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Tricladiaceae fam. nov. (*Helotiales*, *Leotiomyces*)

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Abstract: To resolve the polyphyletic nature of *Solenopezaceae* as it was originally circumscribed, we establish a new family *Tricladiaceae* for those genera originally placed in *Solenopezaceae* that have aquatic hyphomycete-like asexual morphs and/or a sexual morph with glabrous apothecia. These include *Cudoniella*, *Geniculospora*, *Graddonia*, *Halenospora*, *Mycofalcella*, *Spirosphaera*, and *Tricladium*. *Solenopezaceae* is confined to the genera *Lasiobelonium*, *Solenopezia*, *Trichopeziza*, and *Trichopezizella*, all of which have a sexual morph having apothecia with smooth-walled hairs. This taxonomy is supported by a multi-gene analysis using up to 15 genes, with a few of the taxa placed on the basis of a separate ITS phylogeny. *Tricladiaceae* forms a monophyletic clade with a basal sister relationship to *Pleuroascaceae* plus *Helotiaceae*; *Solenopezaceae* forms a monophyletic clade with a basal sister relationship to *Lachnaceae*.

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INTRODUCTION

In their studies on *Lachnaceae*, Hosoya *et al.* (2010) and Han *et al.* (2014) showed that *Trichopeziza*, *Trichopezizella* and *Lasiobelonium* were phylogenetically separate from what they considered the core *Lachnaceae* and that they differed morphologically from *Lachnaceae* in having smooth-walled hairs. On this basis, they excluded these three genera from their concept of *Lachnaceae*, but they did not formally name a separate family because support for them as a monophyletic group was not strong in some of their analyses (Han *et al.* 2014). In his discussion on the *Lachnaceae*, Baral (in Jaklitsch *et al.* 2016) commented that *Trichopeziza* and related genera may deserve a family of their own, based on published phylogenies and differences from *Lachnaceae sensu stricto* in hair and excipular characters, but again, no formal family name was provided for this group of genera. Noting the conclusions of these earlier authors, Ekanayaka *et al.* (2019) erected a new family *Solenopezaceae* to include the genera excluded from *Lachnaceae* by Han *et al.* (2014). However, from the results of their published phylogeny, they expanded the taxonomic breadth of the family to also include a set of genera with morphologically complex asexual spores typical of aquatic hyphomycete fungi. Thus, Ekanayaka *et al.* (2019) included the genera *Geniculospora*, *Graddonia*, *Halenospora*, *Lasiobelonium*, *Mycofalcella*, *Solenopezia*, *Trichopeziza*, *Trichopezizella*, and *Tricladium* in their new family. This was despite the clade referred to as *Solenopezaceae* in the phylogenetic analysis of Ekanayaka *et al.* (2019) having no statistical support.

The multi-gene analysis of Johnston *et al.* (2019) showed that the genera *Mycofalcella* and *Tricladium* belonged to a monophyletic clade at the base of what they accepted as *Helotiaceae*, a position phylogenetically distant from *Trichopeziza*, *Trichopezizella*, and *Lasiobelonium*. These latter three genera formed a monophyletic clade at the base of what Johnston *et al.* (2019) accepted as *Lachnaceae*. Supplementary data in Johnston *et al.* (2019), solely based on ITS sequences, suggested that *Solenopezia* also belongs in the clade with *Trichopeziza* and its relatives, while *Graddonia*, *Halenospora*, and *Spirosphaera* may also belong in the clade with *Mycofalcella* and *Tricladium*. Thus, based on the multigene phylogeny of Johnston *et al.* (2019), *Solenopezaceae sensu* Ekanayaka *et al.* (2019) is polyphyletic. Note that Ekanayaka *et al.* (2019) and Johnston *et al.* (2019) used different sequence data to represent *Solenopezia* — GenBank accession U57991 (ex NYS specimen JHH4169, accepted as *Solenopezia solenia* by Raitviir *et al.* 1991) in the case of Johnston *et al.* (2019) and KY592188 (an ITS2 clone from orchid root from an unpublished study) in the case of Ekanayaka *et al.* (2019). These two sequences represent phylogenetically distant fungi, KY592188 appears to be in the core *Hyaloscyphaceae*, while U57991 is in a clade with *Trichopeziza* and *Trichopezizella* (unpubl. data).

Here we erect a new family *Tricladiaceae* to accommodate the genera of *Solenopezaceae sensu* Ekanayaka *et al.* (2019) that are phylogenetically positioned basally to *Helotiaceae* rather than *Lachnaceae*.

MATERIALS AND METHODS

The DNA sequence data used for analyses in this paper have been collated from existing GenBank accessions, previously unreported aquatic hyphomycete cultures isolated from fresh water in Europe, and previously unreported specimens from native forests in New Zealand that were grown in culture from ascospores from the sexual morph of fungi that preliminary analyses showed to be phylogenetically close to *Tricladium* (Table 1).

DNA was extracted from mycelium in cultures using methods outlined in Baschien *et al.* (2013) and Johnston & Park (2005), genes were amplified using standard primers, and sequenced using Sanger sequencing.

