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NOVELTIES OF PROTOMYCETACEAE IN THE TATRA MTS

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Abstract. Two interesting species of *Protomyces* Unger collected recently in the Tatra Mts are described, illustrated in detail and compared with similar taxa. *Protomyces crepidis-paludosae* Büren on *Crepis paludosa* (L.) Moench is a new species for Slovakia. *Protomyces macrosporus* Unger on *Laserpitium latifolium* L. is a new fungus/host combination in the Carpathians. *Protomyces macrosporus* Unger on *Carum carvi* L. is reported from the first locality in the Slovak part of the Tatra Mts.

Key words: microfungi, biology, ecology, Carpathian Mts, Poland, Slovakia

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INTRODUCTION

Species of the genus *Protomyces* Unger (Ascomycota, Taphrinales, Protomycetaceae) are obligate parasites of flowering plants of the families Apiaceae and Asteraceae. The mycelium of the *Protomyces* species invades the host tissues intercellularly and concentrates mainly around the vascular bundles. The fungus induces hypertrophy and hyperplasia of the infected tissues, and finally leads to the formation of distinct galls or swellings on stems, leaves, flowers, fruits and other aboveground parts of host plants (Büren 1915, 1922). The *Protomyces* species parasitize Apiaceae (e.g., *Protomyces macrosporus* Unger), and Asteraceae (e.g., *Protomyces pachydermus* Thüm on *Taraxacum*, *Protomyces kriegerianus* Büren on *Leontodon, Protomyces buerenianus* Buhr on *Galinsoga, Protomyces cirsii-oleracei* on *Cirsium*). Most of them occur only in Europe. Exception are *Protomyces macrosporus* and *P. pachydermus* which are widespread (Büren 1922; Gjaerum 1964; Reddy & Kramer 1975; Sałata 1979).

Here we describe, illustrate and discuss two interesting species of *Protomyces* found in the Tatra Mts Biosphere Reserve: *Protomyces crepidis-paludosae* Büren, new for Slovakia, and *Protomyces macrosporus* Unger, collected on the rarely reported host plants *Laserpitium latifolium* L. and *Carum carvi* L.

MATERIAL AND METHODS

For identification of the species, both the visible symptoms of the infected plant and the anatomical and morphological characteristics of the fungus were used. Transverse and longitudinal sections from naturally infected leaves or stems were observed in a drop of 50% lactic acid, and 100 ascogenous cells were measured by means of a Zeiss light microscope with microphotography equipment. The text uses the following abbreviations: L – mean ascogenous cell length (µm), W – mean ascogenous cell width (µm), Q – ratio of mean ascogenous cell length and width (L/W ratio), (n = *x/y*) *x* measurements of ascogenous cells from *y* specimens (Niemelä 1998).

The collected material was deposited in the Mycological Herbarium of the Institute of Botany of the Slovak Academy of Sciences, Bratislava (SAV) and the Herbarium of the Department of Botany and Mycology, Maria Curie-Skłodowska University, Lublin (LBL).

RESULTS AND DISCUSSION

Protomyces crepidis-paludosae Büren

Beitr. Kryptogamenfl. Schweiz **5**(3): 58. 1922.

The fungus causes small (1–3 mm) swellings and galls along the main and lateral veins on living leaves of *Crepis paludosa*. Visual infection symp-

toms are recognizable as yellow, pale-brown to dark-brown, barely raised spots around small galls along the main leaf veins (Figs 1 & 2A).

The septate mycelium is very thin, invades the leaf tissue intercellularly, and concentrates along the vascular tissue (Fig. 2B). The ascogenous cells are formed intercalarily as a bulge on the cell of the intercellular mycelium (Fig. 2C, D). They are spherical to roughly spherical, 37–68 × 28–52 µm (most frequently 46–52 × 43–46 µm); L = 49 µm, W = 43 µm, Q = 1.14 (n = 100/1) (Fig. 2E; Table 1).

The ascogenous cell wall is three-layered and pale yellowish-brown. The exosporium and mesosporium are smooth (Fig. 2F), and the endosporium often varies in thickness; altogether these three layers are 6.1 µm thick with short pedicle-like basal appendages (Fig. 2E, F). The mature ascus (vesicle) was not found.

