

## TWO IMPORTANT ASCOMYCETES AND THEIR ANAMORPHS ON TWIGS OF *BETULA PENDULA* IN SLOVAKIA\*

KATARÍNA PASTIRČÁKOVÁ\*\* & MARTIN PASTIRČÁK

**Abstract.** The paper reports two fungal species of Pleomassariaceae new for the Slovak mycobiota: *Pleomassaria siparia* (Berk. & Broome) Sacc. and *Splanchnonema argus* (Berk. & Broome) Kuntze, found on dead twigs of *Betula pendula* Roth. The morphology of the fungi and their coelomycetous anamorphs is described, with photographic documentation.

**Key words:** Ascomycetes, Pleomassariaceae, *Pleomassaria siparia*, *Splanchnonema argus*, Coelomycetes, *Prosthemia betulinum*, *Myxocyclus polycistis*

Katarína Pastirčáková, Branch for Woody Plant Biology, Institute of Forest Ecology, Slovak Academy of Sciences, Akademická 2, SK-94901 Nitra, Slovakia; e-mail: uefezima@hotmail.com

Martin Pastirčák, Plant Production Research Institute, Bratislavská cesta 122, SK-92168 Piešťany, Slovakia; e-mail: uefemapa@hotmail.com

### INTRODUCTION

In Slovakia, *Betula pendula* Roth is a frequently planted tree in parks and other urban areas. It is highly adaptable to degraded environments but is commonly attacked by microfungi and thus is frequently the object of mycological studies. Recent studies have demonstrated damage caused by fungi to *Betula* spp. in Slovakia (Juhásová 2004; Juhásová *et al.* 2005; Bernadovičová 2008). A number of fungal species associated with diseased birch branches and twigs have also been recorded in other countries including Canada, Finland and Scotland (Arnold 1967; Paavolainen *et al.* 2001; Green 2004).

During our studies of the mycobiota of birch twigs we found two fungal species of Pleomassariaceae. Species in this family are saprotrophs on woody substrate, but generally their hosts are restricted. *Pleomassaria siparia* and *Splanchnonema argus*, including their coelomycetous anamorphs, have been reported as new species for the Slovak mycobiota.

### MATERIALS AND METHODS

Dead twigs were cut from *B. pendula* at 20 localities in Slovakia between October 2009 and April 2010. Samples were examined and photographed under an Olympus SZ 61 stereomicroscope and an Olympus BX 51 light microscope. The taxonomic positions of the examined fungi were determined by morphological analysis. Measurements were made in distilled water or lactic acid using oil immersion, and the morphological features of the fungi were described. The morphometric values for the examined fungi were compared with previously published data for the taxa (Barr 1982; Hantula *et al.* 1998; Tanaka *et al.* 2005). Herbarium specimens are deposited at the U.S. National Fungus Collections, U.S.A. (BPI).

### RESULTS AND DISCUSSION

*Pleomassaria siparia* (Berk. & Broome) Sacc.

Syll. Fung. (Abellini) 2: 239. 1883.

*Sphaeria siparia* Berk. & Broome, Ann. Mag. Nat. Hist., Ser. 2, 9: 321. 1852.

*Massaria siparia* (Berk. & Broome) C. Tul. & Tul., Select. Fung. Carpol. (Paris) 2: 232. 1863.

\* This paper is dedicated to Professor Tomasz Majewski on the occasion of his 70<sup>th</sup> birthday.

\*\* Corresponding author.

ANAMORPH: *Prosthemium betulinum* Kunze, Mykologische Hefte (Leipzig) 1: 18. 1817.

Ascomata immersed, subglobose, with a central ostiole, 405–750 µm high, 460–1650 µm in diameter. Asci clavate, 8-spored, 170.0–251.5 × 30–45 µm. Ascospores fusiform, muriform, 47.5–69.5 × 13.0–20.5 µm (mean = 58.5 × 16.0 µm, n = 50), 5–7 transversely septate, with a primary septum mostly submedian, constricted at each septum, yellow to brown, finely echinulate, with a mucilaginous sheath 3.5–9.5 µm thick (Fig. 1).

ANAMORPHIC STAGE. Conidiomata pycnidial, subperidermal, globose to depressed globose, 645–750 µm high, 1420–1800 µm in diameter. Conidiophores unbranched, hyaline, septate. Conidia brown, staurosporous, 56–86 µm in diameter (mean = 71.5 µm, n = 50), with two main arms and 2 or 3 smaller arms. Main arms 37.0–47.5 × 11.5–15.0 µm (mean = 41.5 × 14.0 µm, n = 50), 4–5 transversely septate, with terminal cell hyaline to pale brown. Smaller arms continuous or 1–3 septate, hyaline to pale brown (Fig. 2).