Two sets of analyses were carried out. The specimens with multi-gene data (Table 1) were incorporated into the alignments from Johnston *et al.* (2019), together with *Graddonia coracina* sequences (LSU, 5.8S rDNA, *MCM7*, *RPB1*) from Hustad *et al.* (2011), and the expanded dataset was reanalysed using the same ML methods as Johnston *et al.* (2019). Gene coverage for the specimens from Johnston *et al.* (2019) that belong in the clades labelled *Solenopeziaceae* and *Tricladiaceae* in Fig. 1 are: TNS-F 16472 – SSU, 5.8S rDNA, LSU, *RPB1*, *RPB2*, mtSSU; *H. varicosporoides* JGI genome – SSU, 5.8S rDNA, LSU, *RPB1*, *RPB2*, *RPA1*, *TEF1*, *β-tub*, *MCM7*, *RPB2*, *TFB4*, *RPA2*, *α-tub*, *SF3B1*; CCM F-16599 – SSU, 5.8S rDNA, LSU, *RPB1*, *RPB2*, *TEF1*, *β-tub*; CCM F-10289 – SSU, 5.8S rDNA, LSU, *RPB1*, *RPB2*, *TEF1*; KUS F52218 – 5.8S rDNA, LSU, *RPB2*, mtSSU; TNS-F 16760 – SSU, 5.8S rDNA, LSU, *RPB1*, *RPB2*, *β-tub*; TNS-F 16579 – SSU, 5.8S rDNA, LSU, *RPB1*, *RPB2*, *β-tub*; TNS-F 16667 – SSU, 5.8S rDNA, LSU, *RPB1*, *RPB2*, *β-tub*. GenBank accession numbers for these sequences are provided in supplementary data from Johnston *et al.* (2019, see Manaaki Whenua – Landcare Research data store, <https://doi.org/10.7931/T5YV-BE95>).

A second analysis based on ITS sequences only used the specimens in Table 1, together with specimens selected from close BLAST matches from GenBank that were shown in preliminary analyses to belong in a monophyletic clade sister to *Helotiaceae*, and thus matched the phylogenetic relationships revealed in the multi-gene analysis. As far as possible, the GenBank accessions used in the analysis were selected on the basis that they were type specimens or were specimens identified as the type species of a genus. Additional specimens were included where they helped to clarify the taxonomic limits of the *Tricladiaceae* clade.

The alignments used for each gene are available through the Manaaki Whenua – Landcare Research Data store, as are the models used for each gene in the phylogenetic analyses (<https://doi.org/10.7931/2h44-4n89>).

Morphological data mentioned in the results and discussion were mostly extracted from published descriptions. Original data are provided for some apothecial specimens collected from streams in New Zealand native forests. Dried specimens from the New Zealand Fungarium (PDD) were examined morphologically following rehydration in 3 % KOH. Thin (approximately 10 µm thick) vertical sections of apothecia were obtained using a freezing microtome, the sections mounted in lactic acid for microscopic examination. Hymenial elements were examined in 3 % KOH with Melzer's reagent. Notes and images on the morphology of the specimens examined are available through the Manaaki Whenua – Landcare Research SCD webpages, <https://scd.landcareresearch.co.nz/>. Aquatic

hyphomycete strains were grown on 0.1 % or 2 % malt extract agar. Preparations were made from sporulating agar culture pieces incubated in distilled water at 10–15 °C.

RESULTS

Phylogeny

The multigene analysis generated a tree very similar to the one presented in Johnston *et al.* (2019) (Fig. 1). *Solenopeziaceae sensu* Ekanayaka *et al.* (2019) was shown to be polyphyletic, the core genera *Trichopeziza*, *Trichopezizella* and *Lasiobelonium* forming a monophyletic clade basal to *Lachnaceae sensu* Johnston *et al.* (2019) and the taxa here accepted as *Tricladiaceae* forming a monophyletic clade basal to *Helotiaceae sensu* Johnston *et al.* (2019). Note that a second monophyletic basal clade within *Helotiaceae sensu* Johnston *et al.* (2019) was formally named *Pleuroascaceae* by Untereiner *et al.* (2019).

The ITS phylogeny (Fig. 2) includes many poorly supported branches, but suggests that the morphology of asexual spores (reflected in the generic names of the specimens treated) correlates poorly with phylogeny. Genera such as *Tricladium* and *Helicodendron* are polyphyletic across *Tricladiaceae*. DNA sequences from type material of the genera *Mycofalcella*, *Spirosphaera*, and *Tricladium* confirm that these genera belong in *Tricladiaceae*.

Taxonomy

Tricladiaceae P.R. Johnst. & Baschien, *fam. nov.* MycoBank MB834831.

Etymology: Named after the type genus *Tricladium*, the oldest of the genera accepted in the family.

Diagnosis: Phylogenetically forming a monophyletic clade positioned basally to *Helotiaceae* plus *Pleuroascaceae*, found in wet habitats, asexual morph with complex elongate, curved, branched or coiled conidia, sexual morph with sessile to stipitate, glabrous apothecia with excipulum of non-gelatinous, more or less globose, thin-walled cells, ascus apex hymenoscyphus-like.

Type: *Tricladium* Ingold

Description: Asexual and sexual morphs associated with wet habitats, isolated from foam samples from flowing fresh water, or growing on dead woody or herbaceous substrates in streams, ponds, and lakes. Spores from the asexual morph are complex in shape, branched to worm-like, long fusoid and curved, or forming irregular coils; conidiogenous cells simple or on branched conidiophores, sometimes in sporodochia; conidiogenesis holoblastic, thalloblastic, or proliferating percurrently. Sexual morph with apothecia that are sessile to stipitate, glabrous, pale in colour, centrally attached to substrate, often with a dense pad of basal anchoring hyphae, these hyphae with gelatinous walls; excipulum nongelatinous, cells more or less subglobose, short-cylindric externally toward margin of apothecium, arranged at low angle to receptacle surface; asci with hymenoscyphus-like amyloid ring.

