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ORIGINAL ARTICLE

The complete life cycle of *Petrakia echinata*

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Abstract The teleomorph of *Petrakia echinata* (Peglion) Syd. & P. Syd. on leaves of *Acer pseudoplatanus* is described based on field collections, culture studies and ITS sequencing and was assigned to the genus *Mycodidymella* C.Z. Wei, Y. Harada & K. Katumoto. In addition, a *Mycopappus* anamorph and a presumed spermatial *Phoma* stage were observed and conspecificity with *P. echinata* was confirmed by culture morphology and ITS sequencing. The phylogenetic relationships between the *Mycodidymella* teleomorph of *P. echinata* and related taxa were studied using LSU nDNA sequences. The fungus causes brown spots on the leaves of the host plant, known as "Petrakia leaf blotch of sycamore maple".

Keywords Ascomycota · Phylogeny · Pleosporales

Introduction

The hyphomycete *Petrakia echinata* (Peglion) Syd. & P. Syd. is known as the causal agent of a conspicuous leaf blotch disease on *Acer pseudoplatanus* (Kirisits 2007; Brandenburger 1985). Petrak (1966) reported the occurrence of this pathogen in the Caucasus, Austria and the Czech Republic. Recent records come from Austria (Kirisits 2007), Switzerland (Meier et al. 2008) and southern Germany (K. J. Lang, personal communication, June 2008), where disease incidence has apparently increased during the last decade. During the summer of 2007, *P. echinata* was also found in northern Germany (Lower Saxony), which might be an indication of recent dispersal to northern latitudes (Kehr and Butin 2008). However, the pathogen might also have been overlooked in this region, because the symptoms can be macroscopically confused with a leaf blotch disease caused by *Pleuroceras pseudoplatani* (Tubeuf) M. Monod (Butin 2011).

The life cycle of *P. echinata* has never been studied previously. However, the conidia are short lived (H. Butin, unpublished) and the mode of overwintering was unknown. Here we describe the teleomorph, a *Mycopappus* synanamorph and a presumed spermatial state of the form genus *Phoma*. These findings contribute to a more complete picture of the *Petrakia* leaf blotch disease.

Material and methods

Morphology

A hitherto undescribed bitunicate ascomycete was discovered on leaf litter of *Acer pseudoplatanus* collected in Lower Saxonia, Germany, in April 2008 for the first time. Subsequently, other collections of the fungus were made in the Bavarian forest, Germany, and on two sites in Switzerland. In addition, leaves with *Petrakia* leaf blotch were incubated in gardens in Wolfenbüttel, Germany, and in Zurich, Switzerland, under near natural conditions to induce formation of the teleomorph. Several collections of the presumed *Petrakia* and *Mycopappus* synanamorphs were also made.

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Hand sections of fruit bodies were prepared under a dissection microscope (B3, Wild, Heerbrugg, Switzerland) using a razor blade and viewed in tap water, concentrated lactic acid, or in 0.1 % (w/v) cotton blue in lactic acid to enhance the visibility of fungal structures. For light microscopy, a Zeiss Standard Microscope (Carl Zeiss, Oberkochen, Germany) was used. The spore dimensions were measured in tap water at 1,000 × magnification.

Ascoma-tissue cultures, multi-conidia (collected in autumn) cultures, and mono- and multi-ascospore (ejected from mature fruit bodies in spring) cultures were prepared from various collections on 2 % (w/v) malt extract agar (MEA) amended with 50 mg l^{-1} Terramycin (MEAT, Ahlich et al. (1998)). Strains were incubated on MEA at 20 °C to determine growth rates. From the *Mycopappus* stage, mono-conidium cultures were prepared by incubation of infected leaves in a moist chamber for 2–3 days at room temperature and transferring newly formed propagules to MEAT using a fine needle. Multi-ascospore cultures were also made from an associated *Mycosphaerella* species for comparison.

Inoculation experiment

Fully expanded, detached leaves of *Acer pseudoplatanus* were superficially wounded (a) either by making a ca. 5 mm long tangential cut on a main vein using a scalpel under a dissection microscope without prior disinfection or (b) by scarification with sterile sandpaper after local disinfection with concentrated ethanol. Subsequently, the inoculum (ca. 3×3×3 mm agar cube from a growing colony) was placed upside down on the wounded leaf surface and a drop of sterile demineralized water was added to keep the inoculum moist. The leaves were incubated in a moist chamber at room temperature in diffused daylight or at 18 °C under near UV light (Leach 1971). In addition, cultures were made on autoclaved maple leaf pieces on water agar in Petri dishes and in test tubes with 2 ml water.

Field inoculations were performed on saplings growing in the shade in a garden in Zurich using method b on 31 May 2012. For the first 5 days, the inoculum was covered with a small plastic bag fixed by a clothespin. A small piece of a stiff plastic sheet placed on the abaxial leaf surface was used as support.

All inoculation experiments were repeated thrice to five times on leaves of different saplings.