SPECIMENS EXAMINED. On *Crepis paludosa* (L.) Moench: SLOVAKIA, Tatra National Park, Belianske Tatry Mts, Monkova dolina valley surrounding the Ringliarsky potok River, elev. *ca* 930 m, 27 July 2000 & 29 May 2005, *leg. K. Bacigálová* (SAV); Monkova dolina valley, elev. *ca* 996 m, 14 July 2005, *leg. W. Mułenko* (LBL); Javorová dolina valley, elev. *ca* 1140 m, 20 July 2005, *leg. K. Bacigálová* (SAV).

NOTES. *Protomyces crepidis-paludosae* on *Crepis paludosa* recorded in the Belianske Tatry Mts is a new species for Slovakia. This species was previously found only in Germany, Switzerland (Büren 1922), Norway (Gjaerum 1964) and Poland (Sałata 1979; Sałata *et al*. 1984; Bacigálová *et al*. 2005).

Fig. 1. *Protomyces crepidis-paludosae* Büren on *Crepis paludosa* (L.) Moench (SAV): leaf spots and galls along small parts of main vein on leaf of host plant.

Protomyces crepidis-paludosae and other *Protomyces* species are distinguished on the basis of

Table 1. Comparison of ascogenous cell size in *Protomyces crepidis-paludosae* Büren on *Crepis paludosa* (L.) Moench in Europe.

Collections of *P. crepidis-paludosae*	Ascogenous cell diameter (µm) [most frequent]	Ascogenous cell wall (µm)	Ascus 'vesicles'
Switzerland, Alps, elev. *ca* 1800 m (Büren 1922)	45	not published	60–75 × 50
Norway, elev. up to 750 m (Gjaerum 1964)	20–55	2.5–6.0	not published
Poland, Sudety Mts (Sałata 1979)	27–52 [30–40]	2.0–6.5	60–75 × 50
Tatra Mts (Sałata *et al*. 1984; Bacigálová *et al*. 2005)	38–54 × 26–44	4–6	not observed
Slovakia, Belianske Tatry Mts, elev. *ca* 930 m and 1140 m, *leg. K. Bacigálová* (SAV)	37–68 × 28–52 [46–52 × 43–46]	6.1	not observed

