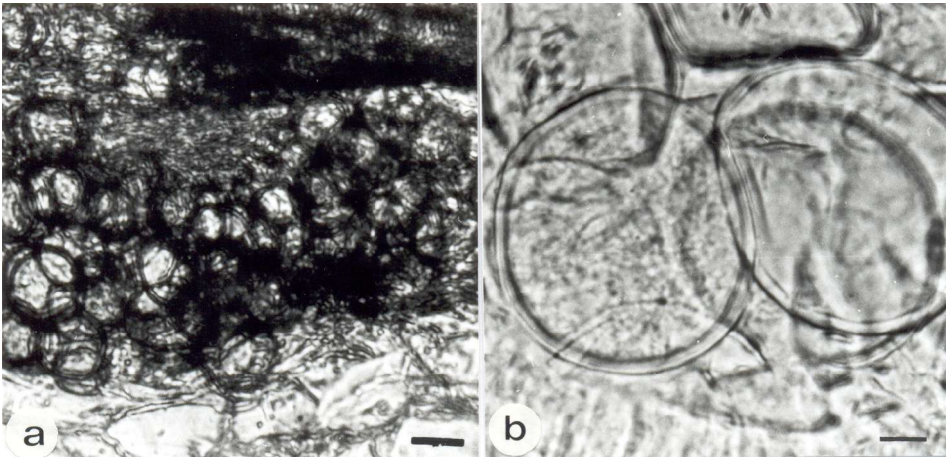


Fig. 3. *Protomyces macrosporus* within the leaves of *Anthriscus sylvestris*
a) relationship of the entire gall with ascogenous cells near vascular tissue.
Bar =100 µm
b) ascogenous cells. Bar =10 µm

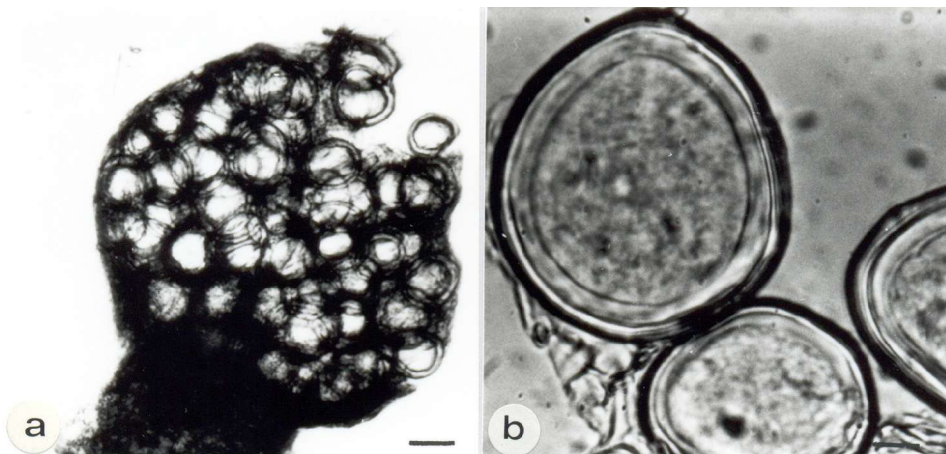


Fig. 4. *Protomyces macrosporus* within the leaves of *Ligusticum mutellina*
a) cross section of the gall. Bar = 100 µm
b)ascogenous cells. Bar =10 µm

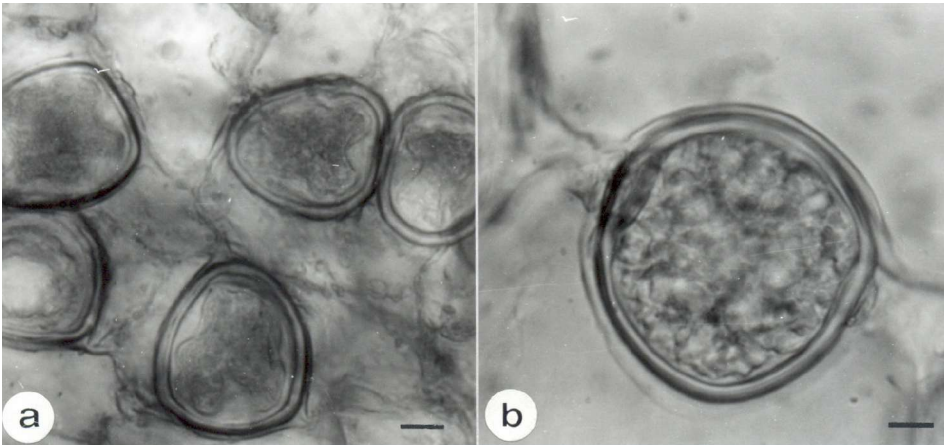


Fig. 5. *Protomyces macrosporus* within the leaves of *Heracleum sphondylium*

- a) relationship of the entire gall with ascogenous cells near vascular tissue. Bar = 100µm
- b) ascogenous cell *in situ*. Remnants of hyphae can be seen showing their intercalary position. Bar = 10µm

