



# Phylogenetic placement of *Leptosphaeria polylepidis*, a pathogen of Andean endemic *Polylepis tarapacana*, and its newly discovered mycoparasite *Sajamaea mycophila* gen. et sp. nov.

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## Abstract

*Polylepis tarapacana* forms one of the highest-altitude woodlands worldwide. Its populations are experiencing a decline due to unsustainable land-use practices, climate change, and fungal infection. In Sajama National Park in Bolivia, *Polylepis tarapacana* is affected by a disease caused by the pleosporalean fungus *Leptosphaeria polylepidis*, recently described in 2005. In this study, the integrative morphological and molecular analyses using sequences from multiple DNA loci showed that it belongs to the genus *Paraleptosphaeria* (Leptosphaeriaceae, Pleosporales). Accordingly, the appropriate new combination, *Paraleptosphaeria polylepidis*, is made. Pseudothecia of *Pa. polylepidis* were found to be overgrown by enigmatic conidiomata that were not reported in the original description of this fungus. Morphological and molecular analyses using sequences from two DNA loci revealed that they belong to an undescribed genus and species in the family Dictyosporiaceae (Pleosporales). The new generic and specific names, *Sajamaea* and *S. mycophila*, are introduced for this unusual fungus.

**Keywords** Andes · Mycoparasite · New genus · New species · Pleosporales · Plant pathogen · *Polylepis* · South America

## Introduction

Along the Andes, dispersed woodlands of *Polylepis* (Rosaceae) constitute a common component of the treeline. *Polylepis* woodlands are characterized by being dominated either mostly or exclusively by representatives of this genus and by being found in areas of difficult access, including rocky outcrops and mountain slopes (Fjeldså and Kessler 1996; Kessler 2006). These woodlands are important habitats for plant and animal species, including several endangered

habitat-specialist bird species (Fjeldså and Kessler 1996) and constitute important sources of firewood, fodder, and medicinal plants for local indigenous communities (Fjeldså and Kessler 1996; Domic et al. 2014; Hurtado et al. 2018). *Polylepis* woodlands are experiencing a rapid decline due to unsuitable land use practices, habitat destruction, and ongoing climate change (Navarro et al. 2005).

*Polylepis tarapacana* is a species that includes small trees and shrubs distributed along the semiarid Andean highlands from Peru to Argentina and Chile (Kessler 1995). The species

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possess morphological (Simpson 1979; Kessler 1995) and physiological adaptations (Rada et al. 2001; Azócar et al. 2007; González et al. 2007) to tolerate frost and desiccation due to extreme environmental conditions, including high solar irradiance, night frosts, and high diurnal temperature variation. In Nevado Sajama in Bolivia, *P. tarapacana* forms one of the world's highest-altitude woodlands, reaching elevations up to 5200 m a.s.l. (Jordan 1980). The species is currently categorized as “Vulnerable” in Bolivia due to firewood extraction, habitat loss, and climate change (MMAYa 2012; Cuyckens et al. 2016). A potentially pathogenic fungus, *Leptosphaeria polylepidis*, constitutes an emerging threat to the permanence of remaining *P. tarapacana* woodlands, as its infection has been attributed to the mortality of several individuals in the Sajama National Park (Coca-Morante 2012). The fungus, originally described from two collections made in 2002 in the Sajama National Park, forms stromatic black knots on the branches of infected individuals of *P. tarapacana* (Macía et al. 2005). Recent evaluations have shown that the infection is widespread in protected areas particularly of the southeast and northeast slopes of Nevado Sajama. However, actions to control the spread of the infection have not been implemented due to a lack of knowledge regarding the life-cycle and pathogenicity of *L. polylepidis*.

The genus *Leptosphaeria*, with the type species *Leptosphaeria doliolum*, resides in the Leptosphaeriaceae within Pleosporales (Zhang et al. 2012; de Gruyter et al. 2013; Ariyawansa et al. 2015). It contains hundreds of described species that in recent molecular analyses were proven to belong to different genera, including *Brunneosphaerella*, *Heterospora*, *Neoleptosphaeria*, *Paraleptosphaeria*, *Plenodomus*, *Pseudoleptosphaeria*, or *Subplenodomus* (Crous et al. 2009; de Gruyter et al. 2013; Ariyawansa et al. 2015). The generic placement of the majority of *Leptosphaeria* species is, however, still uncertain, and their rearrangements into the correct genus need molecular phylogenetic analyses, ideally using freshly collected materials. When describing *Leptosphaeria polylepidis*, Macía et al. (2005) provided a nuc rDNA ITS1-5.8S-ITS2 (ITS) sequence from the holotype material. Comparing this sequence with sequences available at that time in GenBank, they found that *L. polylepidis* was most similar to *Leptosphaeria dryadis*, a parasite of *Dryas octopetala* in arctic and alpine regions of the Northern Hemisphere. *Leptosphaeria dryadis* is currently placed in the genus *Paraleptosphaeria* (de Gruyter et al. 2013). However, it is not clear if this is also the correct genus for *L. polylepidis* because the ITS sequence generated by Macía et al. (2005) was not included in any of the subsequent studies dealing with *Leptosphaeria*-like species; many new sequences of *Leptosphaeria*-like species were generated in recent years that could disprove close relationships between *L. polylepidis* and *Paraleptosphaeria dryadis*, and sequences from more than

one DNA locus can better resolve generic boundaries within Leptosphaeriaceae (Ariyawansa et al. 2015).

Therefore, it is the aim of this study to resolve the phylogenetic and generic placement of *Leptosphaeria polylepidis* by employing integrative morphological and molecular analyses using sequences from multiple DNA loci. During the analyses of freshly collected material, we unexpectedly found that pseudothecia of *L. polylepidis* were overgrown by conidiomata that were not reported in the original description of the fungus. It was not clear if these conidiomata represented an asexual state of *L. polylepidis* or, alternatively, belonged to a mycoparasitic fungus. Therefore, the second aim of this study was to answer this question by applying morphological and molecular analyses using nuc rDNA ITS and LSU sequences.

## Materials and methods

### Specimen sampling and documentation

This study is based on newly collected material of *Leptosphaeria polylepidis*. Four specimens were collected in the Sajama National Park in Bolivia, which is the type locality and the only known place of occurrence of *L. polylepidis*. One of them was partly covered with conidiomata. This specimen was used for detailed morphological/anatomical and molecular analyses. The voucher material is preserved at the Herbario Nacional de Bolivia (LPB) and at the fungal herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (KRAM F). Attempts to obtain cultures failed.

### Morphological analyses

The morphology was examined using standard stereo and light microscopy (Nikon SMZ 800, Nikon Eclipse 80i DIC). Thin sections of ascomata and conidiomata were made manually using a razor blade or with the aid of a freezing microtome Thermo Scientific Microm HM430 equipped with BFS-MP freezing stage and BFS-3MP controller. The sectioned material was examined in distilled water, 10% solution of potassium hydroxide (KOH), or lactophenol cotton blue (LPCB). The amyloidity of fungal structures was tested using Lugol's solution (IKI), or a combination of first KOH and then IKI (KOH/IKI). All measurements were made in distilled water.