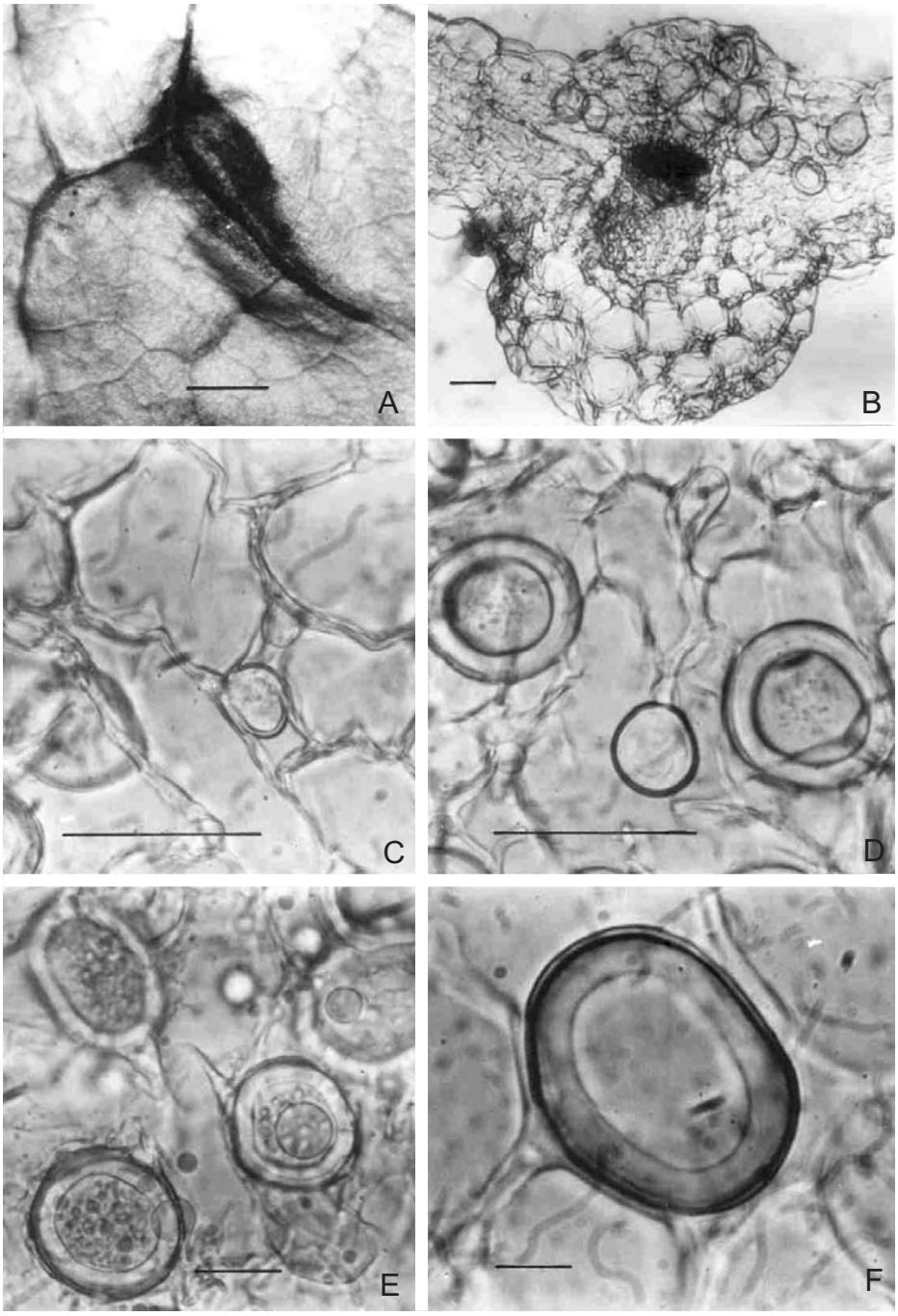

Fig. 2. *Protomyces crepidis-paludosae* Büren within leaves of *Crepis paludosae* (L.) Scop. (SAV). A – galls along leaf vein (scale bar = 1 mm), B – cross section of leaf, mycelium and formation of ascogenous cell along vascular tissue on upper side of leaf (scale bar = 50 μm), C & D – intercellular mycelium and young stages of ascogenous cells showing their apical position on mycelium (scale bar = 50 μm), E – young stages and mature ascogenous cells in *in situ* (scale bar = 20 μm), F – mature ascogenous cell – three-layered cell wall with short pedicle-like basal appendages (scale bar = 10 μm).

Fig. 3. *Protomyces macrosporus* Unger on *Laserpitium latifolium* L. (SAV): leaf spots and galls along leaf vein of host plant.

macroscopic symptomatological characteristics and the size of ascogenous cells, according to the methods used by Büren (1915, 1922) and Sałata (1979). The morphological variability and the size of ascogenous cells in *P. pachydermus* Thüm., *P. cirsii-oleracei* Buhr, *P. kriegerianus* Büren and other species parasitizing Asteraceae led Reddy and Kramer (1975) to synonimize all these taxa with *P. pachydermus*. Our observations do not support Reddy and Kramer's (1975) conclusions. Ascogenous cells of *Protomyces crepidis-paludosae* (on *Crepis*), *P. kriegerianus* (on *Leontodon* and *Hypochoeris*), *P. cirsii-oleracei* (on *Cirsium*) differ from the ascogenous cells of *P. pachydermus* (on *Taraxacum* sp.) (Table 2), and their modes of formation also differ (Bacigálová 2004).

Protomyces macrosporus Unger

Die Exanth. der Pflanzen: 344. 1833.

Visual infection symptoms on *Laserpitium latifolium* are recognizable as white-green to brown, large, hard galls or round callosities within the stem, leaves and fruits tissues (on leaves the galls are usually restricted to areas along the petiole, veins and veinlets) (Figs 3 & 4A). On *Carum carvi* the fungus causes brown-orange galls on stems and leaves (Fig. 5A).

The thick septate mycelium invades the leaf tissue intercellularly and concentrates along the vascular tissue. The ascogenous cells are formed intercalarily on the intercellular mycelium (Figs 4B–D & 5B). On *Laserpitium latifolium* they are spherical to roughly spherical, 46–83 × 40–68 µm (most frequently 59–62 × 55–59 µm); L = 61, W = 54, Q = 1.13 (n = 109/1) (Fig. 4E, F). On *Carum carvi* they are spherical, 37–71 × 31–62 µm (most frequently 46–52 × 43–46 µm); L = 50, W = 45.5, Q = 1.10, (n = 100/1) (Fig. 5C, D). The wall of the ascogenous cell is three-layered and pale yellowish-brown. On *Laserpitium latifolium*

Table 2. Comparison of ascogenous cell size in some *Protomyces* species associated with Asteraceae.