DNA amplification and sequencing

Cultivation and DNA extraction were performed as previously described (Grünig et al. 2003). The ITS regions of the rDNA of the isolates listed in Table 1 were amplified using primers prITS1 and prITS4 (White et al. 1990). In addition, a part of the 28S rDNA of one isolate of the presumed *Petrakia* teleomorph (culture no. HB 090918.1) was amplified using primers LR0R (GTACCCGCTGAACTTAAGC) and LR3 (CCGTGTTTCAAGACGGG) according to Vilgalys (1992). PCR conditions were as described in Grünig et al. (2007) except for the annealing temperature, which was set to 55 °C. Sequencing of purified PCR products was performed by Microsynth (Balgach, Switzerland) using sequencing primer LR0R for the 28S rDNA, and prITS4 for the ITS regions. Sequences were deposited in GenBank (Table 1).

Phylogenetic analysis

Phylogenetic analysis was performed using the partial 28S rDNA (LSU) sequences of the *Petrakia* teleomorph and several pleosporalean species because an initial BLAST search indicated that *P. echinata* belongs to the Pleosporales. Most species included in the phylogeny were chosen based on the comprehensive multi-locus phylogeny of the Pleosporales provided by Zhang et al. (2012). Sequences were obtained from GenBank. *Mycosphaerella punctiformis* served as an outgroup. Maximum likelihood analyses were performed in PAUP (Swofford 1998) using the substitution model identified by MODELTEST v3.7 (Posada and Crandall 1998). Bootstrapping to generate 100 pseudosamples was used for accuracy estimations.

Results

Life cycle

Numerous small black fruit bodies were observed on the adaxial side of litter leaves of *A. pseudoplatanus* with *Petrakia* blotch disease in the forest and after garden incubation during the following spring (Fig. 1a–b), but only a low proportion of specimens was fertile. Ascospore formation in the garden could only be observed in two collections from Germany (specimens no. 2 and 3), whereas incubation trials performed in Switzerland failed. However, numerous mature pseudothecia were observed on litter leaves from a riparian forest in Aristau, Switzerland, in spring 2011 (specimen no. 6). At another site at Zurich with confirmed Petrakia leaf blotch in 2010, the *P. echinata* teleomorph was detected only rarely during the following spring (specimen no. 9). However, *Lachnum rhytismatis* (W. Phillips) Nannf. and *Mycosphaerella* sp. (specimen no. 10) were abundant. The globular, black sub-epidermal ascomata (diam. 50–90 μm) of *Mycosphaerella* sp. occurred on the abaxial leaf side, the ascospores were hyaline, 1-septate and measured 7–9×3–3.5 μm.

Table 1 Cultures of *Petrakia echinata* and *Mycosphaerella* sp. used in the present study. Specimen numbers refer to the last paragraph of the "Results" section

Species	Origin	Original culture designation	CBS number	Genbank number
P. echinata	*Mycodidymella*-multi-ascospore culture from specimen no. 2	HB 090918.1	133072	ITS: JQ655727 28S: JQ691627
P. echinata	*Mycodidymella*-mono-ascospore culture from specimen no. 6	OH 110503.20M1	133070	ITS: JQ691628
P. echinata	*Petrakia*-multi-conidia culture from specimen no. 7	OH 080921.4	133071	ITS: JQ691629
P. echinata	*Mycopappus*-single-propagule culture from specimen no. 8	OH 080907.1a	133069	ITS: JQ691630
Mycosphaerella sp.	Multi-ascospore culture from specimen no. 10	OH 110503.8	133073	ITS: JQ691631