Table 1. New sequences used for the analysis presented in Fig. 1, and new sequences generated for the analysis of ITS sequences presented in Fig. 2.

Species	Voucher*	Country	Substrate	ITS	<i>β-tub</i>	<i>MCM7</i>	<i>TEF</i>	<i>RPB1</i>	<i>RPB2</i>
<i>Anguillospora crassa</i>	DSM 104359	Germany	Stream foam	MK353093	—	—	—	—	—
	DSM 104363	Germany	Stream foam	MK353094	—	MT192242	—	—	MT192255
	DSM 104370	Germany	Stream foam	MK371722	—	MT192240	MT192256	MT192246	—
	CCM F-13483	Slovakia	Sessile apothecia	MT185420	—	MT192241	—	MT192248	—
	CCM F-15583	Slovakia	Sessile apothecia on Angiosperm twiglet	MT185419	—	—	—	—	—
	CCM F-07082	Slovakia	Stalked apothecia	AY204580	—	—	—	—	—
<i>Anguillospora furtiva</i>	ICMP 23618	New Zealand	Sessile apothecia on <i>Juncus</i> sp. dead leaf	MT002397	—	—	—	—	—
	CB L16	Austria	Stream foam	MT185427	—	—	—	—	—
<i>Filosporaella annelidica</i>	CCM F-00681	Slovenia	—	MT185415	—	—	—	—	—
	CCM F-03681	Croatia	—	MT185425	MT192263	MT192244	MT192259	MT192250	MT192252
	CCM F-10195	Croatia	—	MT185426	MT192262	MT192245	MT192258	MT192251	MT192254
	DSM 105083	Germany	Stream foam	MT185424	—	—	—	—	—
<i>Filosporaella</i> sp.	CCM F-11702	UK	Stream foam	MT185416	—	—	—	—	—
<i>Filosporaella</i> sp.	CCM F-11294	—	—	MT185417	MT192261	MT192243	MT192257	MT192249	MT192253
<i>Filosporaella</i> sp.	CCM F-15099	Croatia	—	MT185421	—	—	MT192260	MT192247	—
<i>Filosporaella</i> sp.	CCM F-10789	Croatia	—	MT185422	—	—	—	—	—
<i>Filosporaella</i> sp.	CCM F-11189	Croatia	—	MT185423	—	—	—	—	—
<i>Filosporaella</i> sp.	CCM F-11394	Croatia	—	MT185418	—	—	—	—	—
<i>Helicodendron cf. tubulosum</i>	ICMP 23619	New Zealand	Sessile apothecia on wood in stream	MT002398	—	—	—	—	—
<i>Spirosphaera</i> sp.	ICMP 23620	New Zealand	Sessile apothecia on wood in stream	MT002399	—	—	—	—	—
<i>Spirosphaera</i> sp.	ICMP 23621	New Zealand	Sessile apothecia on saturated wood	MT002400	—	—	—	—	—
<i>Spirosphaera</i> sp.	ICMP 23622	New Zealand	Sessile apothecia on wood in running water	MT002401	—	—	—	—	—

Table 1. (Continued).

Species	Voucher*	Country	Substrate	ITS	β -tub	MCM7	TEF	RPB1	RPB2
<i>Spirosphaera</i> sp.	ICMP 23623	New Zealand	Sessile apothecia on wood in running water	MT002402	—	—	—	—	—
<i>Tricladiaceae</i> sp.	ICMP 23617	New Zealand	Sessile apothecia on wood in stream	MT002396	—	—	—	—	—
<i>Tricladiaceae</i> sp.	ICMP 23624	New Zealand	Sessile apothecia on wood in running water	MT002403	—	—	—	—	—

*CCM – Czech Collection of Microorganisms.

DSM – Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH.

ICMP – International Collection of Microorganisms from Plants; DNA extracts from cultures grown from ascospores from specimens subsequently dried and deposited in the PDD fungarium.

Genera included in the family

Cudoniella

The type species of *Cudoniella*, *C. queletii*, was accepted as a synonym of *C. acicularis* by Dennis (1964), following Boudier (1907). Specimens identified as *C. acicularis*, *C. clavus*, and *C. indica* have DNA sequences accessioned into GenBank. Based on these, *C. acicularis* and *C. indica* are both in *Tricladiaceae*, but phylogenetically distinct within the family (Fig. 2). *Cudoniella clavus* is a member of the core *Helotiaceae sensu* Johnston *et al.* (2019).

Geniculospora

DNA sequences are not available for the type species of *Geniculospora*, *G. inflata*, but cultures identified as *G. grandis* in the CBS database (GenBank MH861735, CBS 261.84) and by Baschien *et al.* (2013; GenBank GQ411354, voucher UMB-176.01) are phylogenetically similar and have been accepted as authentic for the genus by several authors.

Graddonia

A specimen identified as the type species of *Graddonia*, *G. coracina* (GenBank JQ256423, ILLS 60491), is strongly supported as forming a basal clade within *Tricladiaceae* (Fig. 1). Known only from its sexual morph, the lifestyle of this fungus is typical for *Tricladiaceae*, reported from wet habitats (Dennis 1978, Gminder 1993). The sessile apothecia have an excipular structure similar to that found in other *Tricladiaceae* (Dennis 1955) but the asci are reportedly nonamyloid. Placed in *Helotiaceae* by Baral (in Jaklitsch *et al.* 2016), who noted that the paraphyses are typical for this family.