Species	Host plant and locality	Ascogenous cell diameter (µm) [most frequent]	L (µm)	W (µm)	Q	Ascogenous cell wall (µm)
P. crepidis-paludosae	*Crepis paludosa*, Slovakia, Belianske Tatry Mts	37–68 × 28–52 [46–52 × 46–46]	49	43	1.14	6.1
*P. pachydermus**	*Taraxacum tatrense* R. Doll, Slovakia, Vysoké Tatry Mts	26–53 × 16–44 [31–42 × 31–39]	37	33	1.12	3.1
*P. pachydermus**	*Taraxacum officinale* Weber agg., Slovakia, Bratislava, Institute of Botany	25–42 × 23–39 [31–36 × 28–31]	34	30	1.12	3.1
*P. kriegerianus**	*Leontodon hispidus* L., Slovakia, Bratislava, Institute of Botany	34–47 × 28–45 [39–42 × 36–39]	40	37	1.07	4.7
*P. kriegerianus**	*Leontodon hispidus* L., Slovakia, Selenecká dolina valley	28–50 × 25–47 [39–47 × 39–44]	43	39	1.09	4.7
*P. cirsii-oleracei**	*Cirsium oleraceum* (L.) Scop., Poland, Białowieża National Park	30–45 × 24–45 [36–39 × 31–39]	36.5	35	1.04	4.7