To date, the recorded natural hosts of *Pleomassaria siparia* are *Carpinus betulus* L. in Ukraine, *Amorpha fruticosa* L. in Canada, and four species of *Betula* L. (*B. alleghaniensis* Britton, *B. papyrifera* Marshall, *B. pendula*, *B. pubescens* Ehrh.) in North America (Canada), Europe (Denmark, Finland, Lithuania, Netherlands, Poland, Russia, Sweden, United Kingdom) and Asia (Japan) (Farr & Rossman n.d.; Treigienė *et al.* 2007).

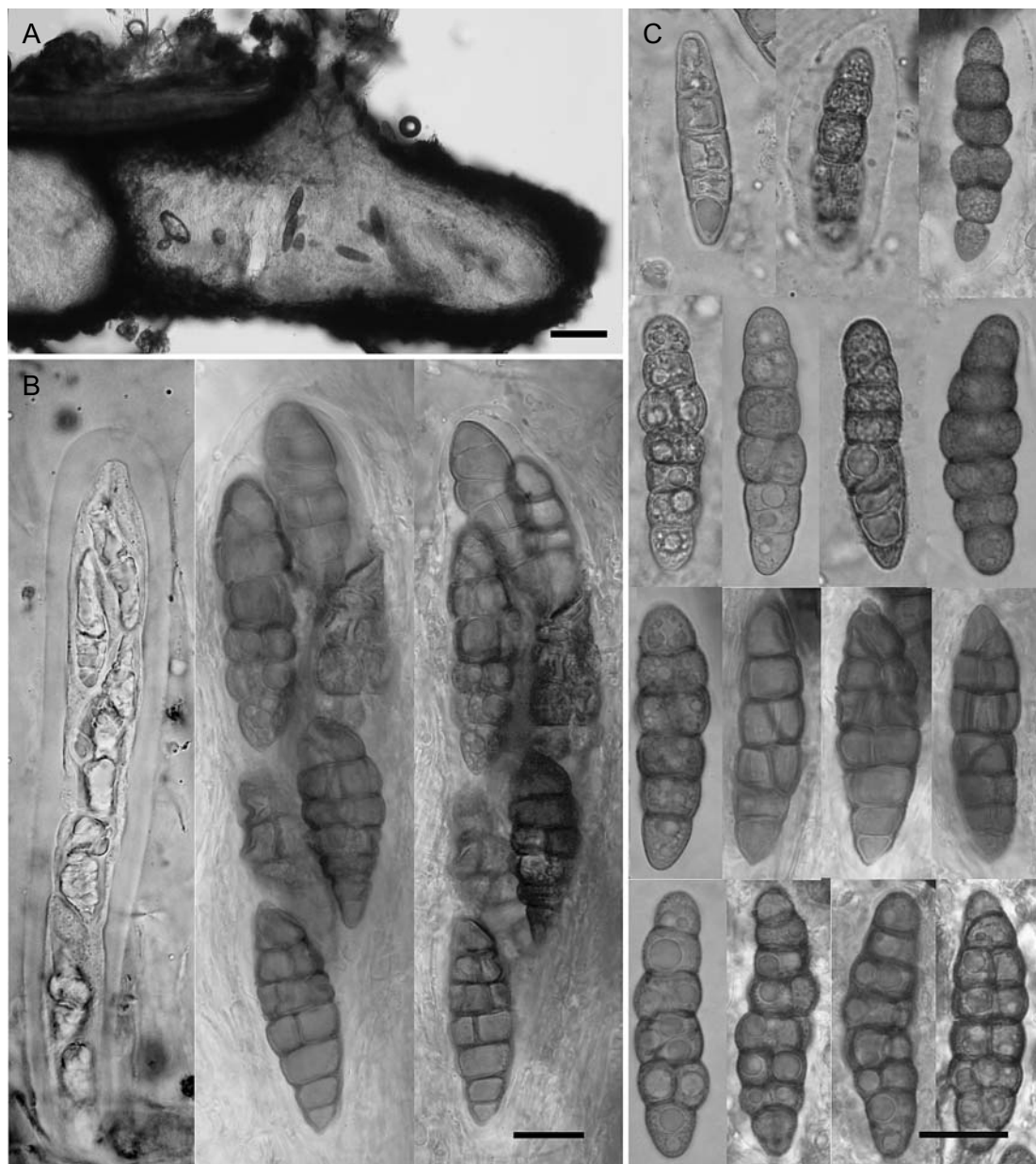
Herbarium specimens deposited in BPI confirm the occurrence of this fungus or its anamorph on *B. pubescens* in the Czech Republic (formerly Czechoslovakia; specimens from the territory of Bohemia and Moravia) and Germany, on *B. pendula* in Ukraine, and on *Betula* sp. in Italy.