### DNA isolation, PCR, and sequencing

Genomic DNA was extracted in three separate extraction rounds, namely one extraction from 10 pseudothecial hymenia of *Leptosphaeria polylepidis*, one extraction from conidial mass obtained from five conidiomata, and one extraction from 15 entire conidiomata of unknown fungus, using the

DNeasy™ Plant Mini Kit or QIAamp DNA Investigator Kit (Qiagen, Germany), following the manufacturer's instructions. For *Leptosphaeria polylepida*, a total of four genetic markers were amplified, namely nuc rDNA 18S (SSU), nuc rDNA ITS1-5.8S-ITS2 (ITS), nuc rDNA 28S (LSU), and a fragment of the translation elongation factor 1-alpha gene (*TEF1*). For unknown conidiomatal fungus, two genetic markers were amplified, namely ITS and LSU. The following primer pairs were used for amplification: NS1-NS24 (White et al. 1990; Gargas and Taylor 1992), ITS1F-ITS4 (White et al. 1990; Gardes and Bruns 1993), LROR-LR7 (Vilgalys and Hester 1990; Rehner and Samuels 1994), and EF1983F-EF12218R (Rehner and Buckley 2005) for SSU, ITS, LSU, and *TEF1*, respectively. Polymerase chain reactions (PCRs) were performed as follows: for *Leptosphaeria polylepida*, 2 µl of DNA extract was used for SSU, ITS, and LSU and 5 µl for *TEF1*, while for unknown conidiomatal fungus, 3 µl of DNA extract was used for ITS and LSU in a total volume of 25 µl PCR reactions; the rest of components were added according to Flakus et al. (2019). Cycling conditions were performed as reported by Rodriguez-Flakus and Printzen (2014) for the nuclear markers and Rehner and Buckley (2005) for *TEF1* with modification of the initial denaturalization to 4 min and final extension to 5 min (M. Mardones, pers. comm.). PCR products were visualized on agarose gels and later purified using ExoSap. The PCR amplicons were sequenced by MacroGen Europe B.V. (Amsterdam, the Netherlands).

## Phylogenetic analyses

Newly generated sequences were trimmed, assembled, and edited using Geneious Pro, version 8.0.5 (Biomatters Inc.). BLAST searches in GenBank (Altschul et al. 1997) were performed to find sequences of most closely related species, revealing affinities of sequences from *Leptosphaeria polylepida* to sequences of the Leptosphaeriaceae and sequences obtained from unknown conidiomatal fungus to sequences of the Dictyosporiaceae. Accordingly, two separate datasets were assembled to resolve the phylogenetic placement of each fungus.

To resolve the phylogenetic position of *Leptosphaeria polylepida*, a four-gene dataset (SSU+ITS+LSU+*TEF1*) was assembled that included sequences generated in this study and sequences from 45 specimens of closely related members of the Leptosphaeriaceae and a member of the Cucurbitariaceae used as an outgroup (selected from Zhang et al. 2012 and Tibpromma et al. 2017; see Table 1). Each single gene dataset was aligned separately using the MAFFT algorithm (Katoh et al. 2005) as implemented on the GUIDANCE web-server (Penn et al. 2010); to remove poorly or ambiguously aligned uncertain columns, a default cut-off score of 0.93 in all single gene alignments were chosen. The single-gene datasets were concatenated to a final alignment using Geneious Pro and consisted of 1023 bp (SSU), 452 bp (ITS), 1326 bp (LSU),

and 915 bp (*TEF1*). Subsequently, the analyses were performed in the CIPRES Scientific gateway portal ([:dito\\_existshttp://www.phylo.org/portal2](http://www.phylo.org/portal2)) (Miller et al. 2010). The optimal partitioning scheme and substitution models for each single-gene alignment were inferred by PartitionFinder 2 (Lanfear et al. 2016). A single partition for SSU and LSU; three partitions for ITS1, 5.8S, and ITS2 regions; and three for each of the codon positions of *TEF1* were found. Substitution models selected under the greedy search algorithm and Akaike information criterion (Lanfear et al. 2016) were TVM+G+I for SSU, ITS2, and LSU; JC+I for 5.8S; and GTR+G for ITS1 and used as priors for Bayesian phylogenetic inference (BI) analyses. Maximum likelihood (ML) and bootstrap support (BS) analyses were performed on each locus separately and concatenated data set by using RAXML-HPC2 on XSEDE with a rapid BS algorithm (Stamatakis 2014), 1000 replicates, and GTRGAMMA substitution model. Bayesian inference (BI) analyses were carried out using Markov Chain Monte Carlo as implemented in MrBayes on XSEDE (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) and were based on 10 million generations, sampling every 1000th tree, two independent parallel runs of four incrementally heated chains (0.15) and discarding the first 0.5 of the sampled trees.

To resolve phylogenetic position of the conidiomatal fungus, a two-gene dataset (ITS+LSU) was assembled, which contained sequences generated in this study and sequences from 28 related members of the Dictyosporiaceae and two sequences of *Periconia igniaria* used as an outgroup (selected from Iturrieta-González et al. 2018 and Yang et al. 2018; see Table 2). Phylogenetic analyses were performed as described above and were based on 820 bp (LSU) and 412 bp (ITS) alignments. A single partition for LSU (GTR+I+G) and three partitions for ITS (GTR+G for ITS1, SYM+I+G for 5.8S, and JC+I+G for ITS2) were selected based on PartitionFinder 2 (Lanfear et al. 2016). The ML and BI analyses were performed with similar parameters as described above, but 20 million generations in four independent parallel runs were sampled and six gradually heated chains used. Relationships receiving bootstrap support (ML-BP) above 70% and 0.95 as Bayesian posterior probability (PP) were considered strongly supported. The resulting trees from both phylogenetic analyses were built in Figtree v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>).

## Results

### Phylogenetic analyses

The phylogenetic analyses of the four-locus (SSU+ITS+LSU+*TEF1*) dataset comprising representatives of the Leptosphaeriaceae (and a member of the Cucurbitariaceae used as an outgroup) resulted in overall similar tree topologies

**Table 1** Species used for inferring the phylogeny of the Leptosphaeriaceae (and a member of the Cucurbitariaceae used as an outgroup) with details of their host, country of origin, strain or voucher information, GenBank accession numbers (ITS, LSU, SSU, *TEF1*) and references