* – in Bacigálová *et al*. 2005

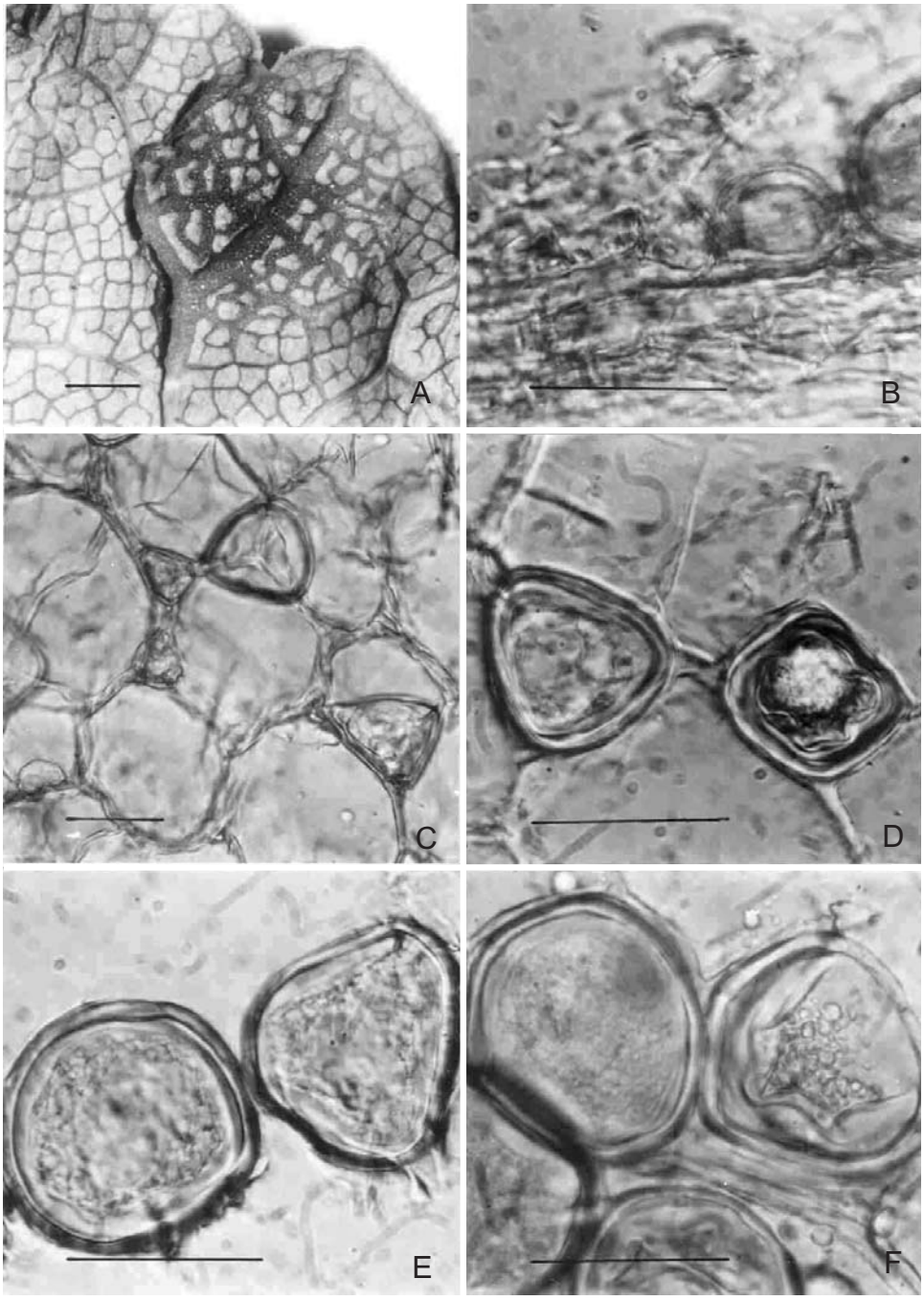

Fig. 4. *Protomyces macrosporus* Unger within leaves of *Laserpitium latifolium* L. (SAV). A – galls along vein on leaves of *Laserpitium latifolium* (scale bar = 1 mm), B – mycelium and young stage of ascogenous cell formation within leaf vascular tissues (scale bar = 50 μm), C & D – young stage of ascogenous cells showing their intercellular and intercalary position on mycelium (scale bar = 50 μm), E – mature ascogenous cells – three-layered cell wall with the other parts of the mycelium cell (scale bar = 50 μm), F – mature ascogenous cells: the spores loosen after the cell wall bursts (scale bar = 50 μm).

Table 3. *Protomyces macrosporus* Unger and their host plants in Slovakia.

Host plant and locality	Ascogenous cell diameter (µm) [most frequent]	L (µm)	W (µm)	Q	Ascogenous cell wall (µm)
Aegopodium podagraria L. Tatra Mts, Monkova dolina valley, elev. *ca* 1050 m, 20 July 2000, *leg. K. Bacigálová* (SAV)	51–86 × 42–67 [61–64 × 58–61]	64.0	57.0	1.12	5.0–6.0
Tatra Mts, Between Skalnaté pleso lake and Lomnické sedlo saddle, elev. *ca* 1781 m, 20 Aug. 1999, *leg. K. Bacigálová* (SAV)	46–77 × 40–74 [62 × 46–52]	62.0	53.0	1.17	4.5–6.5
Tesárske Mlyňany village, elev. *ca* 216 m, 7 Oct. 2005, *leg. K. Bacigálová* (SAV)	43–62 × 40–62 [52–55 × 49–52]	54.5	51.0	1.06	4.5–6.0
Ligusticum mutellina (L.) Crantz, Tatra Mts, Červené vrchy Mt., elev. *ca* 1820 m, 10 Aug. 1999, *leg. K. Bacigálová* (SAV)	47–73 × 41–60 [55–63 × 45–52]	60.0	52.0	1.15	3.0–5.0
