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ORIGINAL ARTICLE

Morphological and molecular characterisation of *Periconia pseudobyssoides* sp. nov. and closely related *P. byssoides*

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Abstract Anamorphic ascomycetes of the genus *Periconia*, occurring on invasive *Heracleum sosnowskyi* and on other native *Apiaceae* plants were examined during this study. On the basis of morphological, cultural characteristics and ITS sequences a new species of *Periconia* closely related to *Periconia byssoides*, is described and illustrated. The new species *Periconia pseudobyssoides*, collected on dead stalks of *Heracleum sosnowskyi*, is characterized by producing brownish verruculose mycelium on malt-extract agar, and differs from *P. byssoides* and other known *Periconia* species by producing reddish-brown, macronematous conidiophores with numerous percurrent proliferations, often verruculose at the apex immediately below the conidial head, verrucose ovoid conidiogenous cells arising directly from the swollen apical cell cut off by a septum from the stipe apex, and spherical, reddish-brown, characteristically ornamented verrucose conidia. Detailed description and illustration of morphological characters, cultural characteristics and ITS sequences barcoding are also provided for *P. byssoides*.

Keywords Anamorphic · Ascomycota · Pleosporales · Taxonomy · ITS · rDNA

Introduction

During a study of microfungi associated with *Heracleum sosnowskyi* Manden. (*Apiaceae*), an invasive plant in Lithuania and in other European countries, 34 species of anamorphic fungi was established, including *Periconia* spp. which frequently occurred. Part of *Periconia* specimens were identified as *P. byssoides* Pers., which is widely distributed on *Apiaceae* and other herbaceous plants, but several specimens differed from *P. byssoides* and other known *Periconia* species by morphological and cultural characters. These specimens represented a separate taxonomic entity which is proposed here as a new species.

Most *Periconia* species are widely distributed terrestrial saprobes and endophytes colonizing herbaceous and woody plants in various geographical regions and habitats (Ellis 1971, 1976; Matsushima 1971, 1975, 1980, 1989, 1996; Rao and Rao 1964; Subramanian 1955; Subrahmanyam 1980; Lunghini 1978; Saikia and Sarbhoy 1982; Muntañola-Cvetković et al. 1997, 1998, 1999; Mel'nik 2000; Minter et al. 2001; Carmarán and Novas 2003), but several species of this genus are economically important and well-studied plant pathogenic fungi that cause various diseases of roots and leaves, like *Periconia circinata* (L. Mangin) Sacc. and *P. macrospinosa* Lefebvre & Aar. G. Johnson (Odvody et al. 1977; Romero et al. 2001). Aquatic saprobes are represented only by three species: *P. prolifica* Anastasiou, *P. abyssa* Kohlm. and *P. variicolor* S.A. Cantrell, Hanlin & E. Silva known from mangrove, marine and hypersaline environments (Tubaki and Ito 1973; Kohlmeyer 1977; Alias and Jones 2000; Prasannarai and Sridhar 2001; Cantrell et al. 2007).

Periconia Tode is a polyphyletic anamorphic ascomycete genus belonging to the *Pleosporales* with a rather complicated taxonomy and unclear phylogenetic affinity (Seifert et al. 2011). Following the main generic characters, *Periconia* is characterized by producing pale to dark brown, smooth or rarely verruculose macronematous (mostly with a stipe and apical conidial head, branched or unbranched), and micronematous (undifferentiated) conidiophores. Conidiogenous cells are monoblastic or polyblastic, discrete, ellipsoidal to spherical directly formed on a stipe or on branches. Sometimes the apex is sterile and conidiogenous

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cells with conidia arise in the lower and basal part of the stipe. The conidiogenous cells give rise to acropetal conidial chains by a holoblastic process (except the basipetal production of the conidial chains in *P. prolifica*), but conidial maturation is basipetal (Cole and Samson 1979; Bunning and Griffiths 1982, 1984). Conidia are catenate, non-septate, spherical, ellipsoidal to oblong, smooth or ornamented, pale to dark brown. According to online databases such as Index Fungorum (CABI 2011) and MycoBank (Robert et al. 2005) the genus includes ca. 183 published names with more than 20 transferred to other genera, but currently only ca. 40 species are recognized as genuinely belonging to *Periconia* (Carmarán and Novas 2003; Kirk et al. 2008).

The genus was erected by Tode (1791) with *Periconia lichenoides* Tode as type species. Afterwards, the type specimen was lost and only Tode's drawings were left available for taxonomical comparison. From Tode's expanded account of *P. lichenoides* it is known that the fungus was found on stems of herbaceous plants belonging to various genera somewhere in Germany. Persoon (1801) described *P. byssoides* Pers. also collected in Germany on stems of unidentified herbaceous plants and very similar to *P. lichenoides*. In the middle of the twentieth century, Mason and Ellis (1953) examined six of Persoon's collections labeled *Periconia byssoides* and noted that *P. lichenoides* is probably conspecific with the latter species or with *Periconia cookei* E.W. Mason & M.B. Ellis and considered *Sporocybe byssoides* Fr. a synonym of *P. byssoides*. Some years later Hughes (1958) revised the genus *Periconia*, and after a detailed comparison of all species he designated *P. byssoides* as lectotype on the basis of the protologue of *Sporocybe byssoides* published by Fries (1932), and this lectotypification was legitimate according to Art. 7.8 as associated with the sanctioning protologue of Fries (Seifert et al. 2011). Modern morphological and cultural characterizations of *P. byssoides* are available in various publications (Mason and Ellis 1953; Ellis 1971; Matsushima 1975) and also in Mycobank (Robert et al. 2005), but corresponding DNA sequence data are still absent. The ribosomal internal transcribed spacer (ITS) gene region is generally applied as universal DNA barcode marker for fungi (Begerow et al. 2010; Schoch et al. 2012). Molecular data of the complete ribosomal ITS gene region of *P. byssoides* based on type or lectotype material is not available in National Center for Biotechnology Information (NCBI) GenBank or elsewhere. Only two ITS data of specimens identified as *P. byssoides,* but both of them not collected in Europe (one from North America (Canada), the other from Australia) are available in Global Mirror System of DNA Barcode Data (GMS-DBD 2010), but the data in this database are unfortunately incomplete, without any ecological and morphological details. The specimens collected in Lithuania (Middle Europe) are undoubtedly closer to the old type and lectotype material collected in Germany and may rather be used for DNA barcoding. The aim of this study was to identify, analyze and characterize *Periconia* isolates obtained from *Heracleum sosnowskyi* and other *Apiaceae* plants in Lithuania based on morphology and cultural features as well as rDNA ITS sequence data, including re-examination and barcoding of *P. byssoides* isolates and discussion of the taxonomic position of the species concerned.