Species	Host	Country	Strain/ voucher	GenBank acc. no.			References
				ITS	LSU	SSU/ <i>TEF1</i>	
<i>Alternariaster bidentis</i>	<i>Bidens sulphurea</i>	Brazil	CBS 134021	KC609333	KC609341	–/–	Alves et al. 2013
<i>Alternariaster bidentis</i>	<i>Bidens sulphurea</i>	Brazil	CBS 134185	KC609334	KC609342	–/–	Alves et al. 2013
<i>Alternariaster centaureae–diffusae</i>	<i>Centaurea diffusa</i>	Russia	MFLUCC 14-0992	KT454723	KT454715	KT454730/–	Ariyawansa et al. 2015
<i>Alternariaster centaureae–diffusae</i>	<i>Centaurea diffusa</i>	Russia	MFLUC 15-0009	KT454724	KT454716	KT454731/–	Ariyawansa et al. 2015
<i>Alternariaster helianthi</i>	<i>Helianthus annuus</i>	Hungary	CBS 199.86	KC609336	KC609343	–/–	Alves et al. 2013
<i>Alternariaster helianthi</i>	<i>Helianthus annuus</i>	–	CBS 327.69	KC609335	KC584369	KC584627/–	Alves et al. 2013
<i>Cucurbitaria berberidis</i>	<i>Berberis julianae</i>	Netherlands	CBS 394.84	–	JX681088	GQ387544/–	Verkley et al. 2014 (ITS); de Gruyter et al. 2010 (LSU)
<i>Heterospora chenopodii</i>	<i>Chenopodium album</i>	Netherlands	CBS 115.96	JF740227	EU754188	EU754089/–	de Gruyter et al. 2013 (ITS); de Gruyter et al. 2009 (LSU, SSU)
<i>Heterospora chenopodii</i>	<i>Chenopodium album</i>	Netherlands	CBS 448.68	FJ427023	EU754187	EU754088/–	Aveskamp et al. 2009 (ITS); de Gruyter et al. 2009 (LSU, SSU)
<i>Heterospora dimorphospora</i>	<i>Chenopodium quinoa</i>	Peru	CBS 165.78	–	JF740281	JF740098/–	de Gruyter et al. 2013
<i>Heterospora dimorphospora</i>	<i>Chenopodium quinoa</i>	Peru	CBS 345.78	–	GU238069	GU238213/–	Aveskamp et al. 2010
<i>Leptosphaeria doliolum</i>	dead stem	United Kingdom	MFLUC 15-1875	KT454727	KT454719	KT454734/–	Ariyawansa et al. 2015
<i>Leptosphaeria doliolum</i>	<i>Phlox paniculata</i>	Netherlands	CBS 155.94	JF740207	JF740282	–/–	de Gruyter et al. 2013
<i>Leptosphaeria doliolum</i>	<i>Urtica dioica</i>	Netherlands	CBS 505.75	JF740205	GQ387576	GQ387515/GU349069	de Gruyter et al. 2013 (ITS); de Gruyter et al. 2010 (LSU, SSU); Schoch et al. 2009 ( <i>TEF1</i> )
<i>Leptosphaeria doliolum</i>	<i>Rudbeckia</i>	Netherlands	CBS 541.66	JF740206	JF740284	–/–	de Gruyter et al. 2013
<i>Leptosphaeria errabunda</i>	<i>Solidago</i>	Netherlands	CBS 617.75	JF740216	JF740289	–/–	de Gruyter et al. 2013
<i>Leptosphaeria errabunda</i>	<i>Delphinium</i>	Netherlands	CBS 125978	JF740217	JF740290	–/–	de Gruyter et al. 2013
<i>Leptosphaeria pedicularis</i>	<i>Pedicularis</i>	Switzerland	CBS 390.80	JF740224	JF740294	–/–	de Gruyter et al. 2013
<i>Leptosphaeria pedicularis</i>	<i>Gentiana punctata</i>	Switzerland	CBS 126582	JF740223	JF740293	–/–	de Gruyter et al. 2013
<i>Leptosphaeria sclerotoides</i>	<i>Medicago sativa</i>	Canada	CBS 144.84	JF740192	JF740269	–/–	de Gruyter et al. 2013
<i>Leptosphaeria sclerotoides</i>	<i>Medicago sativa</i>	Canada	CBS 148.84	JF740193	JF740270	–/–	de Gruyter et al. 2013
<i>Leptosphaeria slovacica</i>	<i>Ballota nigra</i>	Netherlands	CBS 389.80	JF740247	JF740315	JF740101/–	de Gruyter et al. 2013
<i>Leptosphaeria slovacica</i>	<i>Ballota nigra</i>	Netherlands	CBS 125975	JF740248	JF740316	–/–	de Gruyter et al. 2013
<i>Leptosphaeria sydowii</i>	<i>Senecio jacobaea</i>	United Kingdom	CBS 385.80	JF740244	JF740313	–/JF740139	de Gruyter et al. 2013
<i>Leptosphaeria sydowii</i>	<i>Senecio jacobaea</i>	United Kingdom	CBS 125976	JF740245	JF740314	–/–	de Gruyter et al. 2013
<i>Paraleptosphaeria dryadis</i>	<i>Dryas octopetala</i>	Switzerland	CBS 643.86	JF740213	GU301828	KC584632/GU349009	de Gruyter et al. 2013 (ITS); Schoch et al. 2009 (LSU, <i>TEF1</i> ); Woudenberg et al. 2013 (SSU)
<i>Paraleptosphaeria dryadis</i>	<i>Dryas octopetala</i>	Switzerland	CBS 743.86	AF439461	–	–/–	Camara et al. 2002

**Table 1** (continued)

Species	Host	Country	Strain/ voucher	GenBank acc. no.			References
				ITS	LSU	SSU/ <i>TEF1</i>	
<i>Paraleptosphaeria macrospora</i>	<i>Rumex longifolius</i>	Norway	CBS 114198	JF740238	JF740305	–/–	de Gruyter et al. 2013
<i>Paraleptosphaeria nitschkei</i>	<i>Cirsium spinosissimum</i>	Switzerland	CBS 306.51	NR_111621 (=J-F740239)	JF740308	–/–	de Gruyter et al. 2013
<i>Paraleptosphaeria nitschkei</i>	<i>Petasites</i>	Italy	MFLU 13-0644	KP729446	KP729447	–/–	Liu et al. 2015
<i>Paraleptosphaeria polylepidis</i>	<i>Polylepis tarapacana</i>	Bolivia	MA-Fungi 57843 – holotype	AJ786644	–	–/–	Macia et al. 2005
<i>Paraleptosphaeria polylepidis</i>	<i>Polylepis tarapacana</i>	Bolivia	APA-2999	MK795714	MK795717	MK795720/MK831009	This study
<i>Paraleptosphaeria praetermissa</i>	<i>Rubus idaeus</i>	Sweden	CBS 114591	JF740241	JF740310	–/–	de Gruyter et al. 2013
<i>Paraleptosphaeria rubi</i>	<i>Rubus</i>	Italy	MFLUCC 14-0211	KT454726	KT454718	KT454733/–	Ariyawansa et al. 2015
<i>Paraleptosphaeria</i> sp. (as <i>Leptosphaeria</i> sp.)	<i>Phlomis younghusbandii</i>	China	PHY-06	JX401979	JX401985	–/–	Zhang D.W. and Guo S.X. unpubl.
<i>Paraleptosphaeria</i> sp. (as <i>Leptosphaeria</i> sp.)	<i>Phlomis younghusbandii</i>	China	PHY-54	JX401931	JX401999	–/–	Zhang D.W. and Guo S.X. unpubl.
<i>Plenodomus biglobosus</i>	<i>Brassica juncea</i>	France	CBS 127249	JF740199	JF740275	–/–	de Gruyter et al. 2013
<i>Plenodomus biglobosus</i>	<i>Brassica napus</i> var. <i>napobrassica</i>	–	CBS 298.36	AJ550862	GU237980	GU238207/–	Mendes-Pereira et al. 2003 (ITS); Aveskamp et al. 2010 (LSU, SSU)
<i>Plenodomus lupini</i>	<i>Lupinus mutabilis</i>	Peru	CBS 248.92	JF740236	JF740303	–/–	de Gruyter et al. 2013
<i>Plenodomus pimpinellae</i>	<i>Pimpinella anisum</i>	Israel	CBS 101637	JF740240	JF740309	–/–	de Gruyter et al. 2013
<i>Plenodomus salviae</i>	<i>Salvia glutinosa</i>	Italy	MFLUCC 13-0219	KT454725	KT454717	KT454732/–	Ariyawansa et al. 2015
<i>Plenodomus</i> sp. (as <i>Leptosphaeria</i> sp.)	<i>Phlomis younghusbandii</i>	China	PHY-30	JX401955	JX401989	–/–	Zhang D.W. and Guo S.X. unpubl.
<i>Plenodomus visci</i>	<i>Viscum album</i>	France	CBS 122783	–	EU754195	EU754096/–	de Gruyter et al. 2009
<i>Plenodomus wasabiae</i>	<i>Eutrema japonicum</i>	Taiwan	CBS 120119	JF740257	JF740323	–/–	de Gruyter et al. 2013
<i>Plenodomus wasabiae</i>	<i>Eutrema japonicum</i>	Taiwan	CBS 120120	JF740258	JF740324	–/–	de Gruyter et al. 2013
<i>Subplenodomus violicola</i>	<i>Viola tricolor</i>	Netherlands	CBS 306.68	FJ427083	GU238156	–/–	Aveskamp et al. 2009 (ITS); Aveskamp et al. 2010 (LSU)
<i>Subplenodomus violicola</i>	<i>Viola tricolor</i>	New Zealand	CBS 100272	FJ427082	JF740322	–/–	Aveskamp et al. 2009 (ITS); de Gruyter et al. 2013 (LSU)