Halenospora

CBS cultures identified as *Halenospora varia* (AF169303, MF5849 ex Bills *et al.* 1999, incorrectly cited as ATCC 28878), "*Scytalidium*" *multiseptatum* (phylogenetically distant to type specimen of type species, *S. lignicola* (GenBank NR_121314, ex type specimen UAMH 1501 = CBS 233.57)), and *Helicorhoidion* sp. (MH861094, CBS 498.77) have matching ITS sequences

(Fig. 2). DNA sequences from type specimens are not available for any other specimen identified as *Helicorhoidion* or for the type specimens of the other two species. *Halenospora varia* (basionym *Zalerion varium*) is the type species of *Halenospora*. Whether the DNA sequence AF169303 would truly match that from the type of *Zalerion varium* is not known, but it is the DNA sequence data on which Jones *et al.* (2009) based their new genus *Halenospora*. Note that *Zalerion* (type species *Z. maritima*) is a member of the *Lulworthiales*, phylogenetically distant from *Z. varium* (Jones *et al.* 2009).

Helicorhoidion and *Halenospora* are morphologically similar, both forming conidia comprising irregular coils of globose, dark-walled cells and could easily be confused (Hughes 1958, Anastasiou 1963). The *Scytalidium multiseptatum* specimens are likely to have been misidentified. This fungus has very different conidia but forms clumps of dark-walled, globose, chlamyospore-like cells (Holubová-Jechová 1990) that could perhaps be confused with the conidia of *Halenospora*.

Mycofalcella

The ex-type specimen of the type species *Mycofalcella calcarata* (GenBank NR_154165, CCM F-10289) belongs in the *Tricladiaceae* (Fig. 1). A typical aquatic hyphomycete both ecologically and morphologically, with elongate, gently curved conidia (Marvanová *et al.* 1993). Morphologically this genus is very similar to *Anguillospora crassa* except for having a long excentric basal extension of the conidium.

Spirosphaera

An aquatic hyphomycete genus with conidia comprising loosely interwoven, branched, septate coils. The ex-type specimen of the type species *Spirosphaera floriformis* (GenBank NR_138376, CBS 405.52) belongs in the *Tricladiaceae*, but only rDNA sequences are available (Fig. 2). An unnamed species from New Zealand has an asexual morph with a spiro-sphaera-like morphology (Fig. 2), is known from both its asexual (*e.g.*, https://scd.landcareresearch.co.nz/Specimen/ICMP_20907) and sexual morphs (*e.g.*, https://scd.landcareresearch.co.nz/Specimen/PDD_117564), and appears to be common on wood in forest