Materials and methods

Cultural and morphological studies

Specimens of dead stalks of *Heracleum sosnowskyi* and other *Apiaceae* plants were collected from various localities of Lithuania in 2003 and repeatedly in 2011. All specimens were studied by light microscopy (LM); specimens of the new species were also examined by scanning electron microscopy (SEM). Prior to examination, specimens were incubated in moist chambers at room temperature and then studied under a dissecting microscope Nikon SMZ 800; to obtain axenic cultures, conidia were transferred to malt extract agar (MEA) media. Colonies on MEA developed only from specimens collected in 2011. Colony characters and growth characteristic were studied on MEA plates incubated for 2–4 weeks in the dark at 25±1 °C and later after 1 month's growth under light at room temperature to promote sporulation. Growth rates were determined as the average increase in radius in mm per day of three replicate plates. Axenic cultures were subjected to molecular analysis.

Descriptions and illustrations were made from fresh preparations in distilled water and in 25 % lactic acid, LM photomicrographs were made with a digital camera Nikon DS-Fi1 mounted on a Nikon SMZ 800 binocular and with digital camera Pentax *istDS mounted on a Olympus CX41 light microscope at magnifications up to×100. SEM images were obtained using a Hitachi TM1000 electron microscope. Measurements of 50 mature conidia are indicated in the formal (extreme–)A–B–C(−extreme) where A and C are limits of the 95 % interval and B the arithmetic mean. Dimensions (only the extremes) of other structures are given as a range of at least 30 measurements. Colors were determined according to Kornerup and Wanscher (1978). The holotype and other dried specimens, axenic cultures and permanent slides are deposited in the Herbarium of Institute of Botany, Nature Research Centre, Vilnius, Lithuania (BILAS). The nomenclatural novelty is deposited in MycoBank.

DNA extraction, amplification, sequencing and analysis

Genomic DNA was extracted from fresh axenic cultures grown on Malt Extract Agar (MEA) solid medium with NucleoSpin® Plant II Kit (Macherey–Nagel GmbH &Co. KG, Germany) according to manufacturer's instruction using approximately 100 mg wet weight of mycelium. The internal

transcribed spacers 1 and 2 of rDNA, including the 5.8S rDNA, were amplified in 25 μl reactions on TProfessional 96 Gradient Thermocycler (Biometra GmbH, Germany) in the following mixture: ~ 25 ng of template, 0.25 units of Taq polymerase (Thermo Fisher Scientific Baltics, Lithuania), 2.5 μl 10× PCR buffer with KCl and $MgCl_2$, 0.2 mM of each dNTP, 10 μM of primers ITS5 and ITS4 (White et al. 1990). PCR conditions: 5 min at 95 °C as initial denaturation, followed 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 45 s at 72 °C, with final extension of 10 min at 72 °C. Amplicons were visualised under UV light in 1.5 % agarose gels stained with AtlasSight DNA Stain (Bioatlas, Estonia). The PCR products were purified according to the Protocol for PCR Product Clean-up with Exonuclease I and FastAP™ Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific Baltics, Lithuania). Purified PCR products were sequenced by Macrogen (Macrogen Europe, Amsterdam, the Netherlands) on an ABI 3730XL DNA sequencer. PCR products were successfully amplified with the estimated size (603–604 characters). Four different PCR products from each specimen with 2 repeats for each were sequenced from both ends (5' and 3') to confirm the sequence. The rDNA homology searches (BLAST) were performed through the internet at the National Center for Biotechnology Information (National Institutes of Health, Bethesda, USA). Sequences were aligned by using Clustal W, and the phylogenetic tree of the closely related sequences was constructed using the Lasergene software package (DNASTAR, Inc., Madison, USA) by neighbour-joining (NJ). All novel sequences derived in this study were deposited in the NCBI GenBank (Table 1).

Results

Taxonomy

Periconia pseudobyssoides S. Markovskaja & A. Kačergius sp. nov., Figs. 1, 2, 3, 4, 5 and 6.

Table 1 List of fungal taxa and GenBank ITS sequences data used in the phylogenetic analyses

Species/taxon name	Country of origin, reference	GenBank/accession No
Periconia pseudobyssoides S1-11P	Lithuania, this study	KC954161
Periconia byssoides S2-11P	Lithuania, this study	KC954159
Periconia byssoides S3-11P	Lithuania, this study	KC954160
Periconia byssoides DP-10P	Lithuania, this study	KC954157
Periconia byssoides DP-11P	Lithuania, this study	KC954158
Uncultured fungus clone	Germany, Frohlich-Nowoisky et al. 2009	GQ999472
Uncultured fungus clone	Germany, Frohlich-Nowoisky et al. 2009	GQ999522
Uncultured fungus clone	South Korea, Jeon 2012	JX984754
Pleosporales	China, Unterseher and Schnittler 2011	FR864980
Periconia sp.	USA, Rodrigues et al. 2011	HQ608021
Periconia macrospinosa	Canada, Corredor et al. 2010	GU934558
Massarina ignaria	Mexico, Soca-Chafre et al. 2011	GQ377480
Periconia macrospinosa	Hungary, Knapp et al. 2012	JN859364
Periconia macrospinosa	USA, Mandyam et al. 2010	FJ536208
Uncultured Periconia	Austria, Klaubauf et al. 2010	GU055658
Periconia ignaria	Russia, Kolomiets et al. 2008	EU367468
Periconia sp.	USA, Cantrell et al. 2006	DQ336713
Periconiella velotina	South Africa, Arzanlou et al. 2007	EU041781
Periconiella velotina	South Africa, Arzanlou et al. 2007	EU041782
Periconiella velotina	South Africa, Arzanlou et al. 2007	EU041783
Mycosphaerella laricina	The Netherlands, Verkley et al. 2004	AY152590
Mycosphaerella microsora	Germany, Simon et al. 2009	EU167599
Mycosphaerella punctiformis	The Netherlands, Verkley et al. 2004	AY152594
Periconiella levispora	Sri Lanka, Arzanlou et al. 2007	EU041780
Mycosphaerella lateralis	Australia, Crous et al. 2009	GQ852740
Mycosphaerella eumusae	India, Thangavel et al. 2009	GU168024
Pleospora sp.	Peru, Gazis and Chaverri 2010	FJ884149
Mycosphaerella berberis	Germany, Simon et al. 2009	EU167603
Suillus americanus	USA, Wu et al. 2000	AF166502

MycoBank No MB 804763

Periconiae byssoidis similis, sed conidiophoris percurrente proliferantibus, ramulis nullis, infra capitum conidiorum saepe verruculosis, cellulis conidiogenis verruculosis ovatis, ipse ex cellulis apicalibus inflatis cum septis basalibus oriundis et conidiis globosis, aureo- vel rubro-brunneis, singulariter verruculosis, (12–)15–17(−20) μm diam.

Type: Lithuania, Vilnius, 54°45′10″N, 25°16′04″E, on dead stalks of *Heracleum sosnowskyi*, 28 October 2011, leg. S. Markovskaja, BILAS 50334 (holotype), ex- holotype culture BILAS 50334(S1-11P), GenBank accession No KC954161.