APA A. N. Palabral-Aguilera; CBS CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; MA Real Jardín Botánico herbarium, Madrid, Spain; MFLU Mae Fah Luang University herbarium; MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand

obtained from BI and ML analyses. The *Heterospora* clade nested together with *Subplenodomus* in the ML analysis but was sister to the remaining genera of the Leptosphaeriaceae in BI analysis. In general, high bootstrap support values above 70% (ML–BP) and posterior probabilities above 0.95 (PP) were retrieved and supported monophyletic lineages representing

distinct genera as in previous studies (de Gruyter et al. 2013; Ariyawansa et al. 2015; Tibpromma et al. 2017). The Bayesian tree is displayed in Fig. 1. The newly generated *Leptosphaeria polylepidis* sequences clustered with ex-holotype sequence of *L. polylepidis* (ML–BP = 100%, PP = 1) in their well-supported *Paraleptosphaeria* lineage (ML–BP = 78%, PP = 1);

relatedness of *L. polylepidis* and *Paraleptosphaeria dryadis* was weakly supported (ML–BP = 60%). The ITS sequence from the newly generated material and holotype of *L. polylepidis* differed in 6 bp. The SSU, LSU, and *TEF1* sequences were not available from holotype material.

The phylogenetic analyses of the two-locus (ITS+LSU) dataset, including representatives of the Dictyosporiaceae and two sequences of *Periconia igniaria* used as an outgroup, resulted in similar tree topologies. *Dictyosporium* grouped in a single clade together with *Aquadictyospora* in ML analysis but was sister to the *Pseudodictyosporium* lineage in the BI analysis. The Bayesian consensus tree is displayed in Fig. 2. The phylogenetic tree shows 11 monophyletic lineages of the Dictyosporiaceae, corresponding to 10 known genera and one unknown lineage, the latter of which consisted of the conidiomatal fungus from *Leptosphaeria polylepidis*. Results were roughly consistent with previous phylogenetic results (e.g., Iturrieta-González et al. 2018; Yang et al. 2018), except for some differences in branches with low statistical support values mainly at the backbone of the phylogenetic tree. The sequences of the conidiomatal fungus from two independent extraction rounds were identical and of high quality, excluding the possibility of DNA isolation from contaminating fungi, and were nested within the currently circumscribed family Dictyosporiaceae (ML–BP = 100%, PP = 1). They formed a sister group to the members of the genus *Pseudocoleophoma* (ML–BP = 89%, PP = 1). The phylogenetic relationships of the conidiomatal fungus and the *Pseudocoleophoma* lineage with their relatives in the Dictyosporiaceae remain unresolved, due the lack of support in our molecular analyses.

## Taxonomy

***Paraleptosphaeria polylepidis*** (M.J. Macía, M.E. Palm & M.P. Martín) Piątek, Flakus & Rodr. Flakus, comb. nov. (Fig. 3)

Mycobank no. MB832195

**Basionym:** *Leptosphaeria polylepidis* M.J. Macía, M.E. Palm & M.P. Martín, Mycotaxon 93: 402 (2005)

**Ascomata** pseudothecial, developed on stroma. **Stroma** 1–3 mm high, 2–7 mm wide, black, surface with visible individual pseudothecia, usually with base growing inward and intermixed with host tissue. **Pseudothecia** dark brown to black, matt, rough, 300–700 µm high, 300–600 µm wide, globose to subglobose with base usually protruding into ca. 200–1000 µm high stipes that fuse below into stroma; papillae short or lacking. **Peridium** 60–150 µm wide, pale to dark brown (KOH+ greenish grey), consisting of a single stratum, paraplectenchymatous, textura angularis, composed of several layers of isodiametric to slightly elongate thin-walled cells, 15–25 × 9–15 µm. **Hamathecium** persistent, composed of relatively thin, 1.5–4 µm wide, septate, branched and strongly anastomosed pseudoparaphyses. **Asci** 120–250 × 20–45 µm, 5–8-spored, bitunicate, cylindrical-

clavate, slightly curved, with a short stipe, apically widened, with an ocular chamber, IKI–, KOH/IKI–; endosacus KOH/IKI+ orange. **Ascospores** 45–60 × (14–)20–23 µm, pale brown, broadly ellipsoidal, narrower at the top, constricted at septa, uniformly thin-walled, without perispore or gelatinous coat, (2–)3(–4)-septate. **Asexual state** unknown.

**Specimen examined (used for morphology and molecular analyses):** See host fungus in the type of *Sajamaea mycophila*.

**Additional specimens examined:** Bolivia, Oruro, Sajama National Park (=Parque Nacional Sajama), W slope of Sajama's volcano, Quebrada Huaytana, 18°11' S, 68°51' W, elev. ca. 4000–4200 m, on *Polylepis tarapacana*, 1 Feb. 2016, A. Domic (three collections: AD1, AD2, AD5; all in LPB).

***Sajamaea* Flakus, Piątek & Rodr. Flakus, gen. nov.**

Mycobank no. MB832196

**Etymology:** Referring to the place of occurrence of the new genus – slopes of Nevado Sajama, the highest mountain in Bolivia.

**Description:** *Mycoparasitic*. **Conidiomata** pycnidial, uniloculate to multi-loculate, subglobose to irregular in shape, pale brown. **Peridium** composed of several layers of isodiametric to slightly elongate cells in the form of textura angularis. **Conidiophores** hyaline, reduced to phialidic conidiogenous cells. **Conidia** pigmented (pale brown), broadly ellipsoidal, aseptate, smooth, thin-walled, guttulate. **Generic type:** *Sajamaea mycophila* Flakus, M. Piątek & Rodr. Flakus.

***Sajamaea mycophila*** Flakus, Piątek & Rodr. Flakus, sp. nov. (Fig. 4)

Mycobank no. MB832197

**Etymology:** The epithet name *mycophila* refers to the occurrence of this fungus on other fungus.

**Description:** *Mycoparasitic* on *Paraleptosphaeria polylepidis*, causing moderate damages of host ascomata. **Conidiomata** pycnidial, as pale brown galls on host surface, 150–300 µm wide, 120–300 µm high, first erumpent through the outermost layer of host peridium, later almost sessile on pseudothecial clusters of host, uniloculate to multi-loculate, solitary to aggregated, subglobose to irregular in shape, pale brown; better seen when wet. **Peridium** 20–30 µm wide, pale to dark brown, composed of about 3–8 layers of cells with walls up to 2 µm wide, cells isodiametric to slightly elongate, textura angularis. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** 5–11 µm high, 4–8 µm wide, enteroblastic, phialidic, smooth-walled, hyaline, with a small collar, densely outlining inner surfaces of pycnidia. **Conidia** 9–13 × 5.5–7.5 µm, pale brown, broadly ellipsoidal, sometimes slightly narrower at one point, with rounded ends, aseptate, smooth, thin-walled, guttulate. **Sexual state** unknown.