According to Barr (1982), another species of the genus *Pleomassaria* occurs on *Betula* spp., *P. monosperma* (Peck) M. E. Barr in eastern North America. It differs in having large, multiseptate ascospores (up to 35-septate, 7–8 longitudinal septa in most cells) and 1(2)-spored asci.

The morphologically similar anamorphic species *Prosthemium asterosporum* T. Kowalski & Holdenr. was found on *B. pendula* and *Acer*

*pseudoplatanus* L. in Poland (Kowalski & Holdenrieder 1996), and on *Betula* spp. in Switzerland (Barengo *et al.* 2000), Finland (Paavolainen *et al.* 2000) and Japan (Tanaka *et al.* 2005). *Prosthemium betulinum* is very close morphologically to *P. asterosporum*, but the conidia of *P. asterosporum* have all arms equally developed. The connection between *P. siparia* and *P. betulinum* on *Betula* spp. was confirmed by Hantula *et al.* (1998). According to Paavolainen *et al.* (2000), *P. siparia* is composed of two separate biological species indistinguishable by teleomorph morphology: ‘type A’ with anamorphic species *P. asterosporum* and ‘type B’ with *P. betulinum*. Based on morphological similarities, Kamiyama *et al.* (2009) proposed *P. asterosporum* as a synonym of *P. orientale* (Melnik) Kamiyama, Kaz. Tanaka & Melnik occurring on twigs of *Betula* spp.