Etymology: The epithet refers to **Greek** "*pseûdos*", combining form meaning false, pretended, unreal; compound word "*pseudobyssoides*"denoting close or deceptive resemblance to *P. byssoides.*

Morphological characteristic: Colonies in vivo effuse, dark brown to black, hairy. Conidiophores macronematous, arising usually singly, but occasionally 2 or 3(−4) together on stromata, brown (6D7) to reddish brown (8D6-8), 4–6-septate, thick-walled, often with 2–4 enteroblastic, percurrent proliferations. The first proliferation arises from the apical cell of the conidiophore, the subsequent proliferation arises from the next available septum which is below the original proliferation, extending beyond the open end of the proliferations. Conidiogenesis usually accompanied by the formation of one or two transverse septa in the proliferations. Conidiophores often verruculose,

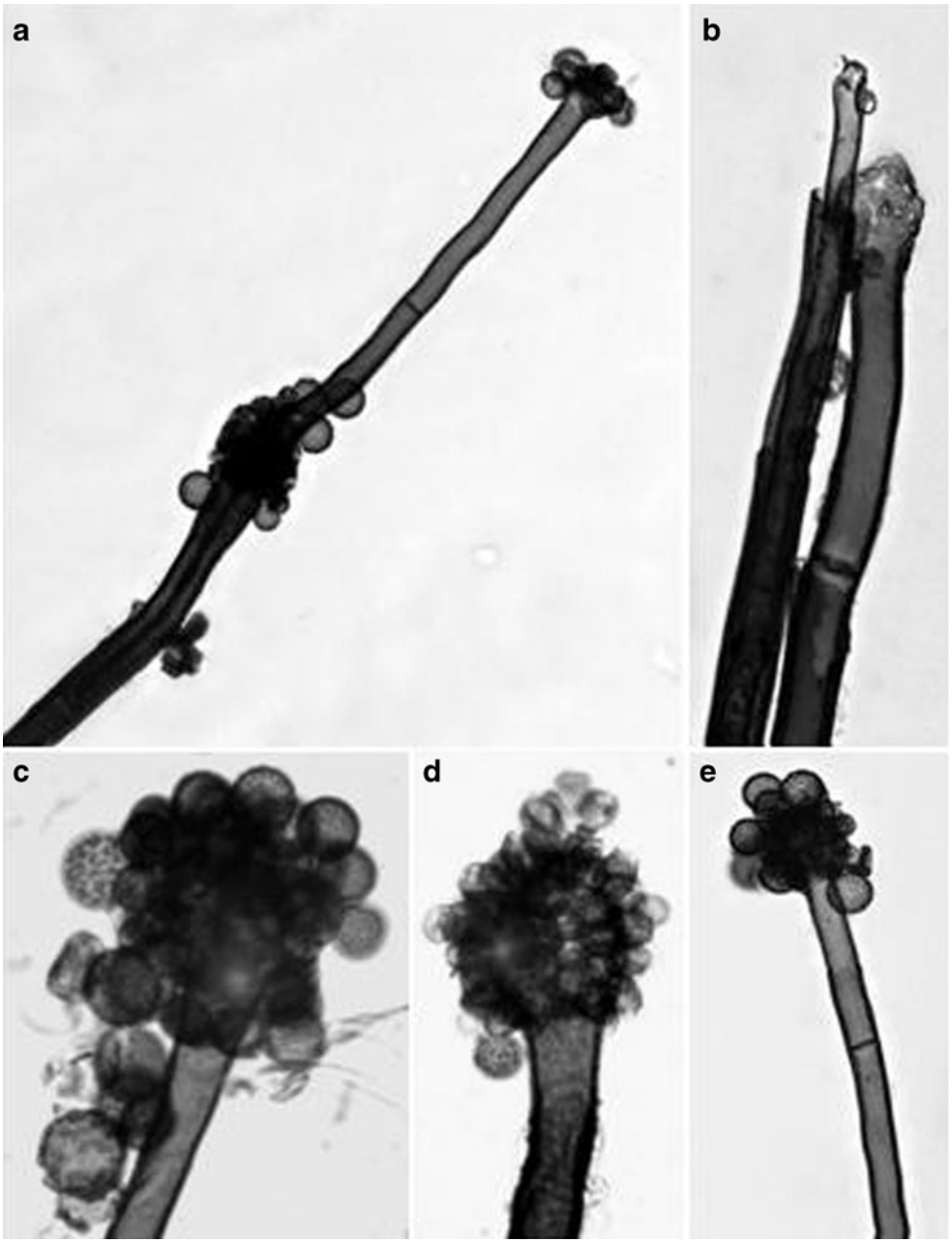

Fig. 1 *P. pseudobyssoides* (from holotype, LM): a, b Proliferation of conidiophores from the septum below original proliferation, and its extension beyond the open end of the first proliferation; c–e Apical part of conidiophores with heads of conidia. Scale bars: a, e=50 μm; b–d=20 μm

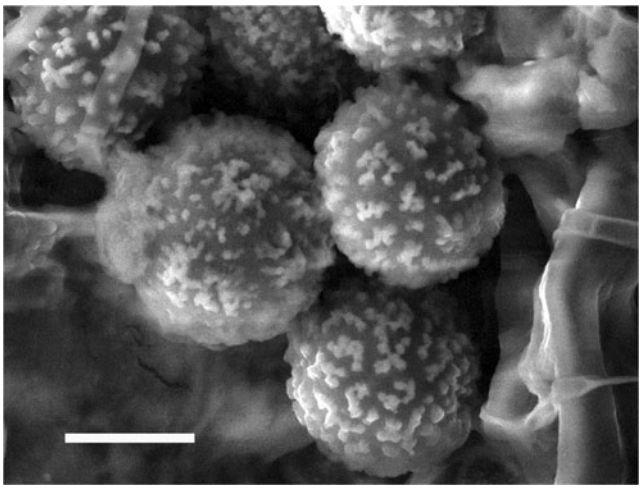

Fig. 2 *P. pseudobyssoides* (from holotype, SEM): Details of the ornamentation of conidia (verrucae joined in irregular lobate or star-shaped narrow crests. Scale bar 10 μm

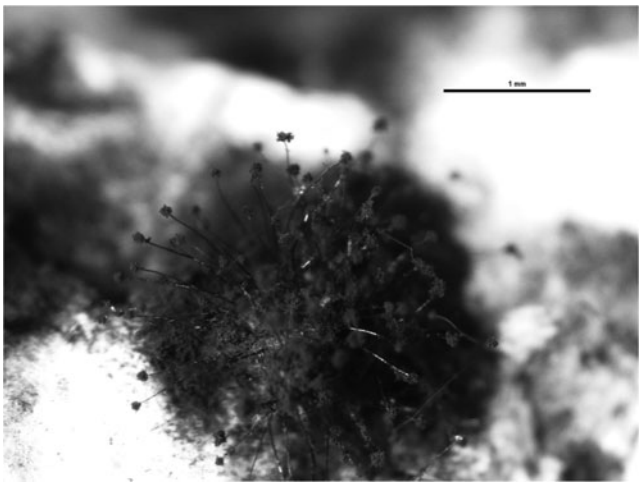

Fig. 4 Conidiophores with conidial heads of *P. pseudobyssoides* on the host plant *Heracleum sosnowskyi.* Scale bar 1 mm

especially at the apical part immediately below the well-defined conidial heads, (8–)12.5–16(−17.5) μm wide in this part, 23–25 μm wide at the base and up to 300–600 μm long. Swollen apical parts and conidial heads of the new proliferating conidiophores are progressively smaller. Brown to reddish brown conidial heads are globose, 30–70 μm diam. Conidiogenous cells are discrete, determinate, usually monoblastic, sometimes polyblastic, ellipsoidal, ovoid to clavate, pale brown (5D5) to reddish brown (8D6), verruculose, 10–12×6–7.5 μm, arising directly from the swollen mid-brown apical cell cut off by a septum from the stipe apex. Apical cell (15–)22–25(−27.5) μm diam. Conidia spherical, golden yellow (5B7) to golden brown (5D7) or reddish brown (8D6), (12–)15–17(−20) μm diam., verrucose when young, mature conidia with specific ornamentation consisting of irregular lobate crests. Conidia born singly