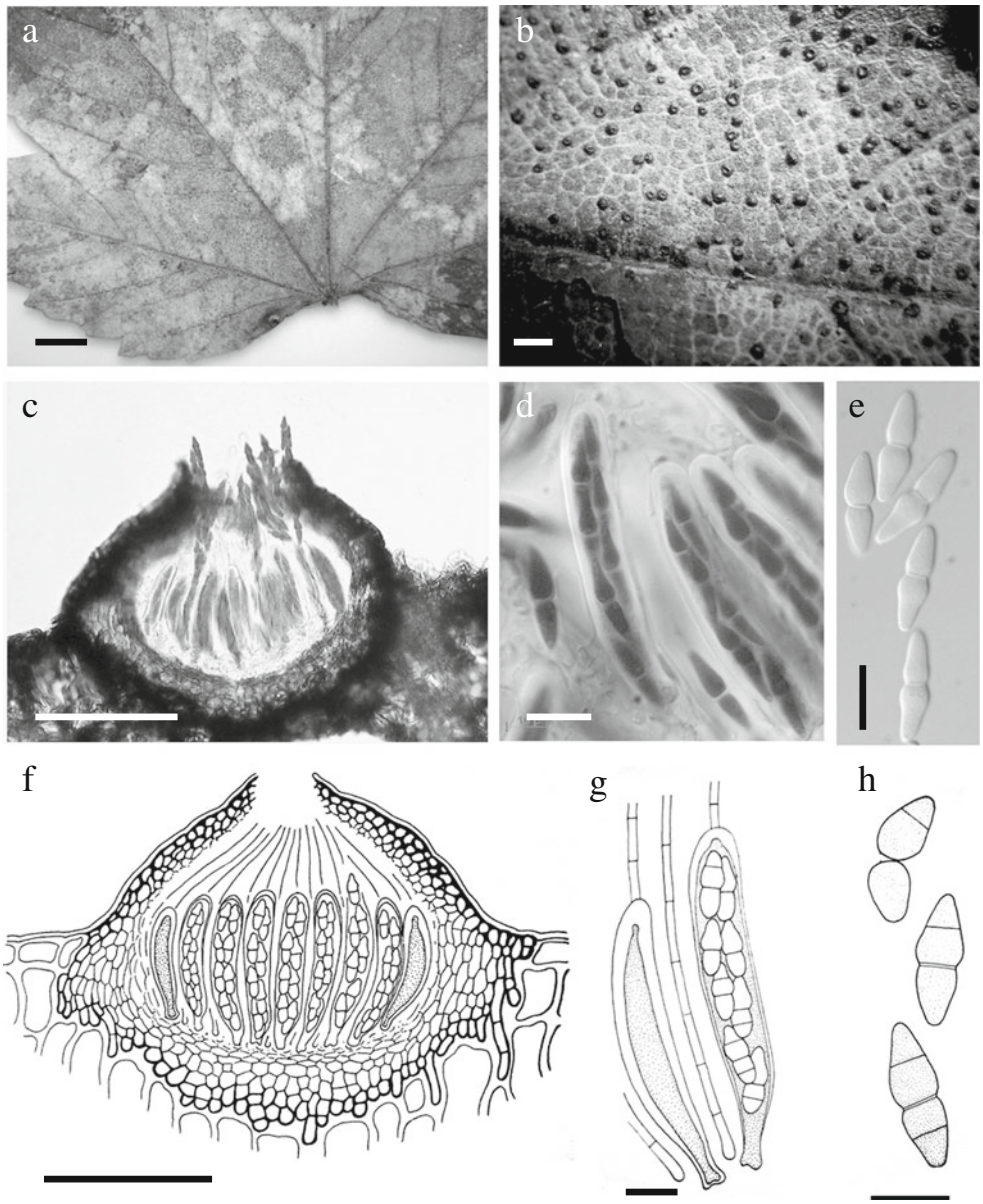

Fig. 1 *Mycodidymella* teleomorph of *Petrakia echinata*. **a** Pseudothecia on a dry litter leaf of *A. pseudoplatanus* (dark areas). **b** Moistened pseudothecia, close-up view. **c** Vertical section through a mature ascoma, **d** asci and ascospores (**c, d** stained with cotton blue in lactic acid). **e** Freshly ejected ascospores. **f** Idealized sectional drawing of a mature pseudothecium, **g** asci and paraphyses, **h** ascospores. Scale bars for **a** = 1 cm; **b** = 1 mm; **c** = 100 μm; **d, e** = 10 μm; **f** = 100 μm; **g, h** = 10 μm

Cultural characteristics and DNA sequences

When *P. echinata* conidia (Fig. 2e) were left to germinate in distilled water at 20 °C, long branched hyphae developed after approximately 4 h from the horn-like projections of the conidia. A few hours later, hyphae developed also from some of the melanized conidial cells. The growth rate on MEA at 20 °C was 0.9–1.4 mm d^{-1}. Colonies were at first white, developed a floccose aerial mycelium with grey tinges within a week and became darker with culture age. Three month old cultures were almost black. Freshly isolated cultures showed a pronounced aerial mycelium with irregular surface (Fig. 3d), whereas subcultures frequently formed flat homogeneous colonies with very sparse aerial mycelium (Fig. 3e). The culture outline was in most cases circular, but sometimes lobed. The reverse of the colonies showed a black coloration with milky-grey tinges. The aerial hyphae were hyaline to dark brown and 3–5 μm thick, and no spores were formed.

Ascospores of the *P. echinata* teleomorph incubated in distilled water at room temperature germinated after 4 hours even when hyaline and still in the ascus. In contrast to multi-ascospore cultures, mono-ascospore cultures grew very slowly during the first 1–2 months and showed growth rates of only 0.15–0.35 mm d^{-1}. However, later on they achieved the same growth rate as multi-ascospore isolates. Colonies originating from ascospores of the newly discovered teleomorph were indistinguishable from colonies originating from *P. echinata* conidia. On MEA and autoclaved maple leaves, the cultures remained sterile, even after incubation under near UV light for 2 months.

Occasionally a *Mycopappus* anamorph (specimen no. 8) was detected before *P. echinata* developed on the same leaf spots (Fig. 2a–d). Cultural characteristics of this fungus were identical to those of *P. echinata*.

The ITS sequences of the cultures HB 090918.1 and OH 110503.20M1 (*Mycodidymella* teleomorph of *P. echinata*), OH 080921.4 (*P. echinata* anamorph) and OH 080907.1a (*Mycopappus* anamorph of *P. echinata*) (Table 1) were identical, indicating that *P. echinata*, the newly discovered *Mycodidymella* teleomorph and the *Mycopappus* synanamorph are different stages of the same fungus. The ITS and the LSU sequence of *P. echinata* revealed 98 % and 99 % homology with the ITS and LSU sequence of *Xenostigmina zilleri* (CBS isolate 115686, Fig. 4), respectively. *X. zilleri* is an anamorphic fungus from *Acer macrophyllum* in North America (Crous et al. 2009; Funk and Dorworth 1988).