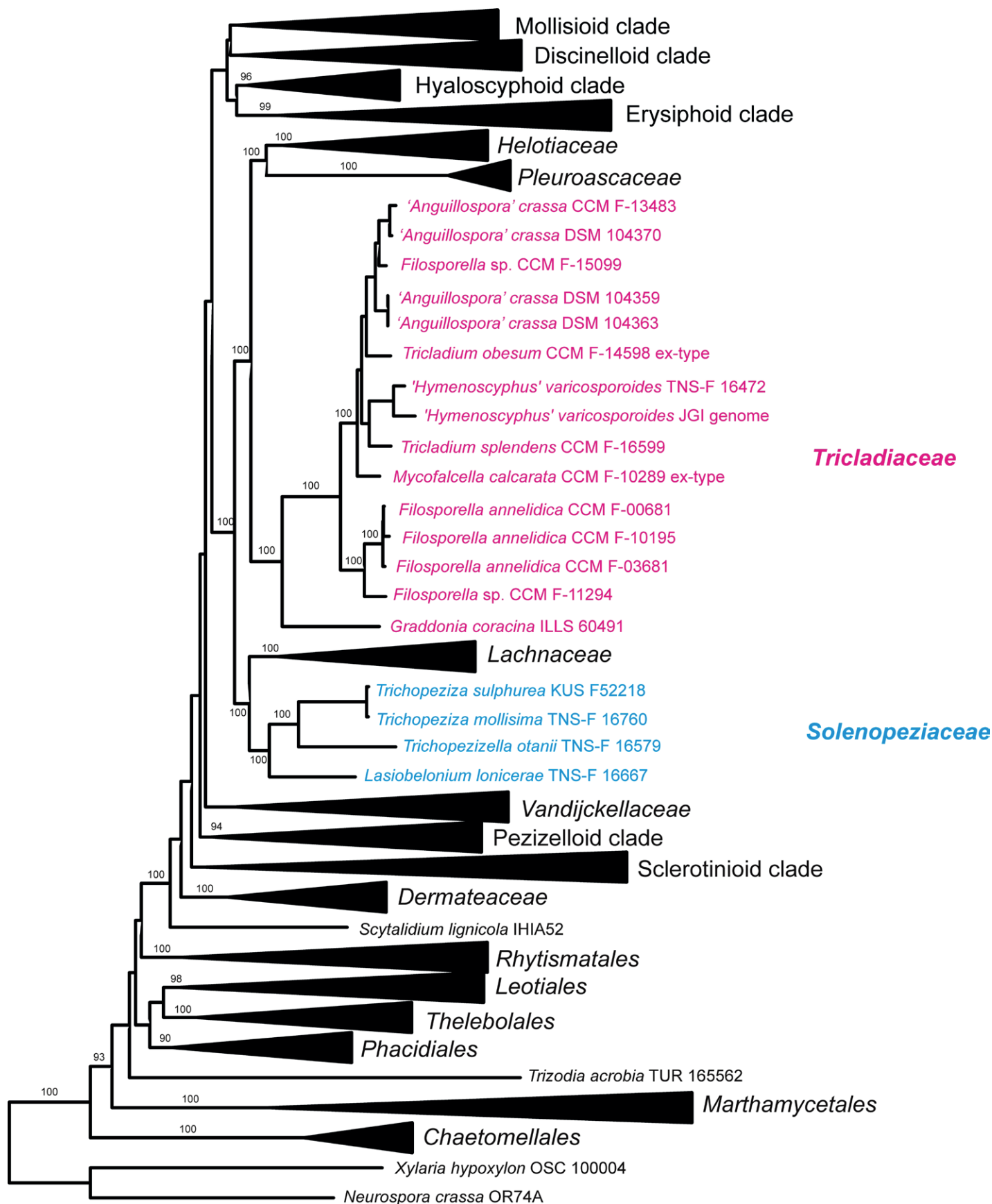


Fig. 1. Maximum Likelihood tree based on concatenated DNA sequences from the 15-gene analysis in Johnston *et al.* (2019, data available from doi.org/10.7931/T5YV-BE95), together with sequences from additional taxa of *Tricladaceae* listed in Table 1 and the *Graddonia coracina* sequences from Hustad *et al.* (2011). Two monophyletic basal clades of *Helotiaceae* *sensu* Johnston *et al.* (2019) have been segregated from a core *Helotiaceae* clade into two additional families, *Pleuroascaceae* (following Untereiner *et al.* 2019) and *Tricladaceae*. The specimens treated are labelled with the names provided by the sequencing authors. Bootstrap values are indicated where > 89 %. The collapsed clades represent the strongly supported order-level and informal supra-familial clades within *Helotiales* accepted by Johnston *et al.* (2019). The *Sordariomycetes* genera *Xylaria* and *Neurospora* are used as outgroups.

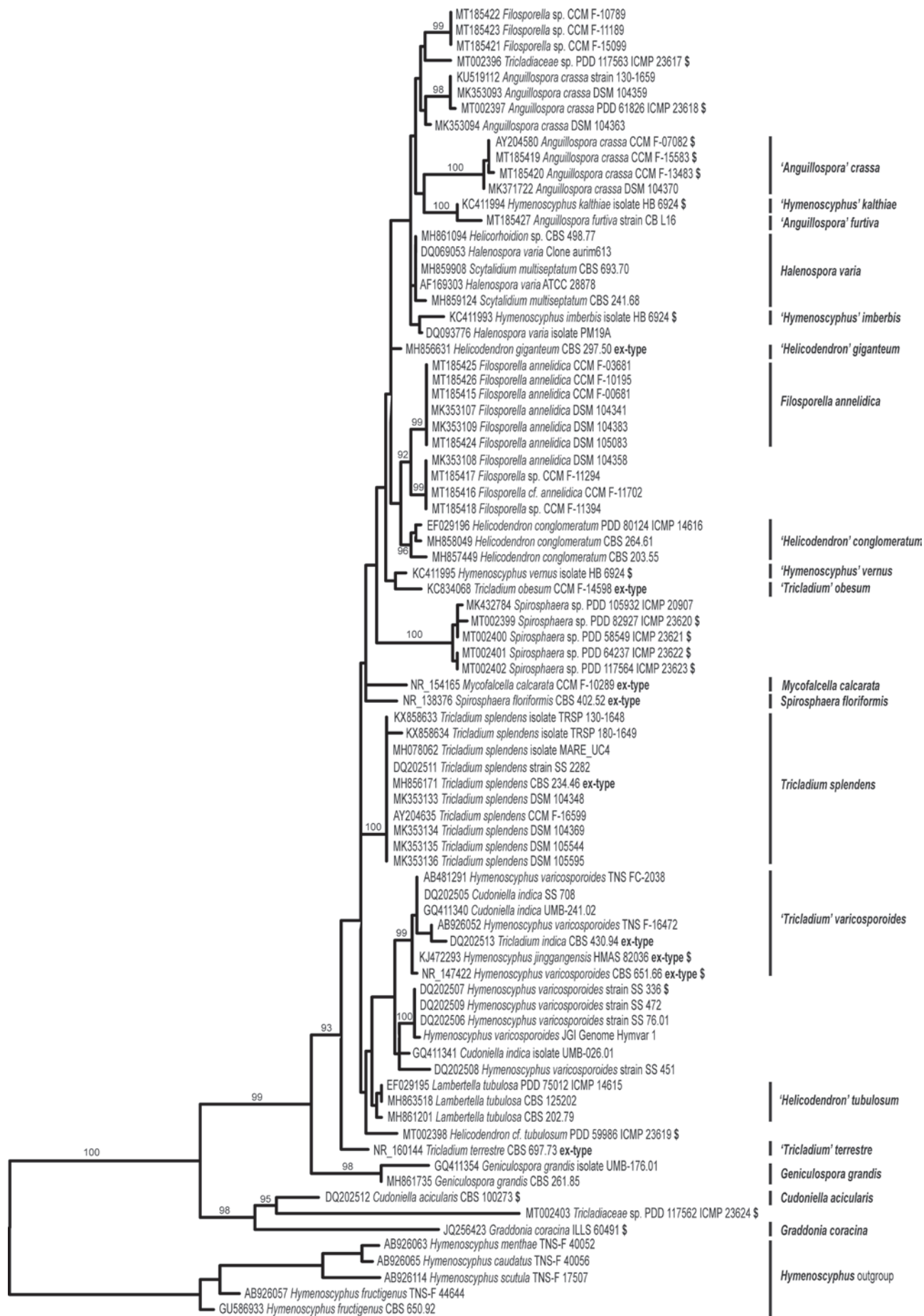


Fig. 2. Maximum Likelihood tree based on ITS sequences from specimens of *Tricladaceae* in GenBank, selected from BLAST searches, together with the newly generated sequences listed in Table 1. Ex-type specimens are indicated, the \$ symbol indicates specimens sequenced from cultures grown from the sexual morph. Specimens are labelled with the name provided with the GenBank record; the names to the right are those accepted in this paper. Bootstrap values are provided where > 90 %. *Hymenoscyphus* (*Helotiaceae*) was selected as the outgroup.

streams. Whether this New Zealand species is congeneric with *S. floriformis* needs additional genes to resolve.