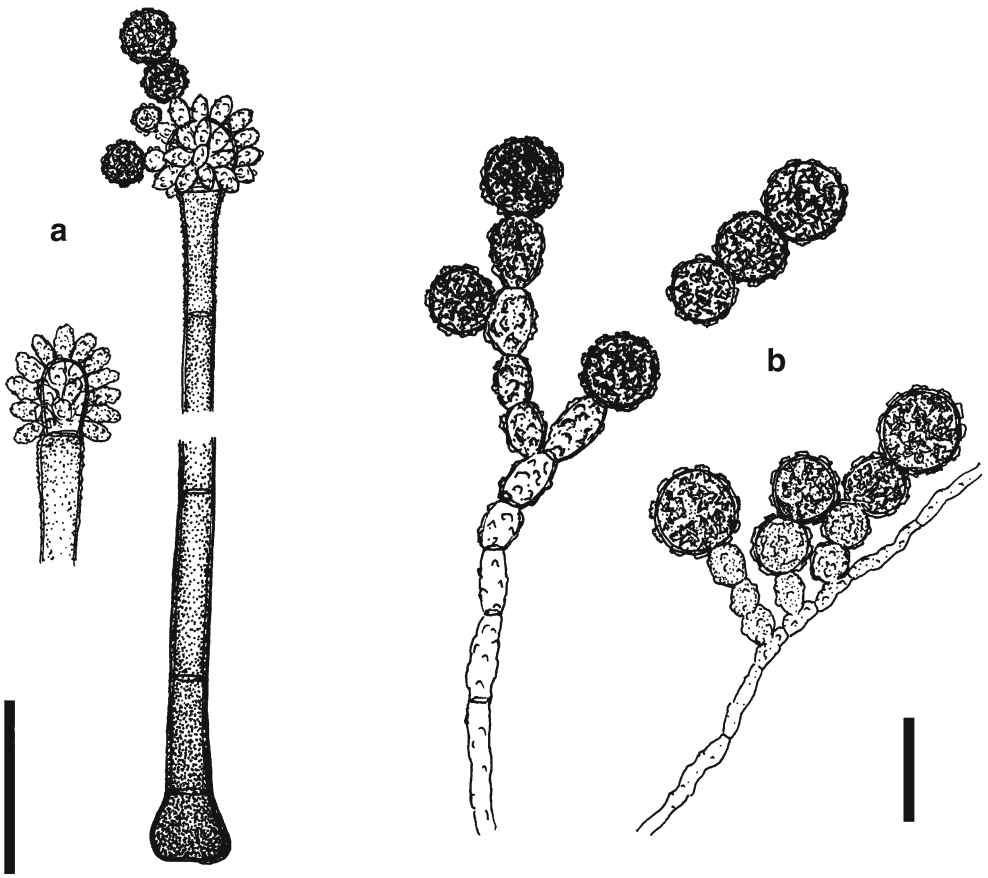

Fig. 3 *P. pseudobyssoides* (from holotype): a Macronematous conidiophores with apical cell and verruculose conidiogenous cells producing chains of verrucose conidia, developing on host plant; b Differing micronematous conidiophores with conidia, developing on malt-extract agar (MEA) medium. Scale bars: a=50 μm; b=20 μm

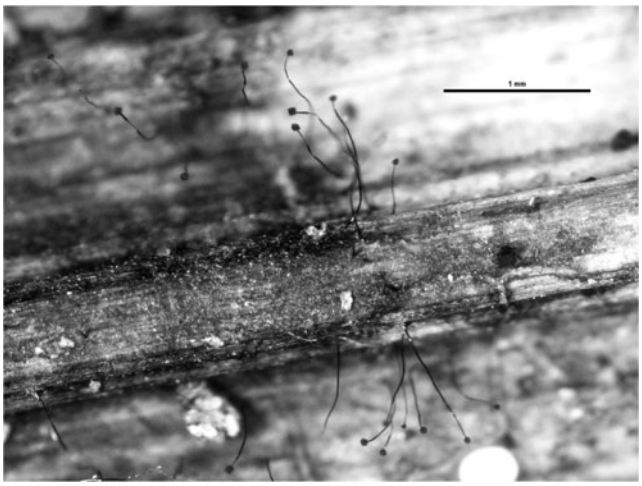

Fig. 5 Tuft of macronematous conidiophores with conidial heads of *P. pseudobyssoides* from culture on MEA. Scale bar 1 mm

or in acropetal chains of 2–4 conidia. Maturation basipetal. Conidiogenesis holoblastic, conidial secession schizolytic. Teleomorph unknown.

Cultural characteristic: Colonies on MEA growing approximately 1.8 mm/day, reaching 50 mm after 20 days at 25±1 °C, cottony with abundant grey (4D1-E1) to olive-brown (4E5), fluffy aerial mycelium, surface in center dark grey (4AF1) with concentric white-grey rings; in reverse colony dark olive-brown (4 F6-4 F8) with concentric dark olivaceous-brown rings, margin even, white to grey (4A1–4B1). Hyaline or greyish green (1C4) verruculose hyphae becoming brownish orange (6C7-8) with pale brown (6D8) verrucae after several weeks. Sporulation occurring on MEA after about 2–3 weeks of incubation as brown clusters of conidia scattered on the surface. Conidiophores simple, micro- and semi-macronematous, unbranched and branched, initially subhyaline or greyish green (1C4), become pale brown (5D5), verruculose, variable in length and constricted at the septae, 20–40×3–6 μm, with numerous