**Specimen examined:** Bolivia, Oruro, Sajama National Park (=Parque Nacional Sajama), W slope of Nevado Sajama,

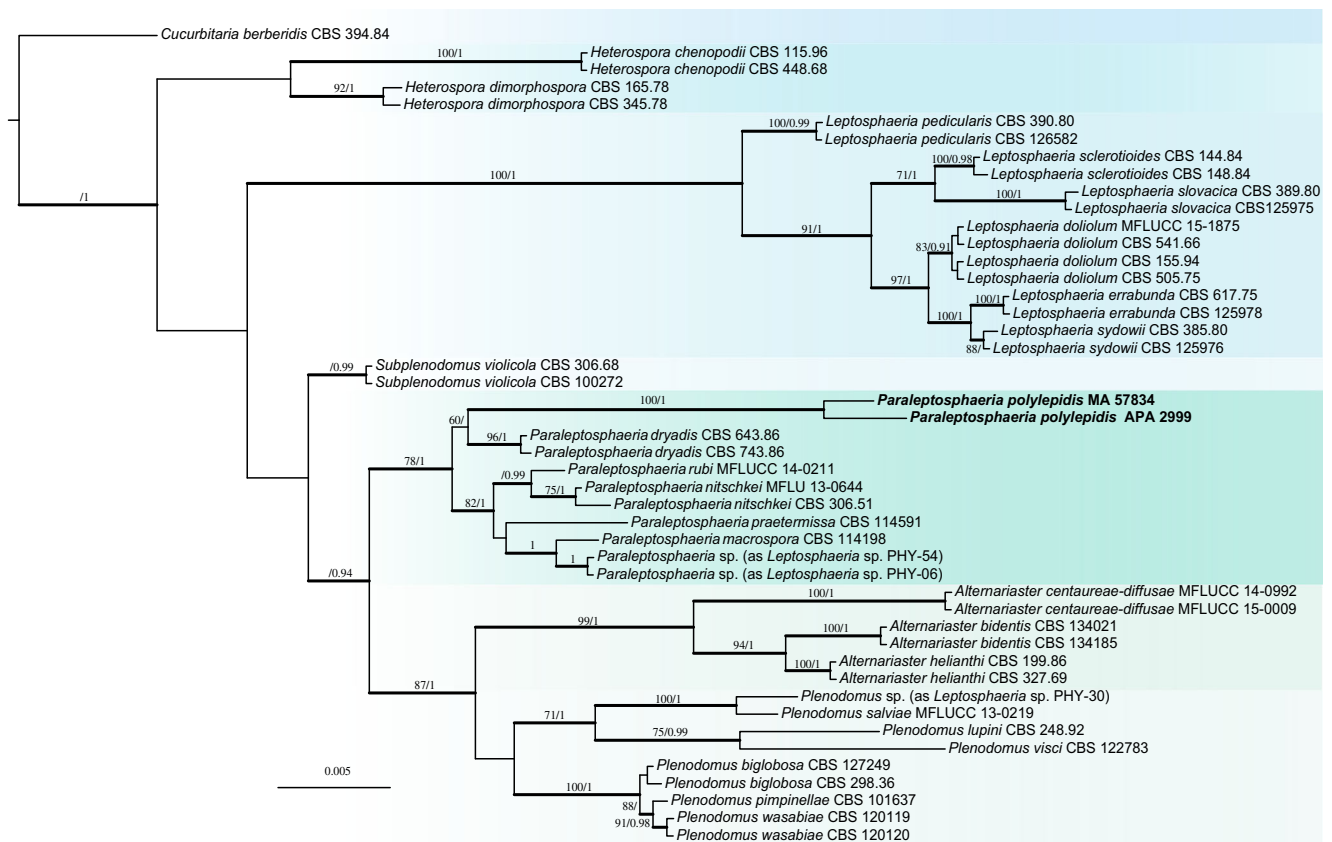
**Table 2** Species used for inferring phylogeny of the Dictyosporiaceae (and *Periconia igniaria* used as an outgroup) with details of their substrate or host, country of origin, strain or voucher information, GenBank accession numbers (ITS, LSU), and references

Species	Substrate/host	Country	Strain/voucher	GenBank acc. no.		References
				ITS	LSU	
<i>Aquadictyospora lignicola</i>	Submerged wood	China	MFLUCC 17-1318	MF948621	MF948629	Li et al. 2017
<i>Cheirosporium triseriale</i>	Submerged wood	China	HMAS 180703	EU413953	EU413954	Cai et al. 2008
<i>Dendryphiella paravinosa</i>	Leaves of <i>Citrus sinensis</i>	Italy	CBS 14128	KX228257	KX228309	Crous et al. 2016
<i>Dendryphiella variabilis</i>	Dead leaf of a lauraceous tree	Cuba	CBS 584.96	LT963453	LT963454	Iturrieta-González et al. 2018
<i>Dendryphiella vinosa</i>	–	Japan (for ITS, unavailable for LSU)	NBRC 32669 (for ITS, strain info unavailable for LSU)	DQ307316	EU848590	Dela Cruz T.E. et al. unpubl. (ITS); Jones et al. 2008 (LSU)
<i>Dictyocheirospora aquatica</i>	Submerged wood	China	KUMCC 15-0305	KY320508	KY320513	Wang et al. 2016
<i>Dictyocheirospora bannica</i>	Dead wood	Japan	KH 332	LC014543	AB807513	Tanaka et al. 2015
<i>Dictyocheirospora rotunda</i>	Submerged dead decaying wood	Thailand	MFLUCC 14-0293	KU179099	KU179100	Boonmee et al. 2016
<i>Dictyocheirospora rotunda</i>	Submerged wood	China	MFLUCC 17-1313 (in GenBank) or MFLUCC 17-1687 (in publication)	MF948625	MF948633	Li et al. 2017
<i>Dictyosporium bulbosum</i>	Dead wood	Japan	yone 221	LC014544	AB807511	Tanaka et al. 2015
<i>Dictyosporium elegans</i>	–	–	NBRC 32502	DQ018087	DQ018100	Tsui et al. 2006
<i>Dictyosporium thailandicum</i>	Submerged wood	Thailand	MFLUCC 13-0773	KP716706	KP716707	Liu et al. 2015
<i>Dictyosporium toruloides</i>	–	–	CBS 209.65	DQ018093	DQ018104	Tsui et al. 2006
<i>Digitodesmium bambusicola</i>	–	–	CBS 110279	DQ018091	DQ018103	Tsui et al. 2006
<i>Gregarithecium curvisporum</i>	Dead culms of <i>Sasa</i> sp.	Japan	KT 922	AB809644	AB807547	Tanaka et al. 2015
<i>Jalapriya inflata</i>	Piece of driftwood	United Kingdom	NTOU 3855	JQ267362	JQ267363	Kirschner et al. 2013
<i>Jalapriya pulchra</i>	Submerged decaying wood	China	MFLUCC 15-0348	KU179108	KU179109	Boonmee et al. 2016
<i>Jalapriya pulchra</i>	Submerged wood	China	MFLUCC 17-1683	MF948628	MF948636	Li et al. 2017
<i>Neodendryphiella mali</i>	Leaf of <i>Malus domestica</i>	Italy	CBS 139.95	LT906655	LT906657	Iturrieta-González et al. 2018
<i>Neodendryphiella mali</i>	Herbivore dung	Spain	FMR 17003	LT993734	LT993735	Iturrieta-González et al. 2018
<i>Neodendryphiella michoacanensis</i>	Soil	Mexico	FMR 16098	LT906660	LT906658	Iturrieta-González et al. 2018
<i>Neodendryphiella tarraconensis</i>	Soil	Spain	FMR 16234	LT906659	LT906656	Iturrieta-González et al. 2018
<i>Periconia igniaria</i>	<i>Triticum aestivum</i>	Switzerland	CBS 379.86	LC014585	AB807566	Tanaka et al. 2015
<i>Periconia igniaria</i>	Bamboo	Papua New Guinea	CBS 845.96	LC014586	AB807567	Tanaka et al. 2015
<i>Pseudocoleophoma calamagrostidis</i>	Dead leaves of <i>Calamagrostis matsumurae</i>	Japan	KT 3284	LC014592	LC014609	Tanaka et al. 2015
<i>Pseudocoleophoma polygonicola</i>	Dead stems of polygonaceous plant	Japan	KT 731	AB809634	AB807546	Tanaka et al. 2015
		United Kingdom	MFLUCC 16-0123	KX576655	KX576656	Hyde et al. 2016