Cultures of *Mycosphaerella* sp. (Table 1), which was frequently associated with the *Petrakia* teleomorph, were somewhat similar to *P. echinata* cultures in the beginning, but showed a distinctly slower growth rate in subcultures (0.3–0.4 mm d^{-1}) and a very different ITS sequence. Moreover, the aerial mycelium of *Mycosphaerella* sp. cultures was composed of more narrow hyphae (1–3 μm) than that of *Petrakia* cultures (3–5 μm), remained whitish-grey in

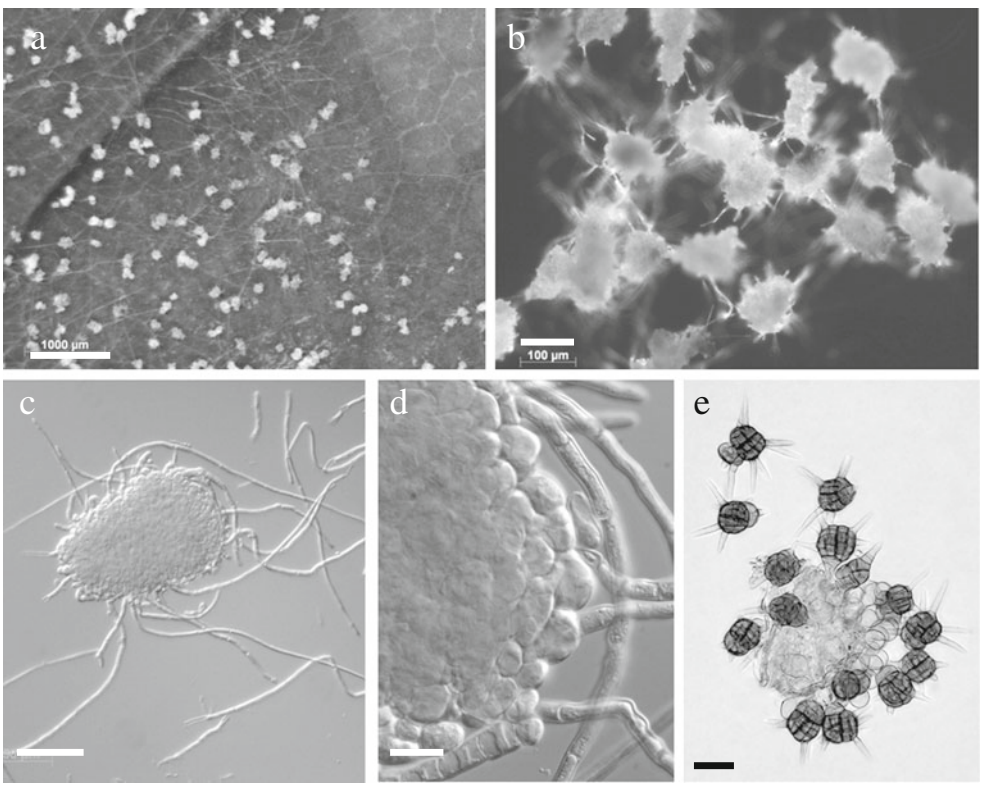

Fig. 2 Anamorphic stages of *Petrakia echinata*. **a–d** *Mycopappus* stage. **e** Sporodochium of *Petrakia echinata* and conidia. Scale bars for **a** = 1 mm; **b** = 100 μm; **c** = 50 μm; **d** = 10 μm, **e** = 20 μm