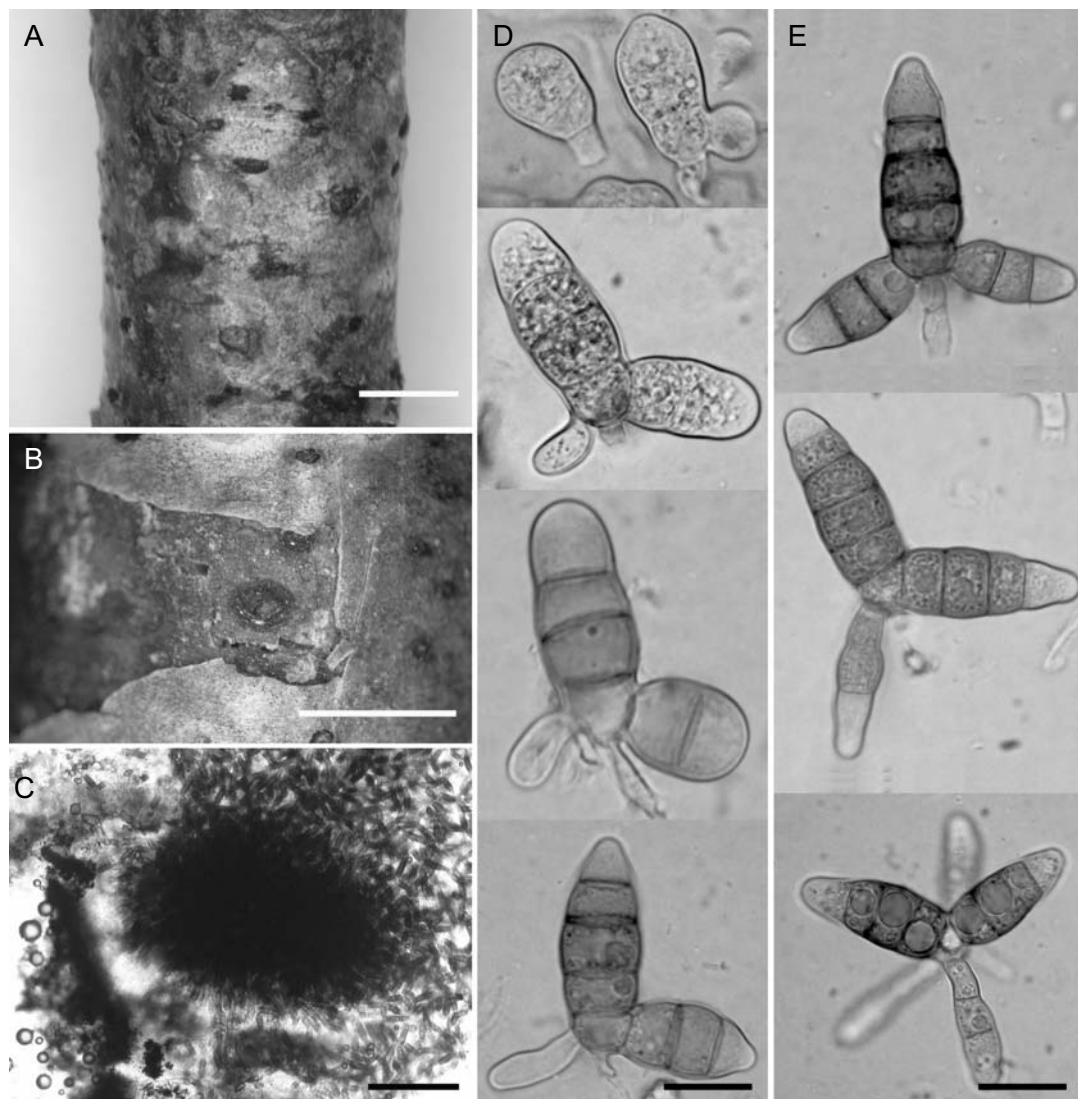
SPECIMENS EXAMINED. On dead twigs of *Betula pendula* – Teleomorph: SLOVAKIA. Hlohovec, 48°26.023'N/17°48.242'E, 3 Nov. 2009, leg. M. Pastirčák (BPI 880436); Važec, 49°04.202'N/19°56.480'E, 1 Dec. 2009, leg. M. Pastirčák (BPI 880435); Šurany, 48°05.179'N/18°10.251'E, 14 Dec. 2009, leg. M. Pastirčák (BPI 880430); Krompachy, 48°55.258'N/20°52.595'E, 2 Jan. 2010, leg. M. Pastirčák (BPI 880432); Leopoldov, 48°26.509'N/17°45.777'E, 5 Feb. 2010, leg. M. Pastirčák (BPI 880429); Piešťany, 48°36.124'N/17°49.234'E, 17 Feb. 2010, leg. M. Pastirčák (BPI 880434); Kapušany near Prešov, 49°02.973'N/21°19.442'E, 7 March 2010, leg. M. Pastirčák (BPI 880433); Kuková, 49°06.586'N/21°27.207'E, 7 March 2010, leg. M. Pastirčák (BPI 880431); Topoľčany, 48°34.020'N/18°12.725'E, 19 Apr. 2010, leg. M. Pastirčák. – Anamorph: SLOVAKIA. Kajal, 48°11.113'N/17°47.043'E, 28 Oct. 2009, leg. M. Pastirčák (BPI 880425); Hlohovec, 48°26.023'N/17°48.242'E, 3 Nov. 2009, leg. M. Pastirčák (BPI 880418); Leopoldov, 48°26.509'N/17°45.777'E, 6 Nov. 2009, leg. M. Pastirčák (BPI 880427); Bratislava, 48°09.420'N/17°09.663'E, 5 Nov. 2009, leg. M. Pastirčák (BPI 880422); Spišské Vlachy, 48°56.099'N/20°48.037'E, 2 Dec. 2009, leg. M. Pastirčák (BPI 880420); Vydriň, 48°59.664'N/20°24.718'E, 2 Dec. 2009, leg. M. Pastirčák (BPI 880415); Nitra, 48°17.835'N/18°04.725'E, 7 Dec. 2009, leg. M. Pastirčák (BPI 880414); Piešťany, 48°36.124'N/17°49.234'E, 11 Dec. 2009, leg. M. Pastirčák (BPI 880428); Galanta, 48°11.229'N/17°43.350'E, 14 Dec. 2009, leg. M. Pastirčák (BPI 880416); Šurany, 48°05.179'N/18°10.251'E,



**Fig. 1.** *Pleomassaria siparia* (Berk. & Broome) Sacc. A – ascoma, B – asci, C – ascospores. Scale bars: A = 100 µm; B & C = 20 µm.

14 Dec. 2009, leg. M. Pastirčák (BPI 880417); Kysak, 48°51.256'N/21°13.529'E, 27 Dec. 2009, leg. M. Pastirčák (BPI 880426); Gíraltovcе, 49°06.771'N/21°30.881'E, 28 Dec. 2009, leg. M. Pastirčák (BPI 880424); Krompachy, 48°55.258'N/20°52.595'E, 2 Jan.

2010, leg. M. Pastirčák (BPI 880421); Kapušany near Prešov, 49°02.973'N/21°19.442'E, 7 March 2010, leg. M. Pastirčák (BPI 880419); Margecany, 48°53.570'N/21°00.895'E, 28 March 2010, leg. M. Pastirčák (BPI 880423).