**Table 2** (continued)

Species	Substrate/host	Country	Strain/voucher	GenBank acc. no.		References
				ITS	LSU	
<i>Pseudocoleophoma typhicola</i>	Submerged stems of <i>Typha latifolia</i>	–	CBS 688.93	DQ018099	DQ018106	Tsui et al. 2006
<i>Pseudodictyosporium elegans</i>	–	–	NBRC 30078	DQ018098	DQ018105	Tsui et al. 2006
<i>Pseudodictyosporium thailandica</i>	Decaying bamboo stem	Thailand	MFLUCC 16-0029	KX259520	KX259522	Hyde et al. 2016
<i>Pseudodictyosporium wauense</i>	–	–	NBRC 30078	DQ018098	DQ018105	Tsui et al. 2006
<i>Sajamaea mycophila</i>	Pseudothecia of <i>Paraleptosphaeria polylepidis</i>	Bolivia	APA-2999 (DNA isolated from conidial mass)	MK795715	MK795718	This study
<i>Sajamaea mycophila</i>	Pseudothecia of <i>Paraleptosphaeria polylepidis</i>	Bolivia	APA-2999 (DNA isolated from conidiomata)	MK795716	MK795719	This study

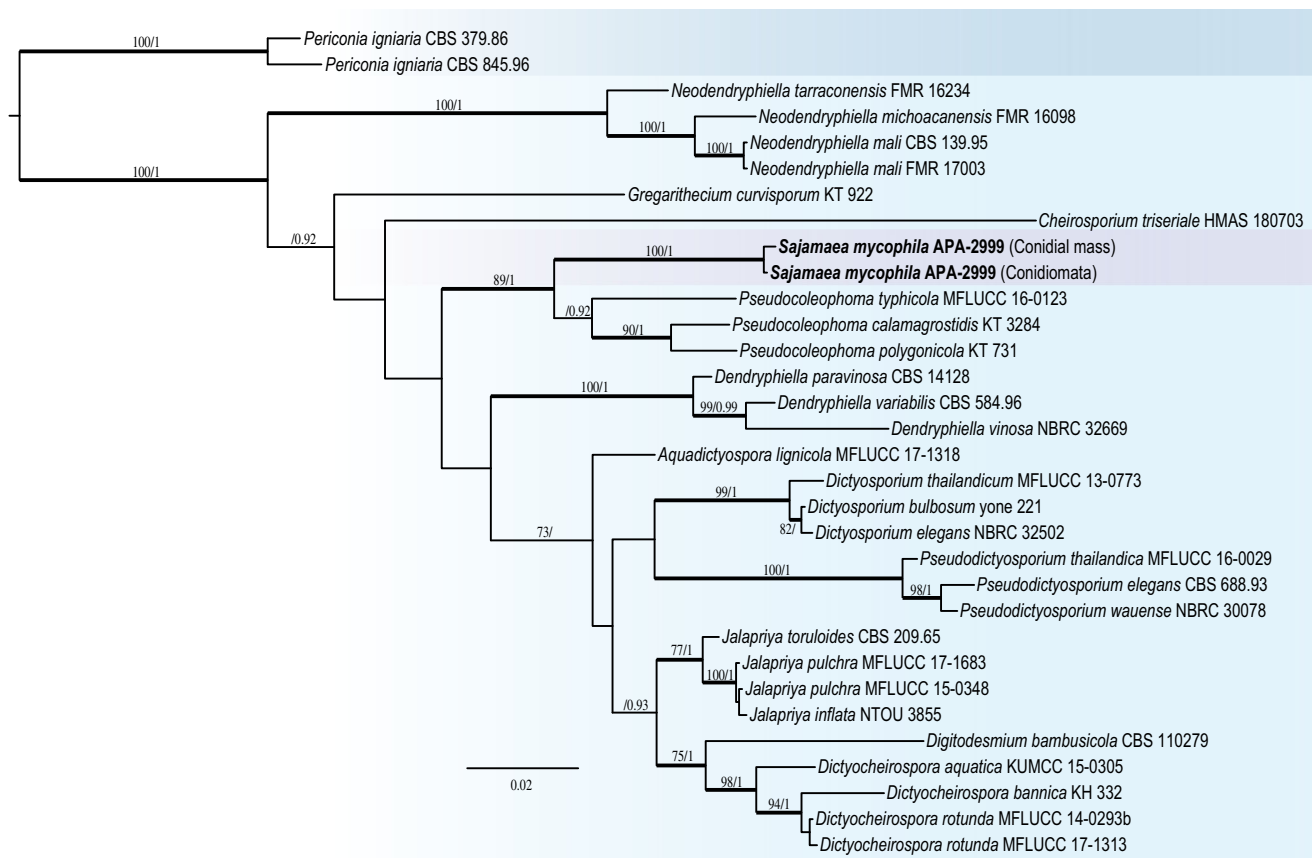
APA A. N. Palabral-Aguilera; CBS CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; FMR Facultat de Medicina i Ciències de la Salut, Reus, Spain; HMAS Herbarium Mycologium Institute of Microbiology Chinese Academy of Sciences, Beijing, China; KH K. Hirayama; KT K. Tanaka; KUMCC Culture collection of Kunming Institute of Botany, Kunming, China; MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NBRC National Institute of Technology and Evaluation, Chiba, Japan; NTOU National Taiwan Ocean University Culture Collection, Keelung, Taiwan; yone H. Yonezawa



**Fig. 1** Bayesian inference consensus tree inferred from a four-locus dataset (SSU+ITS+LSU+TEF1) of the Leptosphaeriaceae clade. Numbers above bold branches indicate bootstrap support values  $\geq 70\%$

from maximum likelihood and posterior probabilities  $\geq 0.95$  from Bayesian inference analyses. *Cucurbitaria berberidis* is used as an outgroup





**Fig. 2** Bayesian inference consensus tree inferred from two-locus dataset (ITS+LSU) of the Dictyosporiaceae clade. Numbers above bold branches indicate bootstrap support values  $\geq 70\%$  from maximum likelihood and

posterior probabilities  $\geq 0.9$  from Bayesian inference analyses. *Periconia igniaria* is used as an outgroup