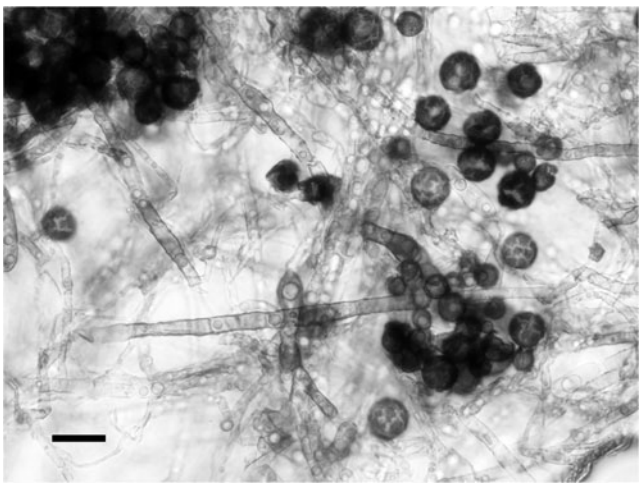

Fig. 6 Micronematous conidiophores and conidia of of *P. pseudobyssoides* from culture on MEA. Scale bar 20 μm

proliferations. Conidiogenous cells discrete, determinate, terminal or lateral, elongate, ovoid to subglobose, mono- and polyblastic, verrucose, pale brown (5D5) to reddish golden (6C7) producing globose, verrucose conidia in acropetal chains (2–3 in number), (12.5–)15–17.5(−20) μm diam., during maturation conidia turn from golden yellow (5B7) to golden brown (5D7) and reddish brown (8D6) with large lobate verrucae joined in crests. Conidia germinate by one subhyaline germ tube and produce greyish green (1C4) to brownish (6C7), verruculose, septate hyphae. Typical macronematous conidiophores with a swollen apex and heads of conidia are formed on the surface of dried MEA media after 1–2 months in groups or tufts (Fig. 5). Morphologically they are similar to conidiophores occurring on the host, but on MEA conidial heads are somewhat larger, up to 80 μm diam.

Ecology: Terrestrial saprobe and probably endophyte found on dead stalks of *Heracleum sosnowskyi*.

Distribution: Known from Lithuania.

Other examined collections: Lithuania, Ignalina distr., Keižiai environs, 55º31′19″N, 26º23′16″E, on dead stalks of *Heracleum sosnowskyi*, 30 July 2003 (BILAS 48786), leg. Z. Gudžinskas, det. S. Markovskaja; Vilnius environs, 54º45′08″N, 25º16′02″E., 6 August 2003 (BILAS 29183), leg. Z. Gudžinskas, det. S. Markovskaja; Vilnius distr., Akmena environs, 55º00′08″N, 25º21′32″E., 21 July 2003 (BILAS 48790), leg. Z. Gudžinskas, det. S. Markovskaja.

Periconia byssoides Pers., *Syn. meth. fung.* (Göttingen), 1: 18 (1801) Figs. 7, 8, 9 and 10.

≡ *Sporocybe byssoides* (Pers.) Fries, *Syst. Mycol*. (Lundae) 3(2): 343 (1832).

≡ *Cephalotrichum byssoides* (Pers.) Kuntze [as '*byssodes*'], *Revis. gen. pl.* (Leipzig) 3(2): 453 (1898).

= *Periconia pycnospora* Fresen., *Beitr. Mykol.* 1: 20 (1850).

Detail description and illustration in Masson and Ellis (1953).

Morphological examination, measurements of conidiophores and conidia revealed that the fungal specimens collected in 2011 on *Heracleum sosnowskyi* and on some other *Apiaceae* plants (*Angelica sylvestris* L., *Conium maculatum* L.) agreed well with the current concept of *P. byssoides* which was based on lectotype material, the protologue (Fries 1832) and the detailed descriptions and illustrations given by Mason and Ellis (1953), Hughes (1958), Ellis (1971) and Matsushima (1975). Therefore, we assumed that they were typical examples of *P. byssoides*, and one of them (BILAS 50335/culture S2-11P) was selected as a representative specimen for cultural characteristic in this study and r-DNA barcoding.

Cultural characteristic: Colonies on MEA growing approximately 1.6 mm/day, reaching 30–40 mm after 20 days at 25°, cottony with abundant white (5A1) to orange-white (5A2), fluffy aerial mycelium, surface in center white (5A1) to pale orange (5A3), in periphery with concentric rings,

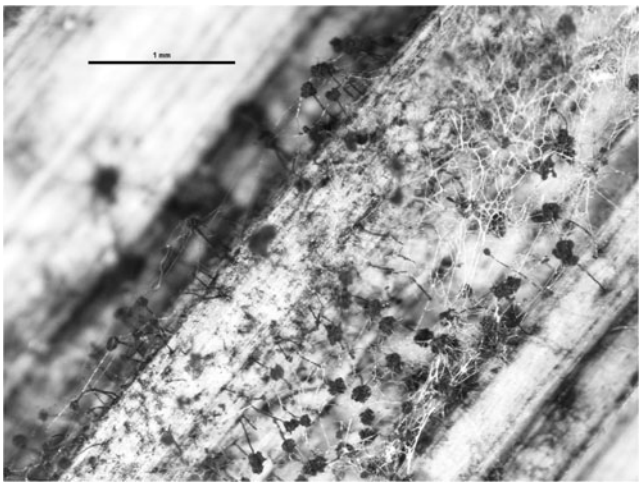

Fig. 7 Conidiophores with conidial heads of *P. byssoides* on host plant *Heracleum sosnowskyi.* Scale bar 1 mm

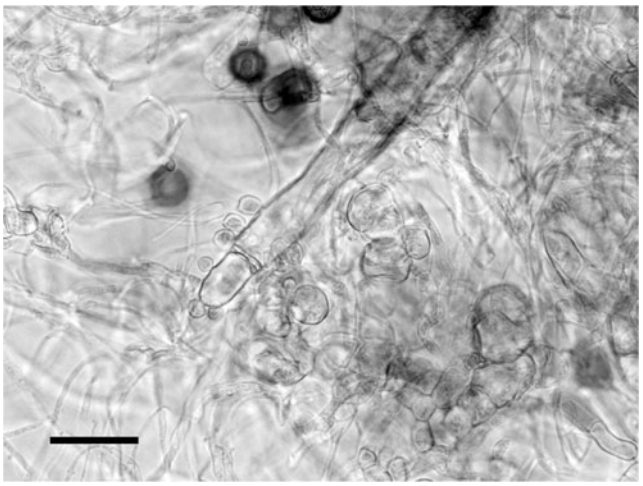

Fig. 9 Immature conidiophore with hyaline apical cell of *P. byssoides* from culture on MEA. Scale bar 20 μm

reddish yellow (4B7) to greyish yellow (4B6) on the surface and olive-brown (4E5) or olive-green (3D7) in deeper layers; in reverse colony dark olive (3E4) to dark olivaceous-grey (3 F2) with concentric dark olivaceous-brown (4E5) rings, margin even, white to grey (4A1–4B1). Hyaline or greyish green (1C4), smooth and verruculose inflated hyphae after several weeks becoming brownish orange (6C7-8) and sometimes verruculose. After 3–4 weeks of incubation on MEA sporulation occurring as brown clusters of conidia scattered on the surface. Observed conidiophores were simple, micro- and semi-macronematous, unbranched and branched, initially subhyaline to brownish (6C6-6D6), verruculose, and variable in length. Conidiogenous cells discrete, determinate, terminal or lateral, subglobose, mono- and polyblastic, smooth to verruculose, pale brown (5D5) producing global verrucose conidia in acropetal chains (3–4 in number), (11.5–)12.5–15(−17) μm diam., conidia pale brown (5D5) to brown (6D7-6E7) verrucose. Typical macronematous conidiophores with a hyaline apical cells and heads of conidia (about 50–110 μm in diam.) formed singly or in small groups pushing through the weft of mycelium on the surface of dried MEA media after a month. They developed from flattened cells, and initially their basal part was greyish green (26D4-28E6), during maturation becoming dark brown (6 F6), but the apical part remained subhyaline or hyaline and was cut off by septa. Conidiogenous cells were formed on an apical cell and in the collar region around the septa, sometimes on short hyaline or subhyaline branchlets. From primary, hyaline, globose conidiogenuos cells numerous secondary conidiogenous cells