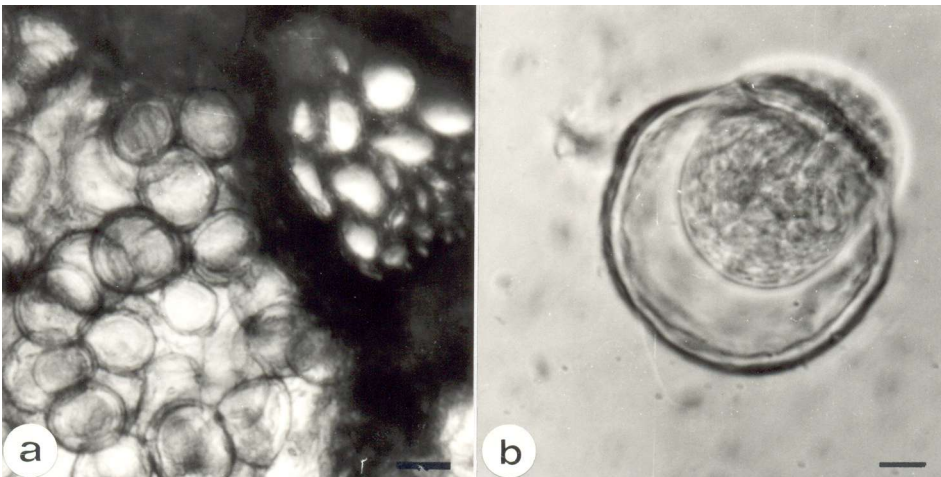


Fig. 6. *Protomyces macrosporus* within the leaves of *Chaerophyllum hirsutum*

- a) relationship of the entire gall with ascogenous cells near the vascular tissue. Bar = 100µm
- b) ascogenous cell in the stage of "vesicule - ascus" emergence. Bar = 10µm

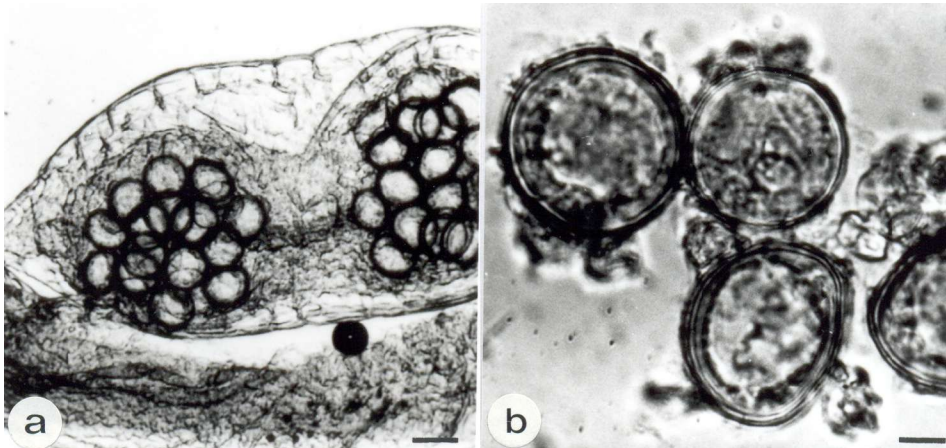


Fig. 7. *Protomyces macrosporus* within the leaves of *Carum carvi*
a) cross section of the galls. Bar = 100 μ m
b) ascogenous cells. Bar = 10 μ m