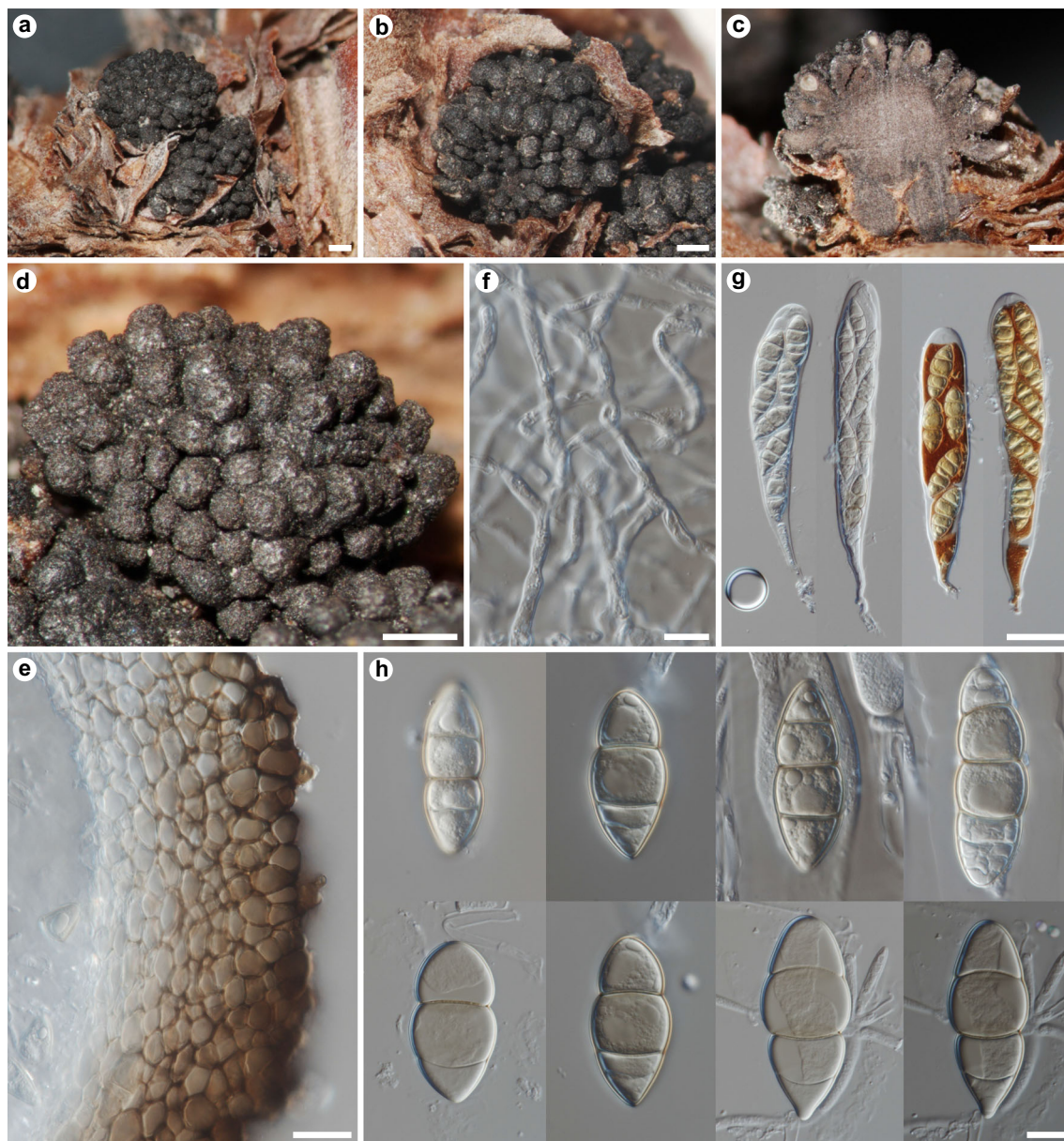
closely located to the permanent monitoring plot “Cohiri,” 19K 512102, 8002927, elev. ca. 4425 m, parasitic on *Paraleptosphaeria polylepidis* growing on *Polylepis tarapacana*, 15 Oct. 2016, A.N. Palabral-Aguilera (APA-2999), C. López & M.I. Gómez (LPB 0003512 – holotype, KRAM F-59659 – isotype).

## Discussion

Molecular phylogenetic analyses, based on the four-gene dataset, placed *Leptosphaeria polylepidis* in a well-supported clade consisting of members of the genus *Paraleptosphaeria*, including the type species *Pa. nitschkei*. The genus *Paraleptosphaeria* has been described by de Gruyter et al. (2013) for *Pa. dryadis*, *Pa. macrospora*, *Pa. nitschkei*, *Pa. orobanches*, and *Pa. praetermissa* that form a distinct monophyletic lineage among Leptosphaeriaceae within Pleosporales. *Paraleptosphaeria* is morphologically similar but clearly divergent genetically from the *Leptosphaeria* lineage. Separateness of *Paraleptosphaeria* from *Leptosphaeria* has been confirmed by subsequent molecular studies

(Ariyawansa et al. 2015; Chen et al. 2015; Liu et al. 2015; Wanasinghe et al. 2016; Tibpromma et al. 2017); additionally, two novel species, *Pa. padi* and *Pa. rubi*, have been described in the genus (Ariyawansa et al. 2015; Tibpromma et al. 2017) after its original establishment. Therefore, prior to this study, seven species were accepted in *Paraleptosphaeria*.

The generic concept of *Paraleptosphaeria* includes species having immersed, solitary or aggregated, thick-walled, ostiolate and unilocular pseudothecia, bitunicate, 8-spored asci, and fusiform, 5–8-septate, hyaline to yellow-brownish ascospores. The asexual state, when present, is coelomycetous consisting of pycnidial, unilocular conidiomata, phialidic conidiogenous cells, and oblong or ellipsoidal, aseptate, hyaline conidia (de Gruyter et al. 2013). *Leptosphaeria polylepidis* generally fits well to this concept, except that its pseudothecia are superficial and developed on stroma. However, this character may be variable in the genus, and, for example, Liu et al. (2015) reported superficial pseudothecia in their material of *Paraleptosphaeria nitschkei*. Therefore, based on molecular evidence, *Leptosphaeria polylepidis* is transferred to *Paraleptosphaeria* (as *Pa. polylepidis*) in this study.