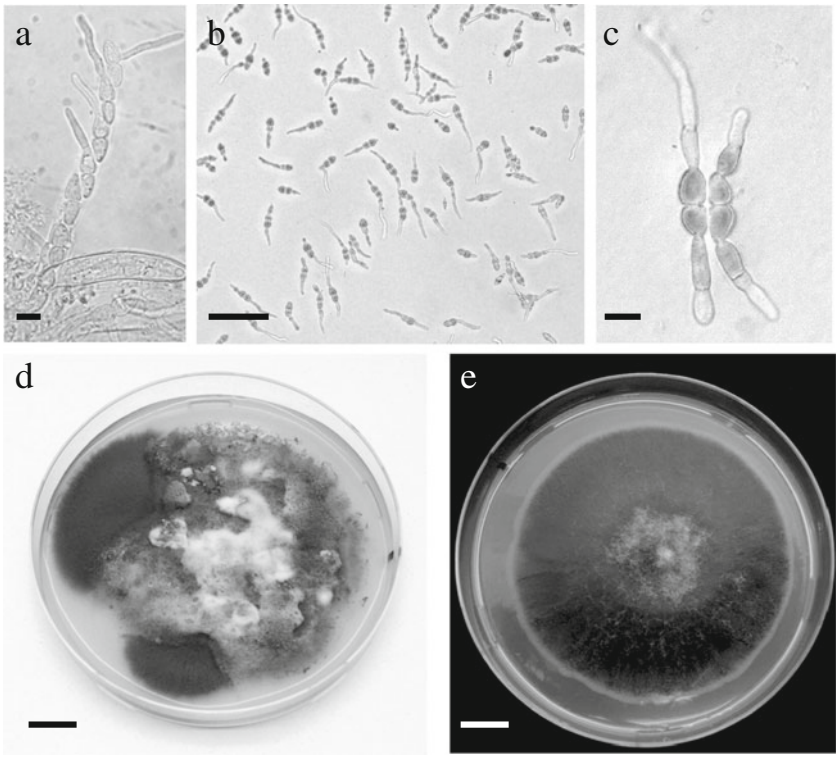

Fig. 3 Ascospores and cultures of the *Mycodidymella* teleomorph of *Petrakia echinata.* **a–c** Germinating ascospores. **d** Three months old, recently isolated single ascospore-culture with cottony aerial mycelium and two flat black sectors. **e** Four week old subculture with predominating flat dark mycelium. Scale bars for **a** = 10 μm; **b** = 100 μm; **c** = 10 μm; **d, e** = 1 cm

subcultures, never developed flat black sectors, showed a dark grey reverse with light grey sectors and developed a *Ramularia*-anamorph after prolonged incubation.

Inoculation experiments on maple leaves

Preliminary inoculations (method a) on detached young leaves (25 April 2012) with a single-ascospore culture (OH 110503.20M1) and a culture from the *Mycopappus* stage (OH 080907.1a*)* yielded conspicuous dark necrotic lesions spreading preferentially along the veins within 2 weeks and numerous conidiomata of the presumed spermatial *Phoma* stage (see below) developed in diffused daylight as well as under near UV light. However, subsequent inoculations on mature detached maple leaves on 31 May 2012 (method b) with the ascospore isolate (OH 110503.20M1) and a culture from *P. echinat*a conidia (OH 080921.4) yielded smaller necrotic lesions, which achieved approx. 10–30 mm diameter within 2 weeks and only occasionally spread along veins. The necrotic lesions were quickly colonized by various hyphomycetes and a *Gloeosporium*-like fungus. Very few conidiomata of the *Phoma* stage were observed in inoculations with both strains, but neither *P. echinata* conidia nor *Mycopappus* propagules were formed neither in daylight nor under near UV light. A third inoculation experiment on 30 June 2012 (method a) produced no visible *Petrakia* infections and all leaves were quickly overgrown by the hyphomycete *Trichothecium roseum*.

Field inoculations with the ascospore (OH 110503.20M1) and the conidial isolate (OH 080921.4) (performed on 31 May 2012) failed completely; after initial development of small lesions, their growth stopped, the necrotic intercostal tissue at the inoculation site disintegrated and the leaves remained green. Controls with malt agar showed the same picture.

Phylogenetic analysis

The phylogenetic tree of the 28S rDNA sequences is characterized by three strongly supported clades (Fig. 4). One clade contains members of the Lophiostomataceae, and another clade members of the Didymellaceae and Phaeosphaeriaceae including *Didymella exigua*, the type of the genus *Didymella* (Corbaz 1956). The third clade mainly contains members of the Melanommataceae, but family membership is not clear for many species, including *P. echinata*. Of the species with a known sexual state, *Pleomassaria siparia*, a member of the Melanommataceae, is the most closely related one, but its morphology deviates from that of the teleomorph of *P. echinata*. Unfortunately, neither living cultures nor sequences of *Mycodidymella* species are available (Shuhei Tanaka, Yamaguchi, Japan, personal communication, 24 August 2011), but the description of the morphology of the genus *Mycodidymella* (Wei et al. 1998) corresponds very well with that of the teleomorph of *P. echinata*.