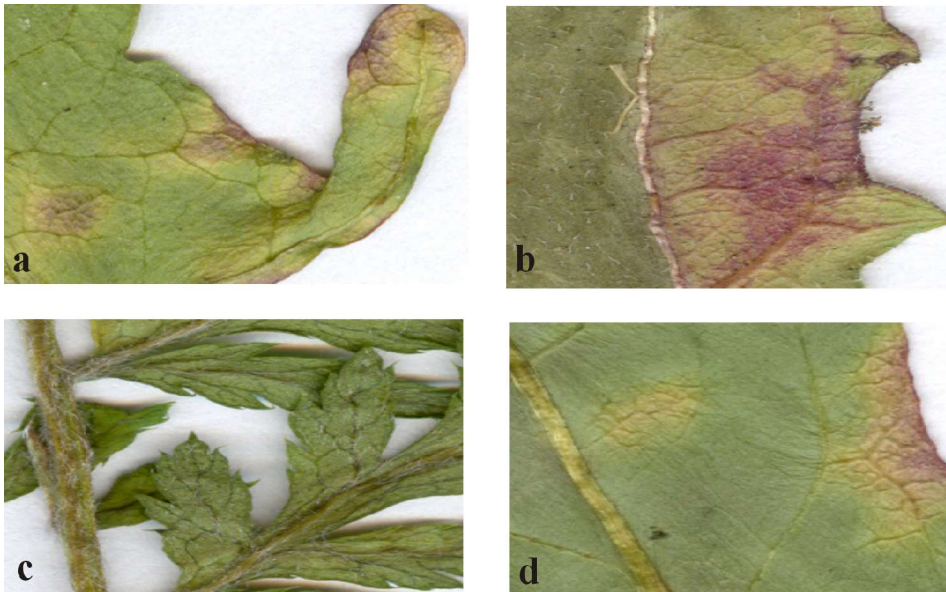


Fig. 8. a) *Protomyces pachydermus*: galls on leaf veins of *Taraxacum officinale*, b) *Protomyces kriegeerianus*: galls on leaf veins of *Leontodon hispidus*, (Bratislava, garden of Inst. of Botany, 2000), c) *Protomycopsis leucanthemi*: galls on leaf veins and stems of *Pyretrum clusii* (Kamenistá dolina, 2003, SAV), d) *Protomycopsis leontodontis*: galls on leaf veins of *Leontodon hispidus* subsp. *danubialis* (Štrbské pleso, 2000, SAV).

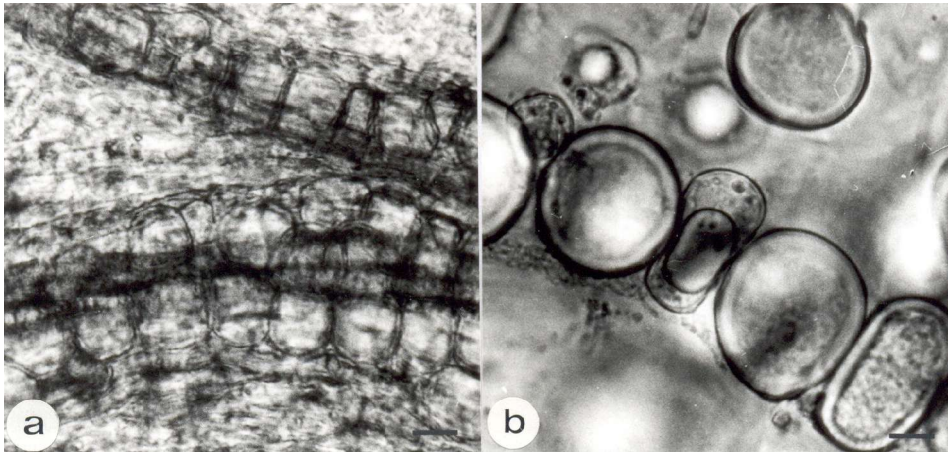


Fig. 9. *Protomyces pachydermus* within the leaf's vascular tissue of *Taraxacum officinale*

- a) cross section of the gall along the leaf vein. Bar = 10 μ m
- b) ascogenous cells in differently matured stage. Bar = 10 μ m