**Fig. 2.** *Prosthemium betulinum* Kunze [anamorph of *Pleomassaria siparia* (Berk. & Broome) Sacc.]. A & B – conidiomata on host, C – conidioma, D – developing conidia, E – conidia. Scale bars: A & B = 0.5 cm; C = 500  $\mu$ m; D & E = 20  $\mu$ m.

***Splanchnonema argus*** (Berk. & Broome) Kuntze

Revis. Gen. Pl. (Leipzig) 3(2): 531. 1898.

*Sphaeria argus* Berk. & Broome, Ann. Mag. Nat. Hist., Ser. 2, 9: 322. 1852.

*Massaria argus* (Berk. & Broome) Fresen., Beitr. Mykol. 2: 59. 1852.

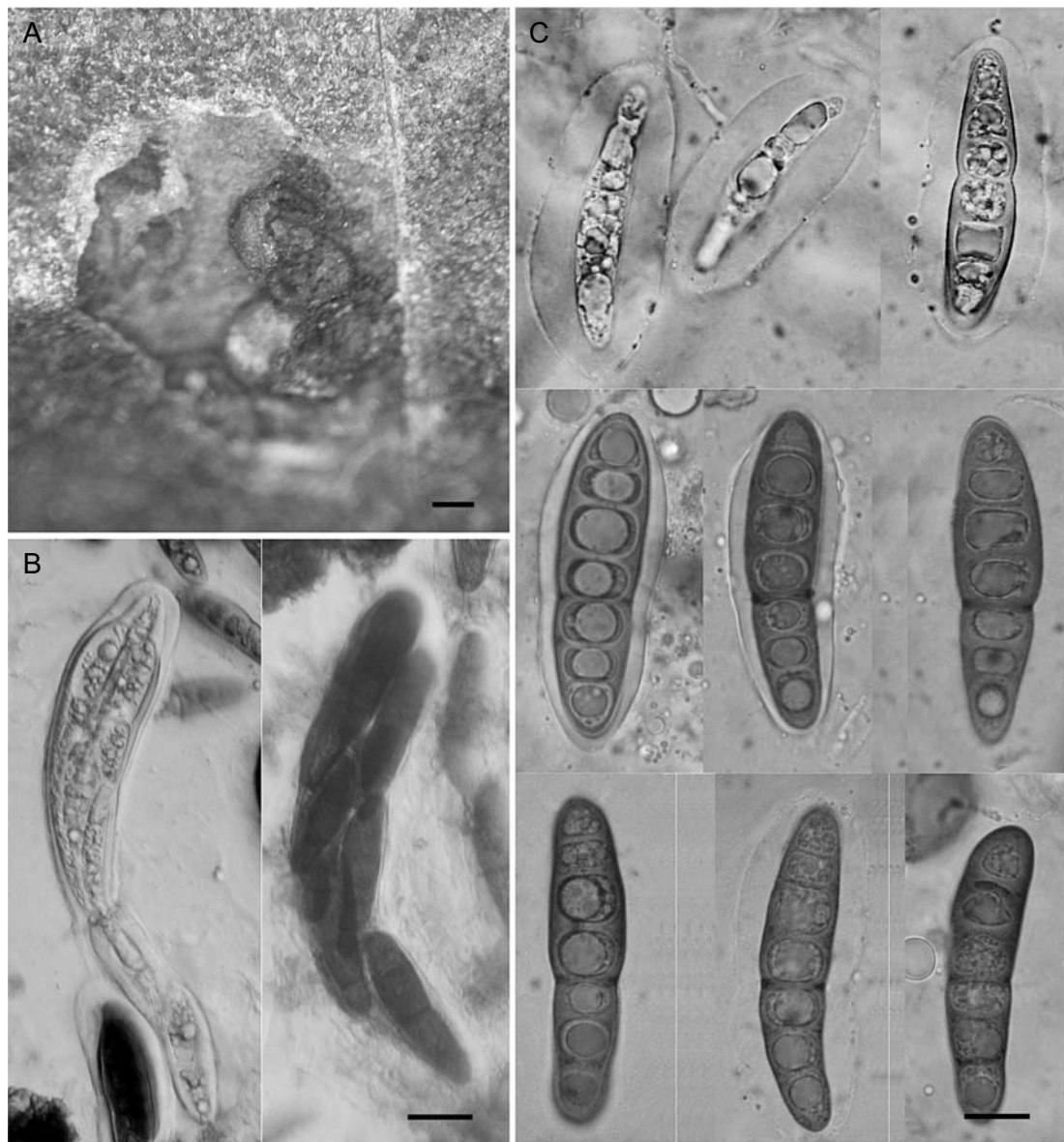
*Massaria niessliana* Rehm (as '*niessleana*'), Hedwigia 21: 84. 1882.