Note that Ekanayaka *et al.* (2019) placed *Spirosphaera* in their *Trizodia-Calloriopsis* clade, but they represented this genus by a specimen identified as *S. minuta*, a specimen phylogenetically distant to the ex-type specimen of the type species, *S. floriformis* (unpubl. data) (Table 2).

Tricladium

The name of the type species, *Tricladium splendens*, has been used for several GenBank accessions. Several of these specimens have multiple genes available and clearly belong in *Tricladaceae*. *Tricladium*, the largest genus of aquatic hyphomycetes, is polyphyletic across *Leotiomycetes* (Campbell *et al.* 2009). Specimens identified as species in this genus are likewise spread across the *Tricladaceae* phylogeny (Fig 2). These include ex-type specimens for *T. obesum* (GenBank KC834068, CCM F-14598) and *T. terrestre* (GenBank NR_160144, CBS 697.73).

The *Tricladium* species in *Tricladaceae* all have dark colonies but conidiogenesis is variable, percurrent in *T. splendens* and *T. obesum*, sympodial in *T. castaneicola* (*Tricladaceae* based on LSU sequences, Campbell *et al.* 2009) and *T. terrestre*, or determinate in *T. indicum*, and this perhaps reflects the polyphyletic nature of the genus as currently accepted in a morphological sense.

The sexual morph of *T. splendens* was described as *Hymenoscyphus splendens* (Abdullah *et al.* 1981). Based on the description of Abdullah *et al.* (1981), it is morphologically similar to the sexual morph of other *Tricladaceae*; see also Discussion.

Comments on other genera

Anguillospora

Anguillospora, the second largest genus of aquatic hyphomycetes, is phylogenetically diverse. The type species, *A. longissima*, is a member of the *Pleosporales* and is now accepted as a synonym of the type species of *Amniculicola*, with this latter genus recommended for protection over *Anguillospora* (Rossman

et al. 2016). Several species placed in *Anguillospora* belong in *Leotiomycetes*, including at least two in the *Tricladaceae*, “*A.*” *crassa* and “*A.*” *furtiva*. The *Anguillospora* species in *Tricladaceae* all have thalloblastic percurrent conidiogenous cells and sigmoid conidia with schizolytic secession. Specimens identified as *A. crassa* that have ITS sequences deposited in GenBank appear to represent several different species (Fig. 2). An authentic specimen of “*A.*” *furtiva* (GenBank AY148107, CCM F-20483, cited in Descals *et al.* 1998) is phylogenetically similar to one of the “*A.*” *crassa* clades (Fig. 2). However, which of the putative “*A.*” *crassa* specimens phylogenetically matches the type, collected from the United Kingdom (Ingold 1958), is not known and epitypification is needed to resolve the taxonomy of this species.

Anguillospora specimens have often been linked to a sexual morph, and some of these have been referred to *Leotiomycetes* genera *Pezoloma* (Descals *et al.* 1998) and *Mollisia* (Webster 1961). Neither of these sexual genera are accepted as *Tricladaceae* (Johnston *et al.* 2019). Based on published descriptions, the *Pezoloma* and *Mollisia* species linked to *Anguillospora* have an apothecial morphology similar to that illustrated for *Spirosphaera* (see https://scd.landcareresearch.co.nz/Specimen/PDD_117564); also see Discussion.

Digby & Goos (1987) reported an *Anguillospora* asexual morph grown from germinated ascospores from the type specimen of *Loramyces juncicola*. This was accessioned as ATCC 46458 and according to the ATCC web pages its genome was sequenced by JGI (<https://www.atcc.org/products/all/46458.aspx#characteristics>). Data from this genome was included in the analyses of Johnston *et al.* (2019) that showed *Loramyces* belongs in a monophyletic clade together with *Mollisia* (*Mollisiaceae*), phylogenetically distant from *Tricladaceae*.

Filosporella

Filosporella spp. fall into two phylogenetically distant groups within the *Leotiomycetes*. The ex-type specimens of *F. fistucella* (GenBank NR_153982, CCM F-13091), *F. exilis* (GenBank

Table 2. Summary of the family level names applied by Ekanayaka *et al.* (2019) and in this paper, for the genera treated here as either *Solenopeziaceae* or *Tricladaceae*.

Genus	Ekanayaka <i>et al.</i> (2019)	This paper
<i>Cudoniella</i>	<i>Helotiaceae</i> ¹	<i>Tricladaceae</i> ²
<i>Geniculospora</i>	<i>Solenopeziaceae</i>	<i>Tricladaceae</i> ³
<i>Graddonia</i>	<i>Solenopeziaceae</i>	<i>Tricladaceae</i>
<i>Halenospora</i>	<i>Solenopeziaceae</i>	<i>Tricladaceae</i>
<i>Lasiobelonium</i>	<i>Solenopeziaceae</i>	<i>Solenopeziaceae</i> ³
<i>Mycofalcella</i>	<i>Solenopeziaceae</i>	<i>Tricladaceae</i>
<i>Solenopezia</i>	<i>Solenopeziaceae</i>	<i>Solenopeziaceae</i>
<i>Spirosphaera</i>	<i>Trizodia-Calloriopsis</i> clade ⁴	<i>Tricladaceae</i> ⁵
<i>Tricladium</i>	<i>Solenopeziaceae</i>	<i>Tricladaceae</i>
<i>Trichopeziza</i>	<i>Solenopeziaceae</i>	<i>Solenopeziaceae</i>
<i>Trichopezizella</i>	<i>Solenopeziaceae</i>	<i>Solenopeziaceae</i>

¹ Represented by AFTOL-ID 166, identified as *Cudoniella clavus*.