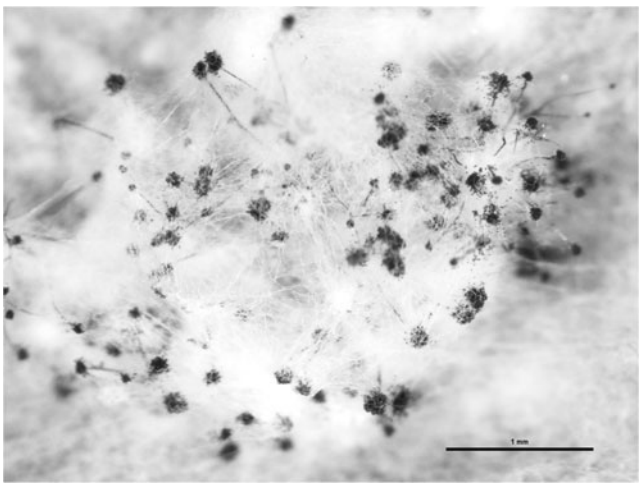

Fig. 8 Macronematous conidiophores with conidial heads of *P. byssoides* from culture on MEA. Scale bar 1 mm

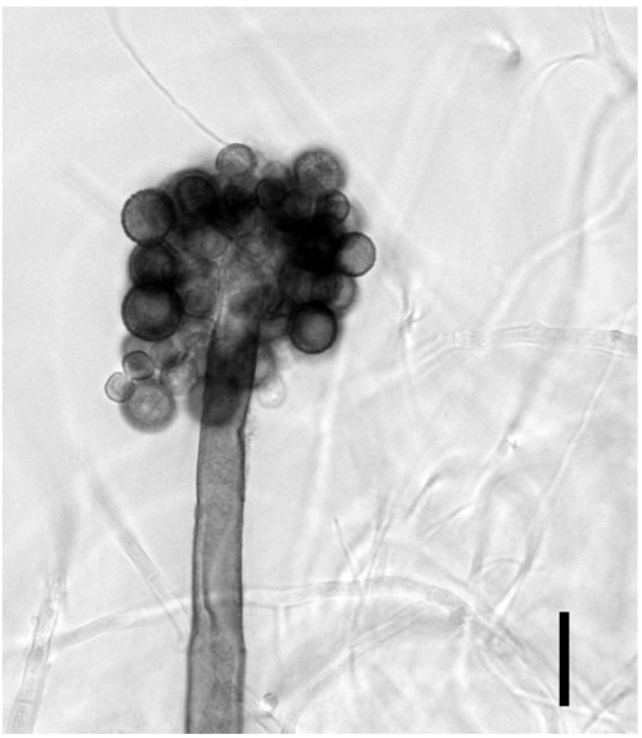

Fig. 10 Mature conidiophore with head of verruculose conidia of *P. byssoides* from a culture on MEA. Scale bar 20 μm

arise, which produce short chains of sphaerical, commonly verruculose but sometimes verrucose, pale brown to brown conidia, 10–15 μm diam. Macronematous conidiophores developing on MEA are morphologically similar to conidiophores occurring in vivo, but on hosts they are usually dark brown with paler brown apical part.

Material examined: Lithuania, Vilnius, Santariškės, 54°44′ 08″N, 25°16′02″E, on dead stalks of *Heracleum sosnowskyi,* BILAS 50335/culture S2-11P, GenBank accession No KC954159, 29 November 2011, leg./det. S. Markovskaja; Lithuania, Vilnius distr., Pranciškonys environs, on dead stalks of *Heracleum sosnowskyi,* BILAS 50336/culture S3-11P, GenBank accession No KC954160, 3 October 2011, leg./det. S. Markovskaja; Molėtai distr., Berža lake enirons, on stalk of *Angelica sylvestris* L., BILAS 50338/culture DP-10P, GenBank accession No KC954157, 22 September 2011, leg./det. A. Kačergius; Lithuania, Molėtai distr., Berža lake environs, on stalk of *Conium maculatum* L., BILAS 50337/culture DP-11P, GenBank accession No KC954158, 22 September 2011, leg./det. A. Kačergius.

Ecology: Terrestrial facultative saprobe on dead stalks of various herbaceous and woody plants, found also on leaf spots.

Distribution: Very common in Europe and both Americas, also known from Asia, Africa and Australia.

Molecular analysis

DNA sequences of five Lithuanian isolates were included in the ITS sequence analysis: four of *P. byssoides* (GenBank accession No KC954158; No KC954157; KC954159; KC954160) consisting of 604 nucleotides and one of *P. pseudobyssoides* (GenBank accession No KC954161) consisting of 603 nucleotides. A total of 28 isolates of *Pleosporales*, of which twelve belonged to *Periconia*, four to *Periconiella*, six to *Mycosphaerella* and other undetermined species (Table 1). *Suillus americanus* (*Basidiomycota*) was designed as outgroup. The phylogenetic tree inferred from this analysis demonstrated two well-supported subclades in strongly supported 'Periconia' clade (Fig. 11). Lithuanian ITS sequences of *P. byssoides* and of new *P. pseudobyssoides* clustered with the environmental ITS sequences from Germany (uncultured fungus clone, GenBank accession No GQ999472 and No GQ999522) and from South Korea (uncultured fungus clone, GenBank accession No JX984754) into separate subclade. Molecular analysis of ITS sequences revealed a close relationship of the new taxon with *P. byssoides*.