Tatra Mts, Batizovská dolina valley, elev. *ca* 1800 m, 19 July 2006, *leg. K. Bacigálová* (SAV)	49–65 × 40–61 [52–55 × 46–52]	55.0	48.0	1.13	4.6–6.1
Tatra Mts, Temné smrečiny forest, elev. *ca* 1580 m, 6 Aug. 1998, *leg. K. Bacigálová* (SAV)	40–65 × 31–55 [49–52 × 46]	52.0	46.0	1.00	4.6–6.1
Chaerophyllum hirsutum L. Tatra Mts, Monkova dolina valley, elev. *ca* 1100 m, 11 Aug. 1999, *leg. K. Bacigálová* (SAV)	48–74 × 45–72 [61–64 × 58–64]	63.0	58.0	1.08	5.0–6.0
Tatra Mts, Bobrovecká dolina valley, elev. *ca* 1100 m, 18 Aug. 2006, *leg. K. Bacigálová* (SAV)	46–74 × 40–65 [62 × 52–55]	60.0	54.0	1.11	4.6–6.1
Heracleum sphondylium L. Tatra Mts, Javorový žľab gully, elev. *ca* 1300 m, 5 Aug. 1999, *leg. K. Bacigálová* (SAV)	32–77 × 32–67 [61–64 × 51–61]	59.0	54.0	1.09	5.0–6.0
Východná village, Čierna dolina valley, elev. *ca* 760 m, 18 July. 2005, *leg. K. Bacigálová* (SAV)	49–71 × 40–62 [62 × 52–55]	58.5	53.0	1.10	4.6–6.1
Anthriscus sylvestris (L.) Hoffm. Bratislava city, Devínska Kobyla hill, Dúbravska cesta Street, 31 Mar. 2003, *leg. K. Bacigálová* (SAV)	50–70 × 48–70 [65 × 65]	65.0	65.0	1.0	4.0–5.0
Carum carvi L. Tatra Mts, Žiarska dolina valley, elev. *ca* 900 m, 13 Aug. 2004, *leg. K. Bacigálová* (SAV)	37–71 × 31–62 [46–52 × 43–46]	50.0	45.3	1.10	2.0–3.0
Terchová village, elev. *ca* 500 m, 16 May 1948, *leg. Zavřel* (BRA)	39–53 × 30–44 [41–45 × 39–44]	50.0	40.0	1.05	2.0–3.0
Laserpitium latifolium L. Tatra Mts, Biela skala Mt., elev. *ca* 1400 m, 19 Aug. 2005, *leg. K. Bacigálová* (SAV) and *leg. W. Mułenko* (LBL)	46–83 × 40–68 [59–62 × 55–59]	61.0	54.0	1.13	6.2