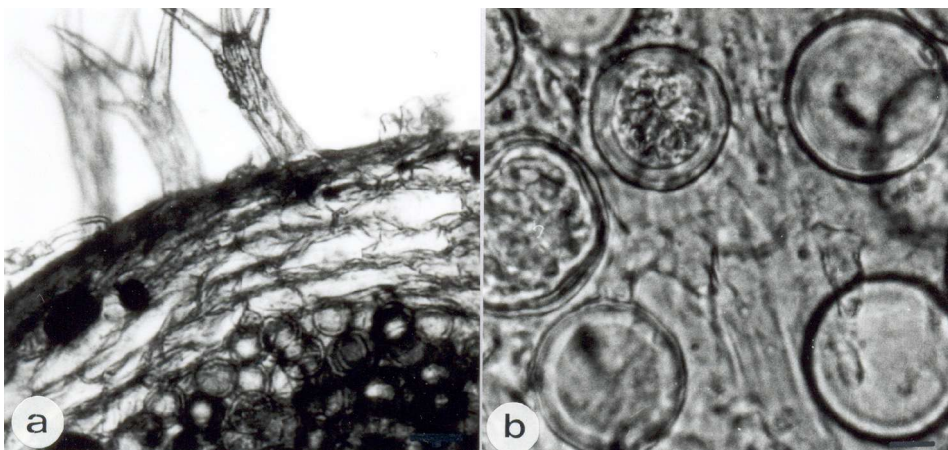


Fig. 10. *Protomyces kriegeerianus* on *Leontodon hispidus*

- a) cross section of the gall on the leaf vein. Bar = 100 μ m
- b) ascogenous cels. Bar = 10 μ m

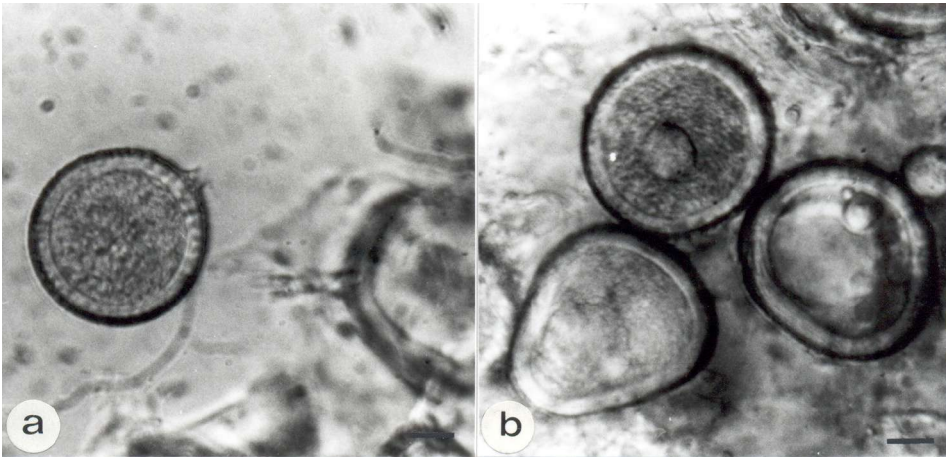


Fig. 11. *Protomycopisia leucanthemi* on *Pyrethrum clusii*

- a) echinaceous ascogenous cell in young stadium. Remnants of hyphae can be seen, showing terminal position. Bar = 10µm
- b) ascogenous cells. The wall of ascogenous cells remains echinaceous also during the abundant stadium. Bar = 10µm

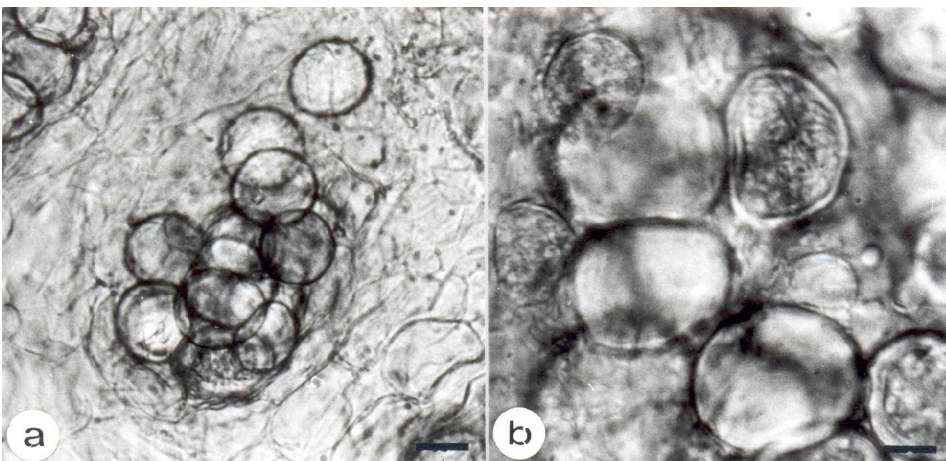


Fig. 12. *Protomycopisia leontodontis* on *Leontodon hispidus* subsp. *danubialis*

- a) cross section of gall on the leaf vein. Bar = 100µm
- b) ascogenous cells. The ascogenous cells are slightly echinaceous during the young stadium. Bar = 10µm.