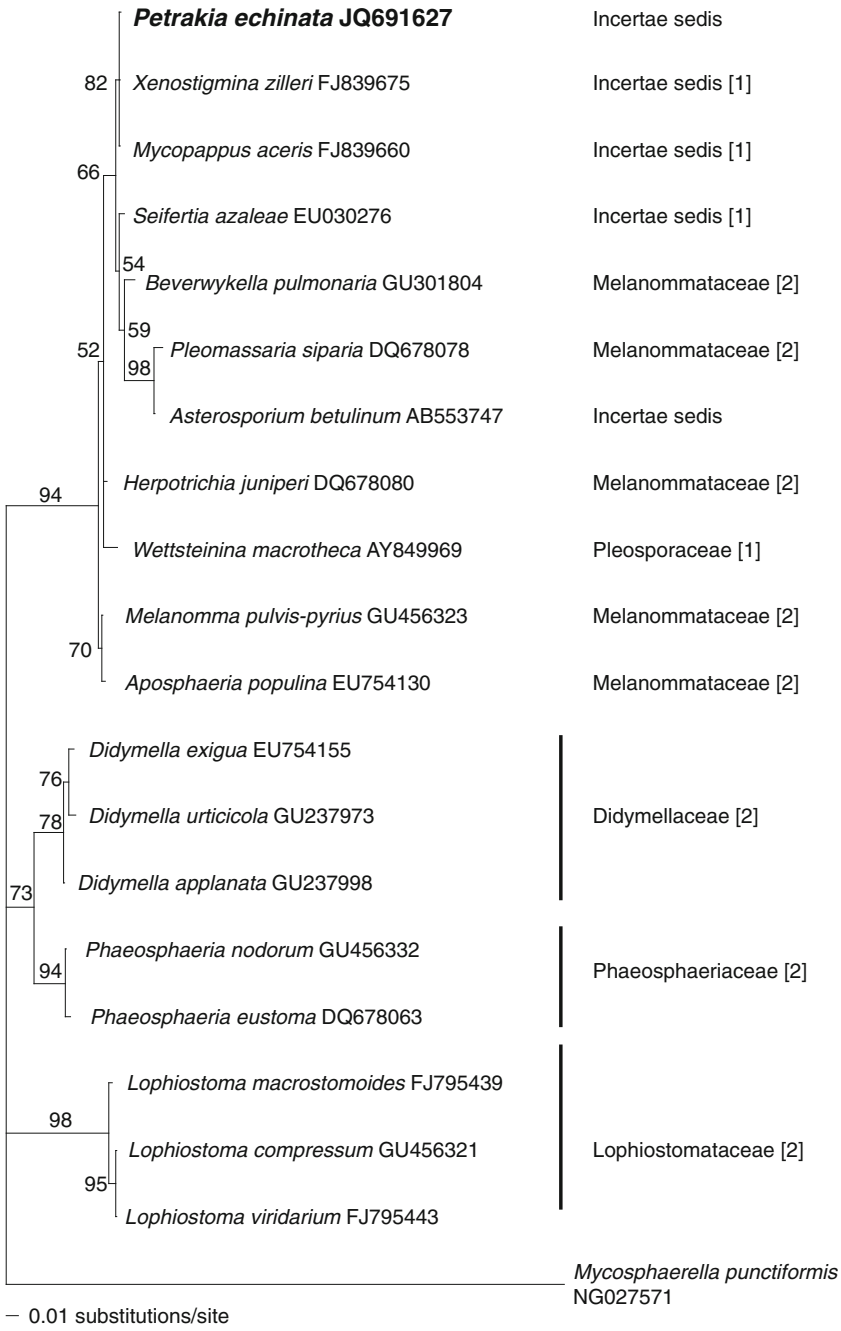

Fig. 4 Bootstrap 50 % majority-rule maximum likelihood consensus tree showing the phylogenetic relationships among *Petrakia echinata* and related species as inferred from partial sequences (560 bp) of the LSU nDNA (100 bootstrap samples). Taxon names are followed by GenBank accession numbers, the sequence labelled in bold was generated de novo. Family names are according to Crous et al. (2009) [1] and Zhang et al. (2012) [2]

Morphology

Mycodidymella teleomorph of P. echinata

Pseudothecia epiphyllous numerous, subglobose or lenticular (in dry condition flattened down from above), black, 150–320 μm wide, 50–150 μm high, subcuticular immersed in the leaf tissue and distended at the upper leaf surface at the tip (Fig. 1a–c). Pseudothecia have round pores without periphyses, occasionally they open by irregular rupturing. The pseudothecial wall is irregularly thickened. The lateral and basal wall is distinctly thickened (35–75 μm) and composed of polyedric and angular cells of up to 15 μm in diameter. The top wall layer is 10–40 μm thick and composed of smaller cells. The outermost layers are dark brown, forming a textura epidermoidea of thick-walled cells. In contrast, the inner cells of the pseudothecial wall are light brown to hyaline and thin-walled (Fig. 1c, f). Bitunicate asci are cylidrical, 50–80×9–12 μm, and line the ascomal cavity. The ascospores are biseriate (Fig. 1d, g). Young mature ascospores are hyaline, fusiform, straight or curved, 17–22×4–7 μm, subacute at the apex with a rounded base and have smooth walls, 1-septate below the middle, deeply constricted at the septum, and the upper cell is wider than

the lower cell (Fig. 1d, g). Old ascospores are slightly brownish, occasionally 3-septate and separating into two two-celled conical parts at the middle septum (Fig. 1h).

Germinating ascospores turn brown in most cases (Fig. 3c), but germination of hyaline spores was also observed (Fig. 3a). Pseudoparaphyses are hyaline, septate, 2–3 μm wide, persisting, unbranched, originating from above and growing downwards between the developing asci, finally becoming attached to the base of the cavity (Fig. 1g).

The anamorph *P. echinata* is characterized by conspicuous macro-conidia and has been described in detail by Petrak (1966) and Kirisits (2007). It forms epiphyllous sporodochia, which are embedded in the leaf tissues with a cone-like stroma and measure 100–150 μm in diameter. In the juvenile stage they are white and globular, composed of pseudoparenchyma. Mature sporodochia are blackish brown, produce globular to ovoid muriform septate, olive brown conidia measuring 16–28×18–22 μm with subhyaline horn-like (10–20×3–5 μm) projections (Fig. 2e).