*Splanchnonema niessliana* (Rehm) Kuntze, Revis. Gen. Pl. (Leipzig) 3(2): 531. 1898.

*Stegonsporium muricatum* Bonord., Handb. Allgem. Mykol. (Stuttgart): 60. 1851.

*Stegonsporium polycystis* (Berk. & Broome) Arx (as '*Steganosporium*'), Gen. Fungi Sporul. Cult., Edn 3 (Vaduz): 228. 1981.

ANAMORPH: ***Myxocyclus polycystis*** (Berk. & Broome) Sacc., Annl. Mycol. 6(6): 559. 1908.

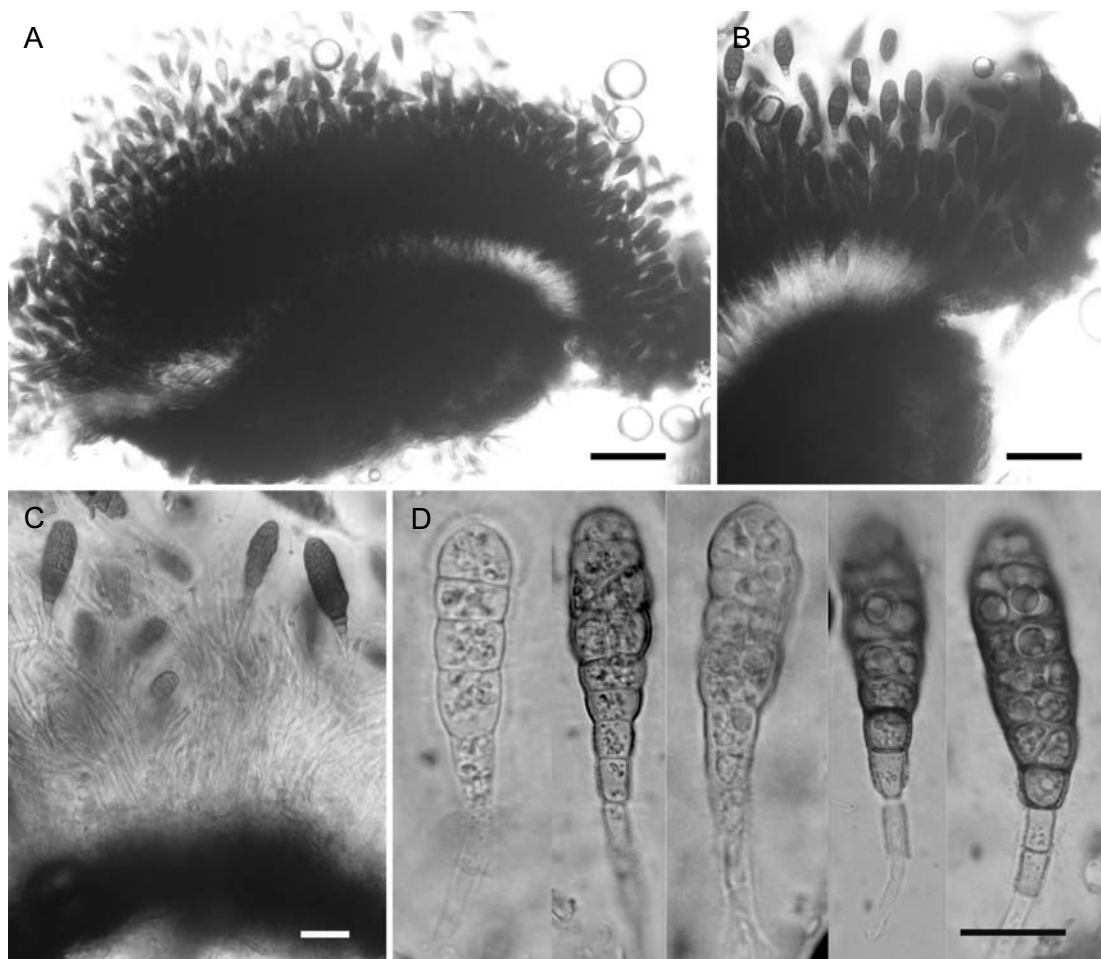


**Fig. 3.** *Splanchnonema argus* (Berk. & Broome) Kuntze. A – ascomata on host, B – asci, C – ascospores. Scale bars: A = 350  $\mu$ m; B = 20  $\mu$ m; C = 10  $\mu$ m.