the exosporium and mesosporium are smooth (Fig. 4E, F) and the endosporium often varies in thickness; altogether these three layers are 6.2 µm thick with short pedicle-like basal appendages (Fig. 4E). On *Carum carvi* the exosporium and mesosporium are smooth, 2 µm thick, and the endosporium is thin and transparent (Fig. 5E); altogether these three layers are 2–3 µm thick. The mature ascus (vesicle) was not found, but we often saw bursting of the ascogenous cell wall (Figs 4F & 5F).

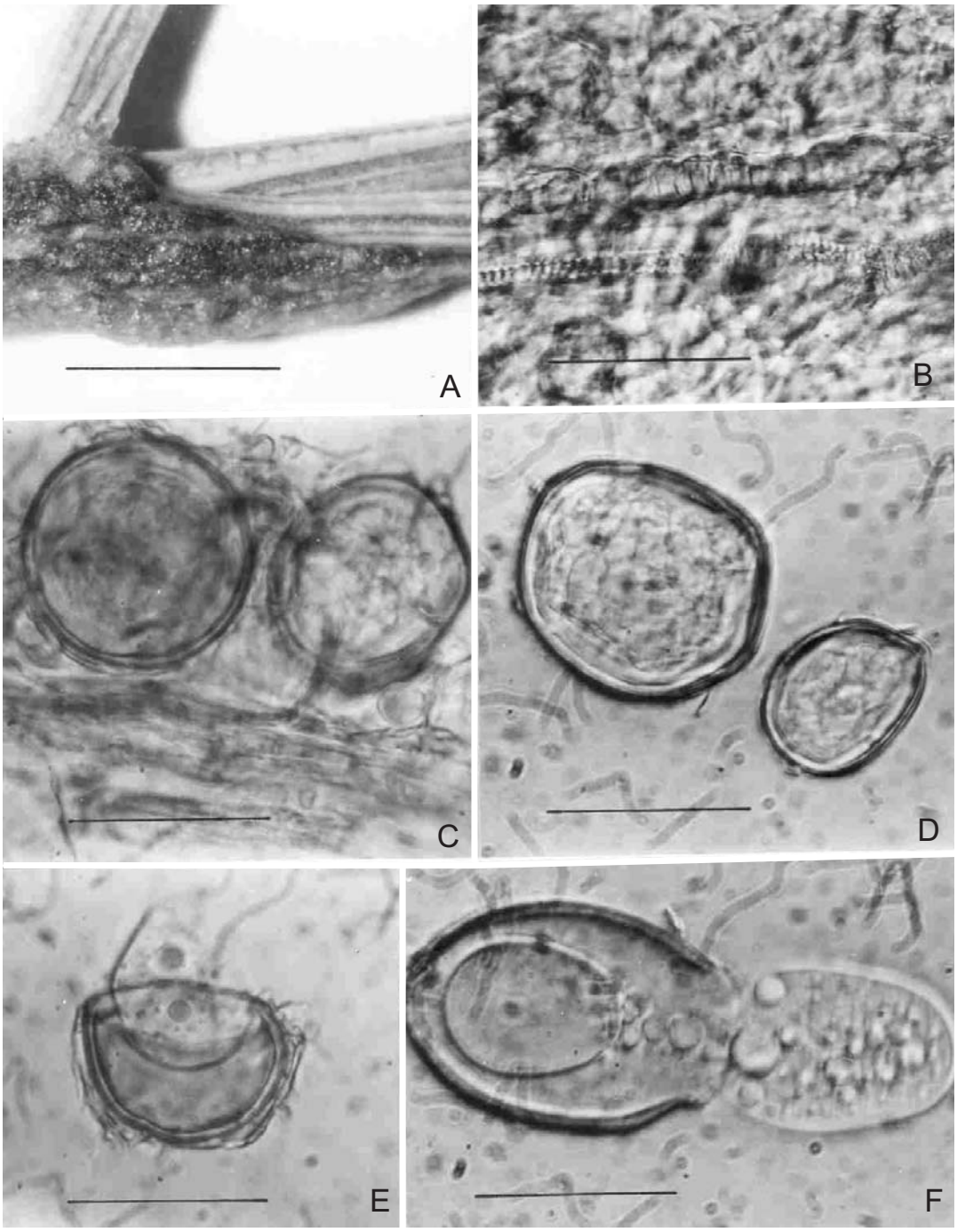

Fig. 5. *Protomyces macrosporus* Unger within leaves and stems of *Carum carvi* L. (SAV). A – gall on steam of *Carum carvi* (scale bar = 1 mm), B – transverse section of infected steam – mycelium of *P. macrosporus* along vascular tissue of *Carum carvi* (scale bar = 50 μm), C – formation of ascogenous cells within vascular tissue (scale bar = 50 μm), D – mature ascogenous cells (scale bar = 50 μm), E – three-layered cell wall of ascogenous cell (scale bar 50 μm), F – vesicle formation (scale bar = 50 μm).

SPECIMENS EXAMINED. On *Laserpitium latifolium* L.: SLOVAKIA, Tatra Mts, Západné Tatry, Sivý vrch Mt., southwest part between the limestone cliffs on the peak of Biela skala Mt., elev. *ca* 1400 m, 19 Aug. 2005, *leg.K. Bacigálová* (SAV), 29 Aug. 2005, *leg. W. Mułenko* (LBL). On *Carum carvi* L.: SLOVAKIA, Tatra Mts, Západné Tatry, Žiarska dolina valley, meadow near trail, elev. *ca* 900 m, 13 Aug. 2004, *leg. K. Bacigálová* (SAV).

NOTES. *Protomyces macrosporus* on *Laserpitium latifolium* is a new fungus/host combination for Slovakia and the Carpathians; previously it was noted on this host only in Switzerland (Büren 1922). *Protomyces macrosporus* on *Carum carvi* has been recorded by Zavřel (BRA, 1948) in the Malá Fatra Mts (Bacigálová 2004) (Table 3) and in the Tatry Zachodnie Mts in Poland. Our record in the Západné Tatry Mts in Slovakia confirms the very rare occurrence of this fungus/host combination. Until now, *Protomyces macrosporus* on *Carum carvi* has been found only in Switzerland (Büren 1915, 1922), Norway (Gjaerum 1964) and Poland (Bacigálová *et al*. 2005).

The taxonomy of *Protomyces macrosporus* has been studied by mycologists for a long time and has evoked numerous discussions. Based on inoculation experiments, Büren (1915, 1922) recognized seven *formae speciales*. No inoculation experiments have been done in any other countries, and the diameter of the ascogenous cells (Table 3) does not support the creation of a new species, so we accepted the name *Protomyces macrosporus*. The tools of molecular biology should clarify many phylogenetic and taxonomic problems (including species concept); Sjamsuridzal *et al*. (1997), for example, used them to construct a phylogenetic hypothesis on evolutionary relationships within Taphrinales, and Bacigálová *et al*. (2003) to characterize *Taphrina* species infecting alder.

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