The *P. echinata* anamorph is occasionally preceded by a *Mycopappus*-stage with approximately 70–180 μm large, more or less globular or somewhat elongated, white, sclerotia-like multicellular structures composed of hyaline globular cells (diameter up to 10 μm) which are formed between arachnoid superficial hyphae with a diameter of 3–4 μm (Fig. 2a–d). After incubation for several days in a moist chamber, these only loosely attached mycelial bodies turned brownish. After transferring these *Mycopappus* propagules to MEA, they developed cultures indistinguishable from *P. echinata*.

In addition, a *Phoma* anamorph is probably also involved in the life cycle of *P. echinata* which is formed in autumn in the necrotic lesions. The conidia of the *Phoma*-synanamorph probably serve as spermatia. The *Phoma*-state can be described as follows: Conidiomata (spermogonia) are pycnidial, scattered, brownish, subcuticular, and half immersed in the upper side of leaves; 100–150 μm diam., subglobose to depressed-globose; apically with round papillate ostiole; pycnidial wall 10–20 μm wide, composed of parenchymatic, thin-walled cells, 4–6 μm across; inner wall of the pycnidial cavity lined by short, 1-celled, hyaline conidiophores and ampulliform conidiogenous cells. Microconidia are non-germinating, hyaline, one-celled, rod-shaped to ellipsoidal, straight or slightly curved, and measure 3.5–6×1.5–2 μm.

Specimens examined: [1] Germany, Lower Saxony, between Eitzum and Räbke, 2.5 km W of the village Räbke, at the northern margin of the Elm range of hills, mixed forest with *Fagus sylvatica* and *Acer pseudoplatanus*, 52°11′18″ N, 10°53′18″ E, 190 m a.s.l., pseudothecia on litter leaves of *Acer pseudoplatanus*, 24 April 2008, leg. H. Butin, ZT Myc 24156. - [2] Germany, Lower Saxony, same collection site as no. 1, *A. pseudoplatanus* leaves with Petrakia leaf blotch, 16 August 2008, incubated outdoors at Wolfenbüttel, Lower Saxony, until June 2009, leg. H. Butin, pseudothecia on litter leaves of *Acer pseudoplatanus* and dried culture HB 090918.1, ZT Myc 24157. - [3] Germany, Bavarian Forest, picnic area Fredenbrücke near Spiegelau, Bavarian Forest, 48°56′16.51″ N 13°27′17.62″ E, 860 m a.s.l., pseudothecia on litter leaves of *Acer pseudoplatanus,* 3 September 2009, incubated outdoors at Wolfenbüttel, Lower Saxony, until 10 July 2010, leg. H. Butin, ZT Myc 24158. - [4] Germany, Bavarian Forest, same collection site as no. 3, pseudothecia on litter leaves of *Acer pseudoplatanus*, 20 June 2010, leg. H. Butin, ZT Myc 24159. - [5] Germany, Bavarian Forest, same collection site as no. 3, *Petrakia echinata* and *Mycopappus* anamorphic states, associated with a presumed *Phoma* anamorphic state on living leaves of *Acer pseudoplatanus*, 11 September 2009, leg. H. Butin, ZT Myc 24160. - [6] Switzerland, Aristau, 47°17′34.16″ N 8°22′59.27″ E, 382 m a.s.l., pseudothecia on litter leaves of *Acer pseudoplatanus*, 28 April 2011, and dried cultures OH 110503.20b and OH 110503.20M1, leg. O. Holdenrieder and L. Paul, ZT Myc 24161. - [7] Switzerland, Zürich, Käferberg, 47°24′29.63″ N 8°31′16.56″ E, 524 m a.s.l., *Petrakia echinata* and *Phoma* anamorphic states on living leaves of *Acer pseudoplatanus*, 21 September 2008, and dried culture OH 080921.4, leg. O. Holdenrieder and L. Paul, ZT Myc 24162. - [8] Switzerland, Zürich, same collection site as no. 7, *Mycopappus*-anamorphic state on living leaves of *Acer pseudoplatanus*, 7 September 2008, and dried culture OH 080907.1a, leg. O. Holdenrieder and L. Paul, ZT Myc 24163. - [9] Switzerland, Zürich, same collection site as no. 7, pseudothecia on litter leaves of *Acer pseudoplatanus,* 30 April 2011, leg. O. Holdenrieder and L. Paul, ZT Myc 24164. - [10] Switzerland, Zürich, same collection site as no. 7, *Mycosphaerella* sp. on litter leaves of *Acer pseudoplatanus*, 30 April 2011, and dried cultures OH 110503.8 and OH 110503.9, leg. O. Holdenrieder and L. Paul, ZT Myc 24165.