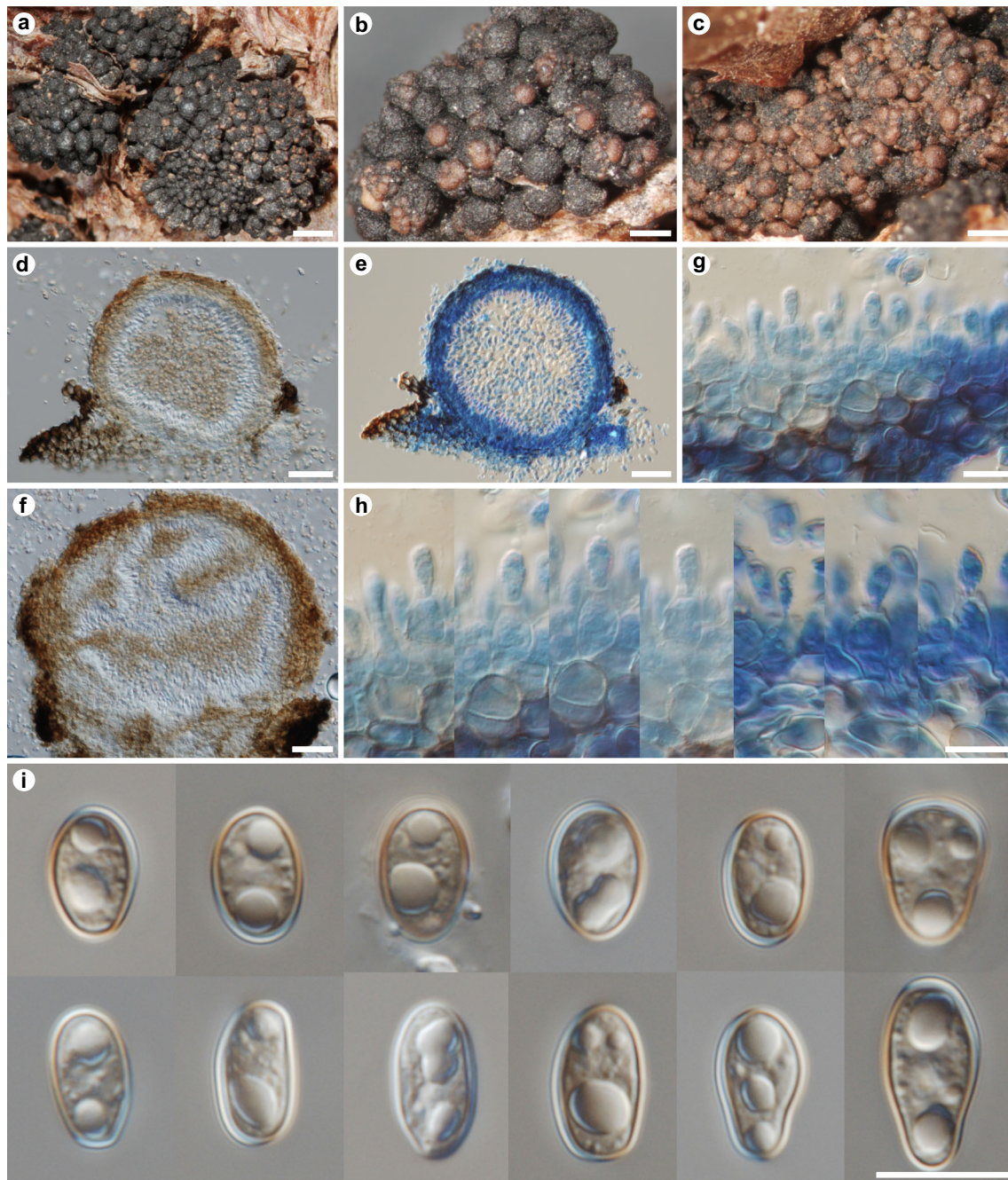


**Fig. 3** *Paraleptosphaeria polylepidis* (KRAM F-59659). **a, b, d** Habit of the crowded ascomata on twig of *Polylepis tarapacana*. **c** Longitudinal section through ascomata and stroma. **e** Paraplactenchymatous peridium from longitudinal section. **f** Hamatecium composed of thin interascal

filaments. **g** Bitunicate asci with 5–8 ascospores inside (left mounted in water, right mounted in KOH/IKI). **h** Ascospores mounted in water. Scale bars: **a–d** 500  $\mu$ m, **e** 25  $\mu$ m, **f, h** 10  $\mu$ m, **g** 50  $\mu$ m

Other than genetically divergent, *Paraleptosphaeria* may be ecologically different from *Leptosphaeria* by having other host specialization. Five of the eight *Paraleptosphaeria* species occur on Rosaceae, and three species on other host families, Asteraceae, Orobanchaceae, and Polygonaceae, while *Leptosphaeria* species tend to inhabit hosts in very diverse families but not Rosaceae (de Gruyter et al. 2013; Ariyawansa et al. 2015; Dayarathne et al. 2015; Liu et al. 2015). The molecular phylogenetic analyses showed that *Paraleptosphaeria polylepidis* was weakly supported as sister species of *Pa. dryadis* supporting earlier finding

by Macía et al. (2005). These authors pointed out that *Pa. polylepidis* and *Pa. dryadis* occur both at high elevations. Their host plants, *Polylepis tarapacana* and *Dryas octopetala* (Rosaceae), are adapted to grow in cold ecosystems. According to our molecular analyses, the branch leading to *Pa. polylepidis* is very long, which may suggest an old evolutionary origin of this lineage. It is, however, also possible that undiscovered closely related *Paraleptosphaeria* species may still exist in poorly studied South American countries. The weakly supported sister-group relationship of *Pa. polylepidis* and *Pa. dryadis* and the circumstance that their hosts



**Fig. 4** *Sajamaea mycophila* (KRAM F-59659). **a–c** Habit of pale brown conidiomata growing on ascomata of *Paraleptosphaeria polylepidis*. **d–e** Longitudinal section of uniloculate conidioma erumpent through the outermost layer of host peridium (**e** mounted in LPCB). **f** Longitudinal section of multi-loculate conidioma showing layers of conidiogenous

cells (hyaline areas) and conidial masses (brown areas). **g** Section through paraplectenchymatous peridium showing conidiogenous cells mounted in LPCB. **h** Conidiogenous cells and young conidia mounted in LPCB. **i** Conidia. Scale bars: **a** 1000  $\mu\text{m}$ , **b–c** 500  $\mu\text{m}$ , **d–f** 50  $\mu\text{m}$ , **g–i** 10  $\mu\text{m}$

are phylogenetically unrelated (Potter et al. 2007) suggests that they evolved from different ancestral species.

The assignment of conidiomata found on the pseudothecia of *Paraleptosphaeria polylepidis* to a new phylogenetic lineage of the Dictyosporiaceae was unexpected as we earlier hypothesized having encountered the asexual state of *Pa. polylepidis*. The sequences obtained from the fungus formed

a strongly supported sister clade to members of the genus *Pseudocoleophoma* but could not be classified in *Pseudocoleophoma* on the basis of different morphological characteristics. The genus *Pseudocoleophoma* has been described for two species, *Ps. calamagrostidis* (generic type) and *Ps. polygonicola*, that produced sexual states on the natural substrates and asexual states in cultures (Tanaka et al.

2015). Two more species have been described later, *Ps. typhicola* forming only asexual state on the natural substrate (Hyde et al. 2016) and *Ps. bauhiniae* producing both asexual and sexual states on the natural substrate (Jayasiri et al. 2019). While the fungus found on the pseudothecia of *Pa. polylepidis* lacks a sexual state and forms uniloculate to multi-loculate pycnidia and pale brown, broadly ellipsoidal conidia, the species of *Pseudocoleophoma* usually produce a sexual state and pycnidia of the asexual state are uniloculate and the cylindrical conidia are hyaline. It also differs ecologically by parasitism on another fungus, while all *Pseudocoleophoma* species are saprobic on dead plant materials. Though we did not check mycelial interactions in culture experiments, the fungus forms pycnidia erumpent through the outermost layer of host peridium and, therefore, is likely mycoparasitic (at least necrotrophic as defined by Sun et al. 2019). Furthermore, the molecular phylogenetic analyses indicated genetic distances between this fungus and *Pseudocoleophoma* comparable to distances between other Dictyosporiaceae clades, assigned to distinct genera (Boonmee et al. 2016). No morphologically similar coelomycete genus was found in Sutton (1980) and in the recent literature; therefore, a new genus and species names, *Sajamaea* and *S. mycophila*, are introduced for this fungus. The family Dictyosporiaceae contains mostly asexual species, while sexual species are very rare, being represented only by *Dictyosporium meiosporum*, *D. sexualis*, *Gregarithecium curvisporum*, *Pseudocoleophoma calamagrostidis*, *P. polygonicola*, and *P. bauhiniae* (Tanaka et al. 2015; Boonmee et al. 2016; Jayasiri et al. 2019). The asexual species are mostly hyphomycetous, often producing characteristic cheirosporous conidia (Tanaka et al. 2015; Boonmee et al. 2016; Iturrieta-González et al. 2018; Yang et al. 2018), but *Sajamaea* and *Pseudocoleophoma* have coelomycetous asexual states, which supports their close phylogenetic relationship.

The parasitism of *Sajamaea mycophila* suggests that this species may be potentially used as a biopesticide that could prevent the development of *Paraleptosphaeria polylepidis* and consequently protect *Polylepis tarapacana* woodlands.

Additional emphases are thus required to re-collect *S. mycophila* and aim at culturing it in order to unravel its mycoparasitic and biopesticidal potentials.

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