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PROTOMYCES CIRSII-OLERACEI (FUNGI, PROTOMYCETALES), A NEW SPECIES FOR POLAND

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Abstract. *Protomyces cirsii-oleracei* Buhr was found on living leaves of *Cirsium oleraceum* (L.) Scop. in the Puszcza Białowieska primeval forest. This is a new species for the Polish mycota. The species is described, illustrated, and compared with similar taxa.

Key words: Protomycetaceae, *Cirsium oleraceum*, Asteraceae, fungi, parasite, taxonomy, Poland

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INTRODUCTION

The species of the genus *Protomyces* Unger (Protomycetales, Ascomycota) are obligatory parasites of plants belonging to Apiaceae, Asteraceae and Cichoriaceae. The mycelium invades the host tissues intercellularly and concentrates mainly around the vascular bundles. They are altered morphologically, and hypertrophy and hyperplasia of the infected tissues results in the formation of distinct galls or swellings on stems, leaves, flowers, fruits and other aerial parts of plants (Büren 1915). Members of the genus *Protomyces* parasitizing Apiaceae (*Protomyces macrosporus* Unger), Asteraceae and Cichoriaceae (*Protomyces pachydermus* Thüm. on *Taraxacum*, *Protomyces kriegerianus* Büren on *Leontodon*, *Protomyces buerenianus* Buhr on *Galinsoga*, *Protomyces kreuthensis* Kühn on *Aposeris*, *Protomyces crepidis-paludosae* Büren on *Crepis*) occur mainly in Europe (Büren 1922; Gjaerum 1964; Reddy & Kramer 1975; Sałata 1979).

The monograph of Polish Protomycetales by Sałata (1979) comprised eight species known to occur in Poland, classified in two genera. Since then only one species has been added to this list, *Protomycopsis arnoldii* Magnus (Bacigálová & Mułenko 2005). Recently, *Protomyces cirsii-oleracei* Buhr, another species new for Poland, was found on *Cirsium oleraceum* (L.) Scop. in the Puszcza Białowieska primeval forest. Until now, this species has been known only from Germany and Switzerland (Büren 1939).

MATERIAL AND METHODS

This study is based on the following specimens: *Protomyces cirsii-oleracei* Buhr – Poland, Wysoczyzny Podlasko-Białoruskie upland, Puszcza Białowieska primeval forest, forest tract no. 464 D, *Tilio cordatae-Carpinetum betuli*, on leaves of *Cirsium oleraceum* (L.) Scop., 11 Oct. 2003, *leg. M. Wołkowycki* (KRAM); *Protomyces kriegerianus* Büren – Poland, Wysoczyzny Podlasko-Białoruskie upland, Białowieża, manor park, on leaves of *Leontodon hispidus* L. and *Leontodon hispidus* subsp. *opimus* (Koch) R. A. Finch & P. D. Sell, 19 May 2004, *leg. K. Bacigálová & W. Mułenko* (SAV); Slovakia, Veľká Fatra Mts, Selenecká dolina valley, on leaves of *Leontodon hispidus* L., 9 June 1994, *leg. K. Bacigálová* (SAV); Devínska Kobyla Mts, Bratislava, Institute of Botany, in garden, on leaves of *Leontodon hispidus* L. and *Hypochoeris radicata* L., 25 June 2003 & 10 June 2004, *leg. K. Bacigálová* (SAV); *Protomyces pachydermus* Thüm. – Slovakia, Devínska Kobyla Mts, Bratislava, Institute of Botany, in garden,