Discussion

Our data clearly show conspecificity of the anamorph *P. echinata* and the newly observed teleomorph. Application of a polyphasic key to the newly described fungus in the database of *Mycosphaerella* and related genera (http://www.mycobank.org/) revealed *Didymella convallariae* (W.E. McKeen & R.C. Zimmer) Arx as the closest match (80 %). However, no species described in Aptroot (2006) showed adequate agreement. *Didymella acerina* H. Fabre, which is known only from the holotype, has been described from *Acer monspessulanum* in France. No material was available for destructive investigation, but photographs of the type were kindly provided by the curator of the Museum Harmas Jean-Henri Fabre, Vaucluse, France. They show

significantly larger globular fruit bodies on decorticated wood, which are clearly different from our fungus on leaves. The teleomorph of *P. echinata* would fit well into the genus *Ohleria* (Pleosporaceae) according to the key of von Arx and Müller (1975), with the difference that the ascomata of *Ohleria* species are superficial on decorticated wood and not on leaves (Samuels 1980). However, *Ohleria* species possess *Monodictys* anamorphs, which are similar to *Petrakia,* but *Monodictys* conidia lack the horn-like projections typical for *Petrakia* species. The morphology of the newly described fungus almost perfectly matched the description of the genus *Mycodidymella* C.Z. Wei, Y. Harada & Katumoto (Wei et al. 1998), except for the color of the ascospores which is hyaline in *Mycodidymella* and occasionally slightly brown in the *P. echinata* teleomorph. Ascospores often become melanized when old but are able to germinate already when they are still hyaline, i.e. melanization is not always a sign of maturity. Thus, we did not consider melanization of the ascospores a criterion to reject *Mycodidymella*. In addition, the so far only known other species in this genus, *M. aesculi* C.Z. Wei, Y. Harada & Katumoto, forms also a *Mycopappus* anamorph (Wei et al. 1998).

The ITS sequences of the *Mycodidymella* teleomorph and its anamorphic *Petrakia*- and *Mycopappus*-states were identical, indicating that they represent different reproductive stages of the same holomorph (Schoch et al. 2012). In addition, despite difficulties with leaf inoculations, strains from the *Mycopappus* stage, *P. echinata* and the teleomorph showed identical behavior on maple leaves. However, lesion development was much better in young leaves compared to mature ones, suggesting ontogenetic resistance of maple to *P. echinata*, as reported also for *Venturia inaequalis* (Kollar 1996).

Xenostigmina zilleri (A. Funk) Crous, currently is the closest known relative of *P. echinata* according to both ITS (98 % similarity, gene bank accession number FJ839640.1) and 28S rDNA (99 % similarity, Fig. 4) sequences. *X. zilleri* was found on living leaves and litter leaves of *Acer macrophyllum* in British Columbia, Canada, and is considered to be the anamorphic state of *Didymella mycopappi* (A. Funk & Dorworth) Crous (Crous 1998) which was originally described as *Mycosphaerella mycopappi* A. Funk & Dorworth (Funk and Dorworth 1988). However, the up to 7 μm wide ascospores of the teleomorph of *P. echinata* are broader than those of *D. mycopappi* (up to 4.5 μm) and an 98 % ITS similarity does not provide sufficient evidence for conspecificity in ascomycetes (e.g. Grünig et al. 2004; Schoch et al. 2012). In addition, the type specimen of *D. mycopappi* is also extensively colonized by a species of *Mycosphaerella* according to Crous (1998). This proximity of the two ascomycetes prompted the same author to cast some doubt on the anamorph-teleomorph connection between *X. zilleri* and *D. mycopappi*, which has never been demonstrated experimentally. No *Xenostigmina*-like fungus is known on *Acer* in Europe. *P. echinata* seems to be a European species as it has been reported from North America only once on *Acer saccharinum* (Greene 1960). This tree is frequently planted in Europe and is locally even invasive (Aas et al. 2010), but *P. echinata* has never been detected on this host in Europe. In addition, inoculation experiments on detached *A. saccharinum* leaves yielded no necrotic lesions (Holdenrieder, unpubl.). Interestingly, both *X. zilleri* and *P. echinata* possess a *Mycopappus*-synanamorph. Thus, the two species are very closely related but not conspecific.

Among the ascomycetes with a known sexual stage, species of *Pleomassaria*, *Wettsteinina*, *Herpotrichia* and *Melanomma* are most closely related to *P. echinata* according to the 28S-rDNA phylogeny (Fig. 4). However, *Wettsteinina* has ovoidal or saccate asci and larger ascospores, and *Pleomassaria* has larger ascospores with transverse and longitudinal septa (von Arx and Müller 1975). In *Herpotrichia* and *Melanomma*, the ascomata are mostly superficial or erumpent in an early stage whereas those of the teleomorph of *P. echinata* are subglobose to lenticular and subcuticular to semi-immersed in the host tissue. In addition, many *Melanomma* species form their ascomata on the surface of decorticated wood. Thus, accommodation of the teleomorph of *P. echinata* in the genus *Mycodidymella* seems justified.

In conclusion, the life cycle of *P. echinata* can be described as follows: Primary infection occurs by ascospores in spring. Presumably, the infections remain dormant during early summer. In late summer, resistance of the leaves progressively decreases and necrotic lesions develop. Depending on suitable microclimatic conditions a *Mycopappus* stage is formed on these lesions. Subsequently, *P. echinata* conidia appear, which, together with *Mycopappus* propagules, are the inoculum responsible for secondary infections. At the same time a *Phoma* stage is formed with spores presumably functioning as spermatia. This life cycle is still hypothetical in parts. In particular, significance of the different stages for infection and the function of the *Phoma* conidia as spermatia deserve further studies.

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