Discussion

Based on morphological and molecular data, *P. byssoides* was identified as a common species on various *Apiacea* plants in Lithuania, but on the invasive *Heracleum sosnowskyi*, together with *P. byssoides*, a similar, but morphologically and genetically well-defined species was found. The new species can be characterized as roughly intermediate taxon between *P. byssoides* and *P. cookei*, which are also known on various *Apiaceae* hosts (Yadav 1966; Matsushima 1975; Mel'nik 2000). All of them produce long, brown, unbranched conidiophores with swollen, brown or dark brown heads of verrucose conidia at the apex, but differ from each other by a complex of features such as color, conidiogenesis mode and ornamentation of conidia. With regard to shape, size and verrucose ornamentation of conidia the new species is also similar to *P. shyamala* A. K. Roy and *P. typhicola* E. W. Mason & M. B. Ellis, but is clearly distinguished by color and conidiogenesis (Table 2, Fig. 1). A comparison of SEM images of these closely allied species showed additional differences in the conidial wall ornamentation. While in *P. pseudobyssoides* it consists of numerous verrucae connected in irregular, lobate or star-shaped narrow crests (Fig. 2), in *P. byssoides* verrucae are connected in more flattened irregular warts (Bunning and Griffiths 1984), whereas in *P. cookei* verrucae are more regular and not fused in warts (Cole and Samson 1979). *P. shyamala* mainly differs in having larger conidia, and *P. typhycola* is distinguished by its conidiophores that produce branches inside the apical head (Table 1). Verruculose ornamentation of conidiophores in their apical parts immediately below the conidial heads, observed in *P. pseudobyssoides* resemble such in *P. shyamala* (Storey 2002), but differ in color (reddish brown in *P. pseudobyssoides* and brown to dark brown in *P. shyamala*). In *P. byssoides*, apical part of conidiophores is usually smooth and subhyaline or light brown, apical cell is elongated and hyaline (not swollen as in *P. pseudobyssoidesi* and *P. cookei*), sometimes with short branchlets around the septa like in *P. shyamala*, conidiogenous cells polyblastic and subhyaline, conidial heads large, up to 110 μm diam. In *P. pseudobyssoides*, conidial heads are more compact (30–80 μm diam.), conidiogenous cells are mostly monoblastic, verruculose, arising directly from the elongated or swollen apical cell of the conidiophore as in *P. cookei*, and apical cell is mostly cut off by septa as in *P. byssoides*. The reddish brown color of mature conidiophores, conidiogenous cells and conidia of the new species resemble *P. atropurpurea* (Berk. & M.A. Curtis) M.A.Litv., *P. saraswatipurensis* Bilgrami and *P. flabelliformis* Munt.-Cvetk., Hoyo & Gómez-Bolea, however, these species strongly differ in various other morphological characters (Bilgrami 1963; Ellis 1971; Muntañola-Cvetković et al. 1999). Percurrent proliferation of conidiophores, which is a taxonomically important feature, is known in *P. byssoides*, *P. cookei* (Mason and Ellis 1953) and in the marine species *P. prolifica*, but the latter sharply differs from *P. pseudobyssoides* in a quite distinct ecology, morphology and conidial ontogeny (Anastasiou 1963; Bunning and Griffiths 1982; Vrijmoed et al. 1982).

A megablast search in GenBank using ITS sequences was mainly uninformative, because data for compared ITS

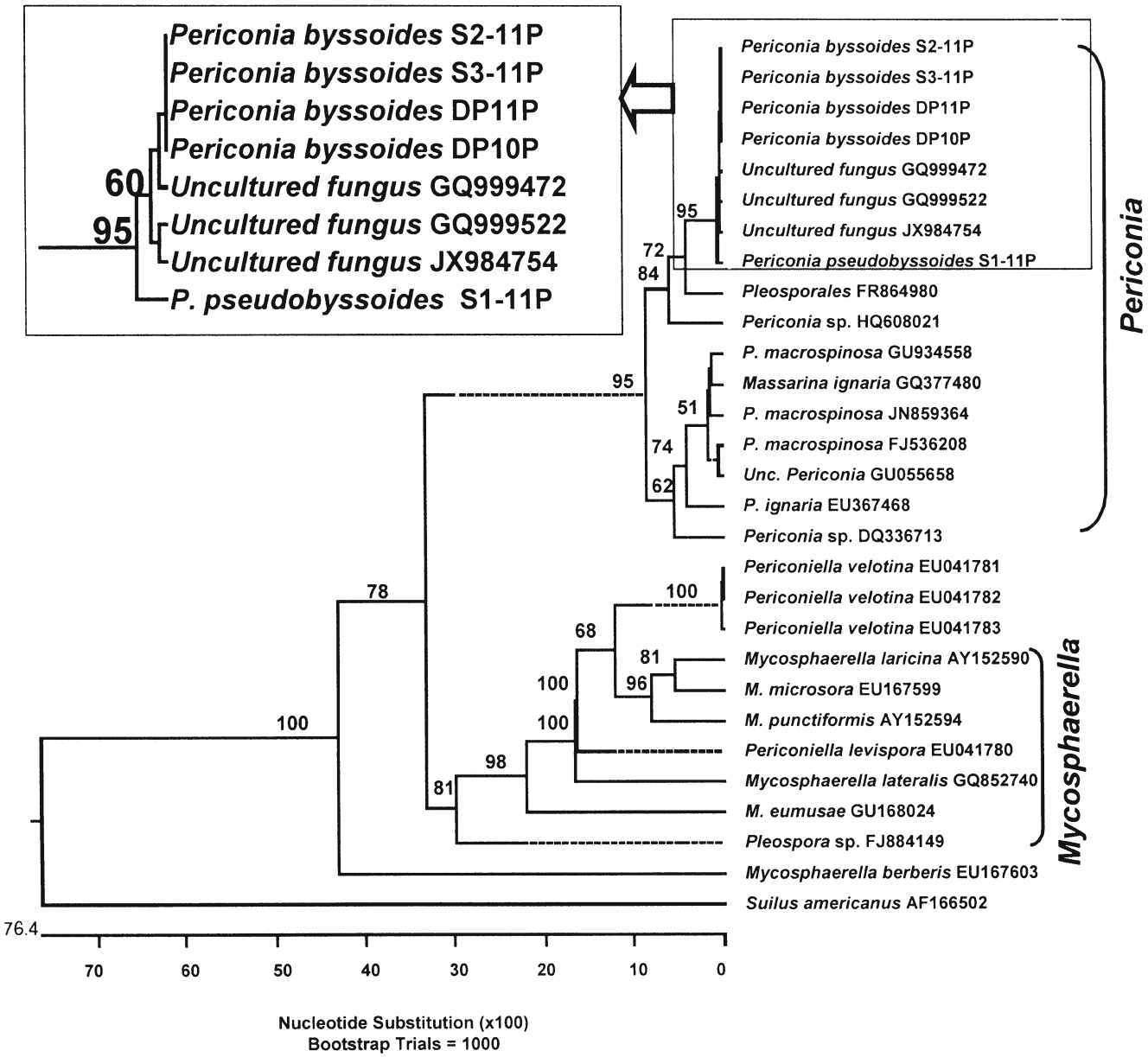

Fig. 11 Neighbour-joining tree formed from ITS region of the rDNA gene, calculated in Lasergene without pairwise corrections. Numbers above branches are bootstrap values obtained from 1000 replications, shown only for branches supported by more than 50 %. Length of the branches is proportional of number of changes

regions were available only for several species of *Periconia* and absent for the most morphologically similar species *P. byssoides* and *P. cookei*. Mostly unnamed sequences, deposited as '*Periconia* sp.' and 'uncultured fungi', were obtained. In GenBank, the closest sister taxa to Lithuanian isolates of *P. byssoides*, with 99 % identities, were 'uncultured fungi', *Pleosporales* sp. and 'Fungal sp.' (Table 3). Other related accessions, though less similar, were *Massarina ignaria* No GQ377480; Identities=442/486 (91 %), Gaps=10/486 (2 %) and uncultured *Periconia* clone No GU055658; Identities=455/506 (90 %), Gaps=11/506 (2 %). Sequences derived from type and lectotype of *P. byssoides*, collected in Germany (Tode 1791; Persoon 1801) do not exist. Notable that sequences derived from Lithuanian collections showed high similarity with two samples of unknown fungi (GenBank accession No GQ999472 and GQ999522) from Germany (Table 3), which in phylogenetic tree nested in *Periconia byssoides* subclade (Fig. 11). Although the molecular differences between Lithuanian specimens are relatively low (the ITS sequence analysis showed a high similarity between the isolates of *P. byssoides* and of *P. pseudobyssoides*; Identities=99 % (576/580), with 1 gap (1/580(0 %)), but their morphological and cultural characters are very distinct. The resolution of pure ITS data is often not sufficient at