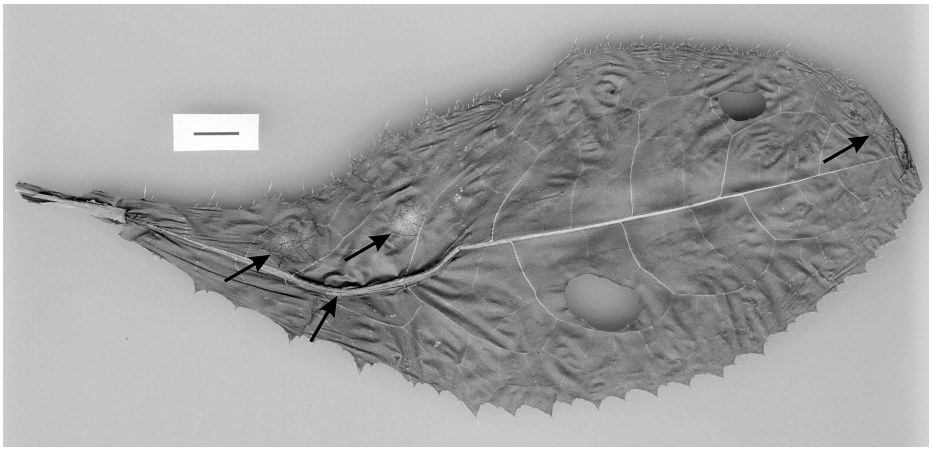

Fig. 1. *Protomyces cirsii-oleracei* Buhr on *Cirsium oleraceum* (L.) Scop. (KRAM): leaf spots, blister-like swellings and galls (arrows) along main vein on leaf of host plant. Scale bar = 1 cm.

on leaves of *Taraxacum officinale* agg., 19 May 1997, *leg. K. Bacigálová* (SAV); Vysoké Tatry Mts, Osterva, 1520 m a.s.l., on *Taraxacum tatrense* R. Doll, 22 July 1991, *leg. K. Bacigálová* (SAV).

For identification of the species, both the visible symptoms of the infected plant and anatomical-morphological characteristics of the fungus were used. Transverse and longitudinal sections from naturally

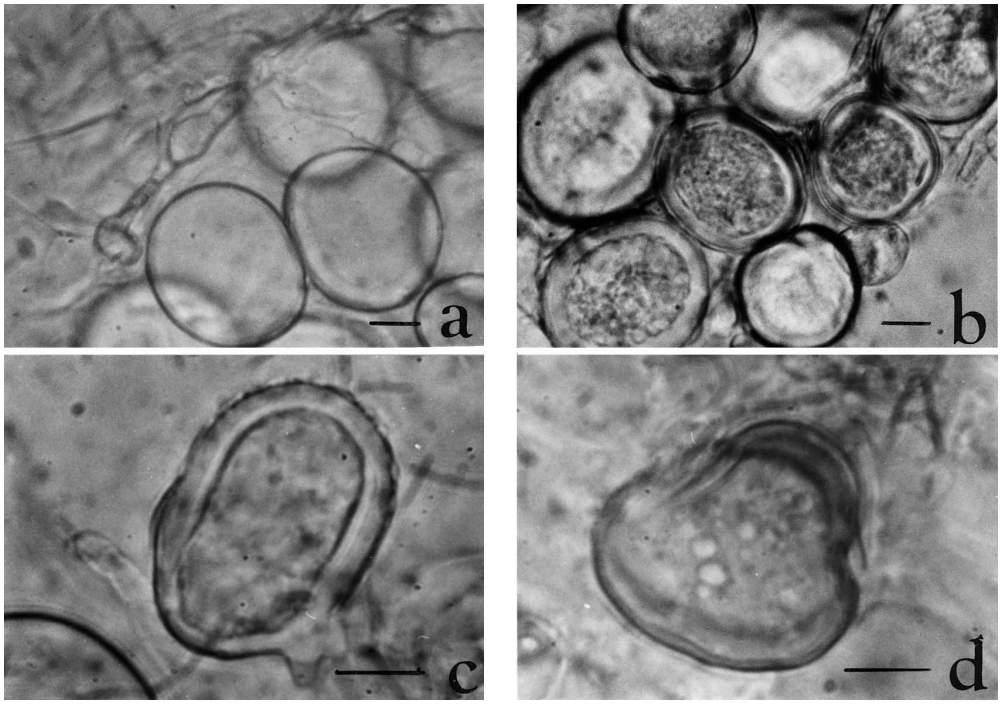

Fig. 2. *Protomyces cirsii-oleracei* Buhr within leaves of *Cirsium oleraceum* (L.) Scop. in LM (KRAM): a – mycelium and young stage of ascogenous cell formation within leaf tissues; b – transverse section of small gall near leaf vein; c – mature ascogenous cell – three-layered cell wall; d – vesicle formation. Scale bars = 10 μm.

infected leaves or stems were observed in a drop of 50% lactic acid and measured by means of a Zeiss light microscope with microphotographic equipment. 100 ascogenous cells were measured. In the text the following abbreviations are used: L – mean ascogenous cell length (arithmetical mean of all ascogenous cells, μm), W – mean ascogenous cell width (arithmetical mean of all ascogenous cells, μm), Q – quotient of mean ascogenous cell length and width (L/W ratio), (n = x/y) *x* measurements of ascogenous cells from *y* specimens (Niemelä 1998).