² Represented by CBS 100273, identified as *Cudoniella acicularis*, synonym of the type species *C. queletii* (Dennis 1964).

³ Species used in analysis is not the type species of the genus.

⁴ Represented by CBS 475.66, *Spirosphaera minuta*.

⁵ Represented by CBS 697.73, ex-type culture of *Spirosphaera floriformis*, the type species of the genus.

KC834046, CCM F-13097) and *F. versimorpha* (GenBank KC834054, CCM F-11194) are within *Gelatinodiscaeae sensu Johnston et al.* (2019). Also in *Gelatinodiscaeae* are specimens referred to the type species of the aquatic hyphomycete genera *Clathrosporium* and *Helicodendron*. On the other hand, specimens referred to *F. annelidica*, together with other currently unnamed *Filospora* spp., belong in *Tricladiaceae* (Figs 1, 2). The specimens identified as *F. annelidica* fall into two clades in Fig. 1. No DNA sequence data are available for the type species of *Filospora*, *F. aquatica*, so the taxonomic position of this genus within *Leotiomycetes* remains unresolved, awaiting epitypification of *F. aquatica*, a species originally described from Tasek Bera in Peninsular Malaysia.

Helicodendron

Specimens identified as the type species of *Helicodendron*, *H. paradoxum*, belong in the *Gelatinodiscaeae* (Johnston et al. 2019). As with several other aquatic hyphomycete genera, the distinctive conidial morphologies on which genera have traditionally been based, have evolved independently many times (Belliveau & Bärlocher 2005, Baschien et al. 2006, Campbell et al. 2009, Tsui et al. 2016). Species with helicodendron-like conidia in the *Tricladiaceae* include the ex-type culture of *Helicodendron giganteum* (GenBank MH856631, CBS 297.50), specimens identified as *Helicodendron conglomeratum*, and specimens identified as *Helicodendron tubulosa* (Fig. 2).

The sexual morph of *Helicodendron tubulosum* was named as *Lambertella tubulosa* by Abdullah & Webster (1981). Several GenBank records have been identified as this species, including one from New Zealand sequenced from cultures grown from conidia of the asexual morph (GenBank EF029195, ICMP 14615). Another New Zealand specimen, sequenced from cultures grown from ascospores from apothecia of the sexual morph (GenBank MT002398, ICMP 23619 ex PDD 59986), is genetically similar. The four specimens have a 98 % match across the ITS region. The apothecial morphology of the New Zealand specimen PDD 59986 matches that described for this species by Abdullah & Webster (1981) and fits the sexual morphological concept of *Tricladiaceae* accepted here. This fungus is clearly not a *Lambertella* (a member of *Rutstroemiaceae*); the most informative name to use for this species for now is "*Helicodendron*" *tubulosum*.

Hymenoscyphus varicosporoides

Sequences from type material of *Hymenoscyphus varicosporoides* (GenBank NR_147422, CBS 651.66), *Hymenoscyphus jinggangensis* (GenBank KJ472293, HMAS 82036), and *Tricladium indica* (GenBank DQ202513, CBS 430.94), show that these are one and the same fungus (Fig. 2). All three have been reported from sexual as well as asexual morphs, *Cudoniella indica* being described as the sexual morph of the earlier named *T. indicum* (Webster et al. 1995).

Descriptions from Tubaki (1966), Webster et al. (1995), and Zhang & Zhuang (2002) show morphologically very similar fungi. Tubaki (1966) referred the asexual morph of his fungus to *Varicosporium* but the type species of this genus, *V. elodeae*, is phylogenetically distant, a member of the *Discinellaceae* (Johnston et al. 2019, as *Discinella-Pezoloma* lineage). The oldest name for this fungus is *Hymenoscyphus varicosporoides* but it is clearly not a *Hymenoscyphus* in the modern sense of this genus. This fungus may deserve to be placed in a new genus but generic relationships within the *Tricladiaceae* remain to be properly resolved, the feature-rich conidial morphology

often homoplastic and most taxa having only rDNA sequences available. The conidia of *H. varicosporoides* and *T. indica* are morphologically typical of *Tricladium*, so for now we provide a new combination in *Tricladium* for the oldest epithet of this fungus.

Tricladium varicosporoides (Tubaki) P.R. Johnst. & Baschien, **comb. nov.** MycoBank MB834832.

Basionym: *Hymenoscyphus varicosporoides* Tubaki, *Trans. Brit. Mycol. Soc.* **49**: 346. 1966.

Synonyms: *Tricladium indicum* Sati & N. Tiwari, *Mycol. Res.* **96**: 229. 1992.

Cudoniella indica J. Webster, Eicker & Spooner, *Nova Hedwigia* **60**: 496. 1995.

Hymenoscyphus jinggangensis Yan H. Zhang & W.Y. Zhuang, *Mycosystema* **21**: 494. 2002.