*Hendersonia polycystis* Berk. & Broome, Ann. Nat. Hist., no. 415. 1850.

Ascomata immersed, scattered to 2–3-crowded, depressed globose, 705–780  $\mu$ m in diameter. Ascomal wall composed of angular brown cell 7.5–11.0  $\mu$ m in diameter. Pseudoparaphyses

1.5–3.0  $\mu$ m thick. Asci clavate, 8-spored, 142.5–196.0  $\times$  30.0–37.5  $\mu$ m (mean = 157.5  $\times$  33.0  $\mu$ m, n = 50). Ascospores cylindrical to clavate, 52.0–66.5  $\times$  13–16  $\mu$ m (mean = 59.5  $\times$  14  $\mu$ m, n = 50), 6–7-septate (mostly 3+1+2), with primary septum submedian, brown, with mucilaginous



**Fig. 4.** *Myxocyclus polycystis* (Berk. & Broome) Sacc. [anamorph of *Splanchnonema argus* (Berk. & Broome) Kuntze]. A & B – conidioma, C – conidiophores, D – conidia. Scale bars: A & B = 100  $\mu\text{m}$ ; C = 35  $\mu\text{m}$ ; D = 20  $\mu\text{m}$ .

sheath 3–9  $\mu\text{m}$  thick, constricted at primary septum (Fig. 3).

**ANAMORPHIC STAGE.** Conidiomata acervular, immersed to erumpent, scattered, cupulate to trapezoid in longitudinal section, 270–570  $\mu\text{m}$  high, 600–750  $\mu\text{m}$  wide at base. Wall composed of hyaline to pale brown prosenchymatic cells, partly with angular cells, with dark brown margin. Conidiophores filiform, hyaline, septate, 78.5–168.5  $\mu\text{m}$  long, 2.0–3.5  $\mu\text{m}$  thick, with slime coating. Conidia cylindrical to clavate, muriform, 40–75  $\times$  14.5–18.5  $\mu\text{m}$  (mean = 59.5  $\times$  17.0  $\mu\text{m}$ ,

n = 50), with (6–)7–8(–9) transverse septa, yellow to brown, echinulate, with mucilaginous sheath 3.5–9.5  $\mu\text{m}$  thick (Fig. 4).

The fungus has been found on *Morus alba* L. in Ukraine and on several species of the genus *Betula* (*B. ermanii* Cham., *B. lenta* L., *B. nigra* L., *B. occidentalis* Hook., *B. papyrifera*, *B. pendula*, *B. platyphylla* Sukaczew, *B. pubescens*) in North America (Canada, U.S.A.), Europe (Lithuania, Poland, Russia, Scotland, Sweden, United Kingdom), Asia (China, Japan) and Australia (Farr & Rossman n.d.; Chlebicki & Treigiene 1995).

Herbarium specimens deposited in BPI confirm that this fungus occurred on *B. pubescens* in the Czech Republic (formerly Czechoslovakia; specimens from the territory of Moravia) and Germany, and on *Betula* sp. in Denmark. The specimen of *Massaria argus* (BPI 619648, not seen) collected by F. Hazslinsky in the former Czechoslovakia, Eperjes (Prešov), is from the territory of Slovakia, but the substrate host is undetermined.

Another species of *Splanchnonema*, *S. scoriadeum* (Fr.) M. E. Barr was found on *Betula* spp. in Europe (England, Scotland, Sweden) and eastern North America (Barr 1982; Farr & Rossman n.d.). The species is distinguishable from *S. argus* by its ascomata erumpent from the host periderm and 1-septate ascospores (septum submedian).

SPECIMENS EXAMINED. On dead twigs of *Betula pendula* – Teleomorph: SLOVAKIA. Kluknava, 48°56.458'N/20°56.145'E, 3 Jan. 2010, *leg. M. Pastirčák* (BPI 880440). – Anamorph: SLOVAKIA. Važec, 49°04.202'N/19°56.480'E, 1 Dec. 2009, *leg. M. Pastirčák* (BPI 880446); Richnava, 48°55.534'N/20°54.913'E, 2 Dec. 2009, *leg. M. Pastirčák* (BPI 880449); Nitra, 48°17.835'N/18°04.725'E, 7 Dec. 2009, *leg. M. Pastirčák* (BPI 880448); Kysak, 48°51.256'N/21°13.529'E, 27 Dec. 2009, *leg. M. Pastirčák* (BPI 880442); Giraltovec, 49°06.771'N/21°30.881'E, 28 Dec. 2009, *leg. M. Pastirčák* (BPI 880441); Kropachy, 48°55.258'N/20°52.595'E, 2 Jan. 2010, *leg. M. Pastirčák* (BPI 880443); Kluknava, 48°56.458'N/20°56.145'E, 3 Jan. 2010, *leg. M. Pastirčák* (BPI 880447); Kapušany near Prešov, 49°02.973'N/21°19.442'E, 7 March 2010, *leg. M. Pastirčák* (BPI 880445); Margecany, 48°53.570'N/21°00.896'E, 28 March 2010, *leg. M. Pastirčák* (BPI 880444).