For scanning electron microscope (SEM) studies, squashed fragments of plant tissue with ascogenous cells were mounted on clean glass and affixed to an aluminum stub with double-sided transparent tape. The stubs were sputter-coated with carbon using a CRESSINGTON sputter-coater and viewed with a Hitachi S-4700 scanning electron microscope at a working distance of *ca* 12–13 mm.

Results and discussion

Protomyces cirsii-oleracei Buhr (Figs 1–3 & 4c) Arch. Ver. Freund. Naturgesch. Mecklenburg, N. F. **10**(1935): 41. 1936.

The fungus causes swellings and galls on living leaves of *Cirsium oleraceum* (L.) Scop. (Asteraceae) along the main and lateral veins. Intensive fungus infections on the leaves are easily recognizable yellow spots with networks of swollen veins and veinlets (Fig. 1). The galls are limpid, white green to yellow, often 2–20 mm long in diameter.

The septate mycelium is very thin, invades the leaf tissue intercellularly, and concentrates along the vascular tissue (Fig. 2a). The ascogenous cells are formed intercalarily on the intercellular

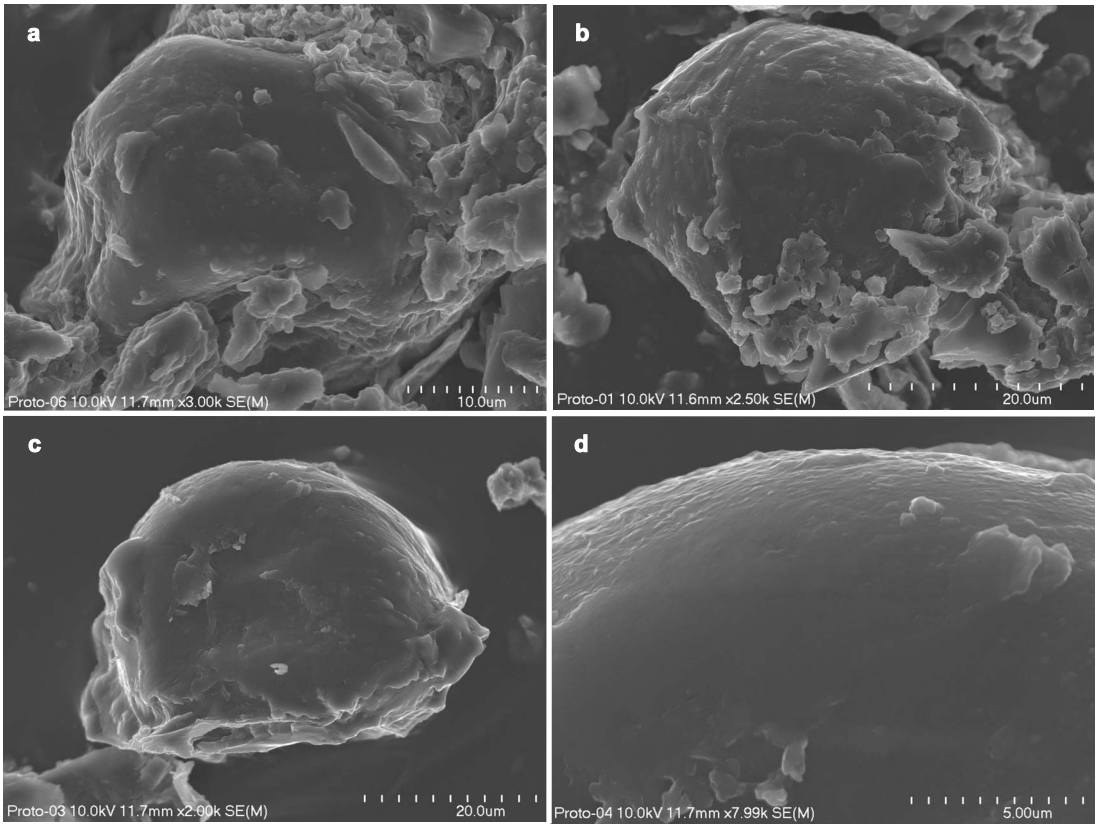

Fig. 3. *Protomyces cirsii-oleracei* Buhr on *Cirsium oleraceum* (L) Scop. (KRAM): a–d – ascogenous cells in SEM.