Table 2 Morphological comparison between *P. pseudobyssoides* and similar *Periconia* species

Species	Conidiophores	Conidia	Color of Conidia	Habitat
P. byssoides (Mason and Ellis 1953; Ellis 1971; Matsushima 1975)	Macronematous and proliferating with elongate apical cell and with branchlets	Spherical, verrucose 10–12(17) μm in diam.	Pale brown to brown	Terrestrial saprobe
Periconia pseudobyssoides	Macronematous, with numerous proliferations and swollen apical cell without branchlets	Spherical, verrucose, (12)15–17(20) μm in diam.	Golden brown to reddish-brown	Terrestrial saprobe
P. cookei (Mason and Ellis 1953; Ellis 1971)	Macronematous, with proliferations and swollen apex without apical cell	Spherical, verrucose 13–16 μm in diam.	Brown to dark brown	Terrestrial saprobe
P. shyamala (Ellis 1971; Storey 2002)	Macronematous, with elongate apical cell and branchlets	Spherical, verrucose (16)18–22(25) μm in diam.	Brown to dark brown	Terrestrial parasite or saprobe
P. typhicola (Ellis 1976)	Macronematous, branched inside the head	Spherical, verrucose 11–17 μm in diam.	Pale brown to brown	Terrestrial saprobe
P. prolifica (Anastasiou 1963; Vrijmoed et al. 1982)	Micronematous, semimicronematous with proliferations	Subglobose, smooth, 7.5–15 μm in diam.	Subhyaline to pale brown	Marine saprobe

species level, above all in assemblages of closely related species as recently it was strikingly demonstrated for *Cercospora* (Groenewald et al. 2012). 99 % congruence in ITS data may indicate a single or different species, but based on obvious morphological differences between *P. byssoides*, *P. cookei* and the new taxon isolated from *Heracleum sosnowskyi* it is justified to describe the latter as separate species. Combined multigene DNA phylogenetic analyses based on another gene sequences (LSU, β-tubulin gene, histone, translation elongation factor 1-α) currently is unable due to lack of DNA information of *Periconia* species in GenBank.

The phylogenetic tree constructed on the basis of ITS sequences together with the data available from GenBank accessions (Table 1), shows that all Lithuanian isolates together with unidentified environmental isolates clustered in a well-supported '*P. byssoides*' subclade with high (95 %) bootstrap value (Fig. 11). Notably, analysis of ITS sequences revealed that Lithuanian isolates of *P. byssoides* were more similar to environmental isolates from air samples in Germany (Frohlich-Nowoisky et al. 2009) and in South Korea (Jeon 2012) than to *P. pseudobyssoides* isolate. It is also noteworthy that morphologically extremely different pathogenic *Periconia macrospinosa* clustered in separate subclade, together with *P. igniaria* E.W. Mason & M.B. Ellis (teleomorph *Massarina igniaria* (C. Booth) Aptroot)) and some undetermined *Pleosporales* and *Periconia* spp. (Fig. 11).

ITS sequences of two isolates deposited under the name *P. byssoides* (DAOM226813 from Australia and 2WL1IIA4 from Canada) in the Global Mirror System of DNA Barcode Data (GMS-DBD 2010) showed insufficient identity with our isolates and between each other. The congruence between the Australian and Canadian isolates of *P. byssoides* same as between Lithuanian isolates and the Australian isolate (DAOM226813) is only 85.79 %, whereas the dissimilarity between the isolate from Canada (2WL1IIA4) and those from Lithuania is even higher (71.69 % congruence), suggesting that the Australian and Canadian isolates were based on misidentifications, i.e., they do not belong to *P. byssoides* and represent some other species.

Sexual stages (teleomorphs) are only known for two species assigned to *Periconia*. They belong to different genera and moreover to different orders suggesting that the genus *Periconia* is polyphyletic in its current circumscription. The teleomorph of *Periconia igniaria* was originally placed in *Didymosphaeria* (*D. igniaria* C. Booth, *Pleosporales*, Ellis 1971), whereas the teleomorph of *Periconia prolifica* was described as *Halosphaeria* (*H. cucullata* (Kohlm.) Kohlm., *Halosphaeriales,* Kohlmeyer 1972), but during the last

Table 3 List of closest to *P. byssoides* by ITS sequences sister taxa from GenBank

Taxon	GenBank No	Identities	Gaps
Uncultured fungi	GQ999472	600/601 (99 %)	0/601 (0 %)
Uncultured fungi	GQ999522	599/601 (99 %)	0/601 (0 %)
Uncultured fungi	JX984754	603/604 (99 %)	0/604 (0 %)
Pleosporales sp.	FR864980	487/493 (99 %)	2/493 (0 %)
Fungal sp.	JN207280	510/514 (99 %)	0/514 (0 %)
Massarina ignaria	GQ377480	442/486 (91 %)	10/486 (2 %)
Uncultured Periconia clone	GU055658	455/506 (90 %)	11/506 (2 %)

decades the taxonomic positions of these teleomorphs have been changed several times. *Didymosphaeria igniaria* C. Booth is currently placed in the genus *Massarina* (*Massarinaceae*, *Pleosporales,* Aptroot 1998) and *Halosphaeria cucullata* in *Okeanomyces* (*Halosphaeriaceae, Microascales*, Pang et al. 2004). *Periconia prolifica* should be excluded from *Periconia* because the fungus is not only distinct by molecular data but differs in having other mode of conidiogenesis characterised by basipetal production of conidial chains, whereas all true *Periconia* species produce acropetal conidial chains. Therefore, *P. prolifica* should better be referred to as *Okeanomyces*, especially in the context of today's discontinuation of dual nomenclature of pleomorphic fungi.

Results of this study proved the taxonomical position of the anamorphic ascomycetous fungus *Periconia byssoides*, which is close to or identical with the type species of *Periconia*, and the new species *P. pseudobyssoides* among true representatives of *Periconia* belonging to *Pleosporales*, *Pleosporomycetidae, Dothideomycetes, Pezizomycotina, Ascomycota* (Mycobank, Robert et al. 2005). Nowadays the taxonomical position of most species is doubtful and requires clarification. A comprehensive taxonomic revision of the whole genus, including molecular analyses, is urgently necessary.

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