There are no previous records of *Pleomassaria siparia*, *Splanchnonema argus* and their coelomycetous anamorphs on *B. pendula* in Slovakia. Our collections represent the first records of these fungi in Slovakia.

ACKNOWLEDGEMENTS. We are grateful to the anonymous reviewer for valuable remarks on the manuscript. This study was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences, project no. 2/0149/10, and by the Slovak Research and Development Agency under contract no. APVV-0421-07.

## REFERENCES

- ARNOLD R. H. 1967. A canker and foliage disease of yellow birch. *Canad. J. Bot.* **45**: 783–801.
- BARENGO N., SIEBER T. & HOLDENRIEDER O. 2000. Diversity of endophytic mycobiota in leaves and twigs of pubescent birch (*Betula pubescens*). *Sydowia* **52**: 305–320.
- BARR M. E. 1982. On the Pleomassariaceae (Pleosporales) in North America. *Mycotaxon* **15**: 349–383.
- BERNADOVIČOVÁ S. 2008. Importance of *Cytospora* damage in relation to health state of birch trees in urban greenery – demonstrated by example of the Nitra town. *Folia Oecologica* **35**(2): 1–8.
- CHLEBICKI A. & TREIGIENE A. 1995. Notes on Pyrenomycetes and Coelomycetes from North Lithuania. *Acta Mycol.* **30**: 95–119.
- FARR D. F. & ROSSMAN A. Y. n.d. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved April 27, 2010, from <http://nt.ars-grin.gov/fungalDATABASES/>.
- GREEN S. 2004. Fungi associated with shoots of silver birch (*Betula pendula*) in Scotland. *Mycol. Res.* **108**(11): 1327–1336.
- HANTULA J., HALLAKSELA A. & KURKELA T. 1998. Relationship between *Prosthemium betulinum* and *Pleomassaria siparia*. *Mycol. Res.* **102**(12): 1509–1512.
- JUHÁSOVÁ G. 2004. Poškodenie briez parazitickými mikroskopickými hubami. *Zahradníctví* **7**: 48–49.
- JUHÁSOVÁ G., BERNADOVIČOVÁ S., IVANOVÁ H., ADAMČIKOVÁ K. & KOBZA M. 2005. Damage of species of the genus *Betula* by parasitic fungi. In: I. LUKÁČIK & J. ŠKVARENINOVÁ (eds), *Autochtónna dendroflóra a jej uplatnenie v krajine*, pp. 274–278. Technical University, Zvolen (in Slovak with English summary).
- KAMIYAMA M., HIRAYAMA K., TANAKA K. & MEENIK V. A. 2009. Transfer of *Asterosporium orientale* to the genus *Prosthemium* (Pleosporales, Ascomycota): a common coelomycetous fungus with stellate conidia occurring on twigs of *Betula* spp. *Mycoscience* **50**: 438–441.
- KOWALSKI T. & HOLDENRIEDER O. 1996. *Prosthemium asterosporum* sp. nov., a coelomycete on twigs of *Betula pendula*. *Mycol. Res.* **100**: 1243–1246.
- PAAVOLAINEN L., HANTULA J., HALLAKSELA A. & KURKELA T. 2001. Dynamics of crown thinning and mycobiota on fallen twigs of mature birches (*Betula pendula*) in Finland. *Scandinavian Journal of Forest Research* **16**: 246–252.
- PAAVOLAINEN L., KURKELA T., HALLAKSELA A. & HANTULA J. 2000. *Pleomassaria siparia* is composed of two biological species, both of which occur in dead twigs of *Betula pendula* and *B. pubescens*. *Mycologia* **92**: 253–258.

- TANAKA K., OOKI Y., HATAKEYAMA S., HARADA Y. & BARR M. E. 2005. Pleosporales in Japan (5): *Pleomassaria*, *Asteromassaria*, and *Splanchnonema*. *Mycoscience* **46**: 248–260.
- TREIGIENĖ A., MARKOVSKAJA S. & BAGDŽIŪNAITĖ A. 2007. Micromycetes associated with *Betula* in Lithuania. *Botanica Lithuanica* **13**(3): 181–196.

Received 13 May 2010