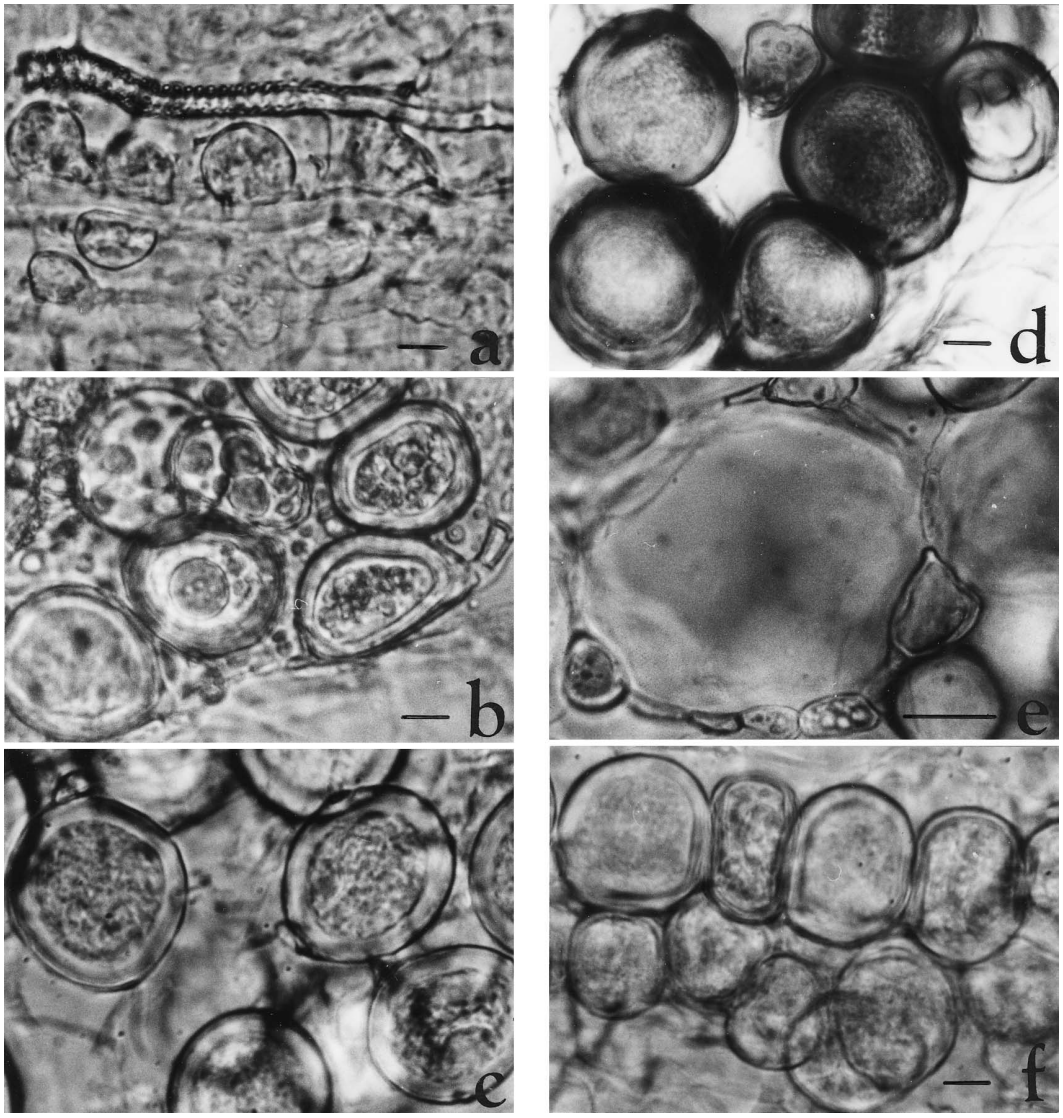

Fig. 4. Ascogenous cells of *Protomyces* species within host tissues: a – mycelium and young stages of formation of *Protomyces kriegerianus* Büren ascogenous cells along vascular tissue in leaf of *Leontodon hispidus* L. (Białowieża, SAV); b – formation of *Protomyces kriegerianus* Büren ascogenous cells along vascular tissue in leaf of *Leontodon hispidus* L. (Bratislava, SAV); c – ascogenous cells of *Protomyces cirsii-oleracei* Buhr within leaf tissue of *Cirsium oleraceum* (L.) Scop. (Puszcza Białowieska primeval forest, KRAM); d – formation of *Protomyces kriegerianus* Büren ascogenous cells within vascular tissue in leaf of *Hypochoeris radicata* L. (Bratislava, SAV); e – mycelium and young stages of formation of *Protomyces pachydermus* Thüm. ascogenous cells within leaf tissue of *Taraxacum officinale* agg. (Bratislava, SAV); f – ascogenous cells of *Protomyces pachydermus* Thüm. formed near each other within leaf tissue of *Taraxacum officinale* agg. Scale bars = 10 μm.

mycelium. They are spherical to roughly spherical or elliptic, 30–45 × 24–45 μm (most frequently 36–39 × 31–39 μm), L = 36.5 μm, W = 35.0 μm, Q = 1.4 (n = 100/1) (Fig. 2b-c). The ascogenous cell wall is three-layered and pale light yellowish-brown. The exosporium is smooth (Fig. 3a-d), the mesosporium smooth, and the endosporium often varies in thickness; altogether these three layers are

Table 1. Comparison of the size of ascogenous cells of some *Protomyces* species associated with Asteraceae and Cichoriaceae (PL – Poland, SK – Slovak Republic).

Species of *Protomyces*	Host plant and locality	Ascogenous cell (μm)	L (μm)	W (μm)	Q	Ascogenous cell wall (μm)
P. cirsii-oleracei	*Cirsium oleraceum* Białowieża Primeval Forest (PL)	30–45 × 24–45 (36–39 × 31–39)	36.5	35	1.04	4.7
P. cirsii-oleracei	*Cirsium oleraceum* (Büren 1939)	30–43	no data	no data	no data	3.7
P. kriegerianus	*Leontodon hispidus* Białowieża (PL)	31–48 × 27–44 (37–39 × 34–36)	38	34	1.11	4.7
P. kriegerianus	*Leontodon hispidus* Białowieża (PL)	31–53 × 28–44 (37–39 × 31–36)	39	35	1.11	4.7
P. kriegerianus	*Leontodon hispidus* Selenecká dolina valley (SK)	28–50 × 25–47 (39–47 × 39–44	43	39	1.09	4.7
P. kriegerianus	*Leontodon hispidus* Bratislava, Institute of Botany (SK)	34–47 × 28–47 (39–42 × 36–39)	40	37	1.07	4.7
P. kriegerianus	*Hypochoeris radicata* Bratislava, Institute of Botany (SK)	31–52 × 23–48 (39–44 × 39–42)	41	38.5	1.12	4.7
P. pachydermus	*Taraxacum officinale* agg. Bratislava, Institute of Botany (SK)	25–42 × 23–29 (31–36 × 28–31)	34	30	1.12	3.1
P. pachydermus	*Taraxacum tatrense* Vysoké Tatry Mts (SK)	26–53 × 16–44 (31–42 × 31–39)	37	33	1.12	3.1

4.7 μm thick, with apical papilla and short pedicle-like basal appendages (Fig. 2b-c). When the ascogenous cell begins to germinate, the cell wall splits, allowing the vesicle to protrude (Fig. 2d). The mature ascus (vesicle) was not found.

Büren (1915, 1922, 1939) distinguished *Protomyces pachydermus*, *P. kriegerianus*, *P. cirsii-oleracei* and other species of *Protomyces* on the basis of differences in pathogenicity, in the size of ascogenous cells, and in the size of the ascus – the 'vesicle'. On the other hand, the morphological variability and the size of ascogenous cells in *P. pachydermus*, *P. cirsii-oleracei*, *P. kriegerianus*, *P. kreuthensis* and other species parasitizing Asteraceae and Cichoriaceae led Reddy & Kramer (1975) to synonymize all these taxa with *Protomyces pachydermus*. Our observations do not support Reddy & Kramer's (1975) conclusions. As seen in Table 1 and Fig. 4b-d, the ascogenous cells of *P. cirsii-oleracei* on *Cirsium* and *P. kriegerianus* on *Leontodon* and *Hypochoeris* from Białowieża (Poland) and Slovakia are very similar, but they differ from the ascogenous cells of *Protomyces pachydermus* on *Taraxacum* (Fig. 4f). *P. cirsii-oleracei*, *P. kriegerianus* and *P. pachydermus* also differ in terms of the differentiation of ascogenous cells (Figs 2a, 4a, 4c). Taxonomic studies based on molecular methods should help elucidate these taxonomic problems.

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