Sister to *T. varicosporoides* in Fig. 2 is a clade based on specimens reported as *Hymenoscyphus varicosporoides* by Sivichai et al. (2003) (GenBank accessions DQ202506, DQ202507, DQ202509) and the genome Hymvar from the JGI MycoCosm portal. These two sister clades are about 3 % divergent across the ITS region and probably represent different species. Sivichai et al. (2003) noted morphological differences between the conidia of specimens from Thailand they identified as *Hymenoscyphus varicosporoides* and the Webster et al. (1995) description of the South African *Cudoniella indica*. Fungi with apothecia morphologically similar to *H. varicosporoides* and conidia morphologically similar to *T. indicum* appear to be common around the world. A molecular comparison of large numbers of isolates is needed to resolve the phylogenetic, morphological, and biogeographic limits of these species.

DISCUSSION

Johnston et al. (2019) took a deliberately conservative approach to erecting new higher taxa within *Leotiomycetes*. This reflected a lack of DNA sequence data for the type species of many genera, a lack of sequence data from type specimens, uncertainty around identification of some of the specimens sampled, and a lack of supporting morphological study of taxa treated in the molecular phylogenetically defined clades. Thus, their concept of *Helotiaceae* included three monophyletic subclades. The core clade included several genera treated by Baral (in Jaklitsch et al. 2019) as *Helotiaceae* — *Bryoscyphus*, *Dicephalospora*, *Glarea* (represented by ex-type specimen of the type species), *Hymenoscyphus*, and *Phaeohelotium*. One of the basal subclades contained the morphologically divergent genera *Connersia* and *Pleuroascus* (traditionally placed in *Pseudeurotiaceae* because of their cleistothecial ascomata), and this clade has since been formally named *Pleuroascaceae* (Untereiner et al. 2019). A second basal subclade contained fungi with aquatic hyphomycete-like asexual spores in genera such as *Tricladium* and *Mycofalcella*, and it is this clade that is here formally named as *Tricladiaceae*. In addition to the genera treated in the multi-gene analysis of Johnston et al. (2019), this family contains the genera *Cudoniella*, *Geniculospora*, *Halenospora*, *Spirosphaera* and *Graddonia*. The phylogenetic position of *Filospora* within *Leotiomycetes* remains uncertain, because species placed in the genus are positioned in multiple family-level clades and sequence data from the type species are lacking.

Ecologically, the aquatic hyphomycete asexual morphs of *Tricladiaceae* have been reported from stream foam and aquatic leaf litter but also have been isolated as root endophytes, e.g., *Tricladium splendens* (Fisher & Petrini 1989) and *A. crassa* (Sati & Belwal 2005). Conidia of *T. splendens* and *Tricladium castaneicola* were reported from tree holes and from stemflow (Gönczöl & Révay 2003, 2006, Karamchand & Sridhar 2008). Other species such as *Helicodendron conglomeratum*, *Lambertella tubulosa*, and *Spirosphaera floriformis* have asexual morphs with helicoform conidia. These fungi with helicoform conidia differ both morphologically and ecologically from the aquatic hyphomycetes. They are known as the aero-aquatic hyphomycetes and unlike aquatic hyphomycetes, they thrive in standing or slow-moving waters, with rather poor access to oxygen and their conidia have air-trapping shapes, resistant against submerging, adapted to dispersal on the water surface (Baschien *et al.* 2006).

Several of the fungi within *Tricladiaceae* are known from their sexual morph. These have been referred variously to *Graddonia*, *Hymenoscyphus*, *Lambertella*, *Mollisia*, and *Pezoloma* spp. (e.g., Dennis 1955, Webster 1961, Abdullah *et al.* 1981, Abdullah & Webster 1981, Gminder 1993, Descals *et al.* 1998, Baral *et al.* 2013). Although the macromorphology and anatomy of the excipulum of the specimens with sessile apothecia often recalls *Mollisia*, the asci are more typical of *Helotiaceae* (the family to which *Hymenoscyphus* belongs) than *Mollisiaceae*. The asci have an hymenoscyphus-like amyloid pore (a thin-walled tube often confined to the inner half of the wall), whereas *Mollisia* has a calycina-like amyloid pore (the apical part of the tube thicker-walled and laterally flaring) (Baral *et al.* 2015, Johnston *et al.* 2014). Sometimes reported from culturing studies (e.g., Webster 1961), the sexual morph has also been found in nature on woody substrates at wet sites or in running water (Table 1, Abdullah *et al.* 1981, Descals *et al.* 1998). Typically the apothecia are white to pale yellow to orange, more or less glabrous, sessile to stipitate, attached to the substrate centrally, and often with a well-developed pad of dark-walled, gelatinous hyphae at the base of the apothecium, ectal excipulum non-gelatinous, cells thin-walled, usually globose to subglobose. The gelatinous tissue was observed at the base of the apothecia of several of the specimens from New Zealand (unpubl. data) and was also mentioned for *Anguillospora furtiva* apothecia by Descals *et al.* (1998). Of the sexual morph genera, only *Graddonia* belongs in *Tricladiaceae*. The other fungi, if they have a named asexual morph, are for now best referred to using the generic names that were based on their asexual morphs. As the taxonomy of *Tricladiaceae* becomes better resolved, some may require new generic names.

The analyses presented here help clarify the limits of *Solenopezaceae*. When erecting *Solenopezaceae*, Ekanayaka *et al.* (2019) included two phylogenetically distinct sets of genera, those that we propose here as *Tricladiaceae*, along with those that we accept as “core” *Solenopezaceae* — *Lasiobelonium*, *Solenopezia*, *Trichopeziza*, and *Trichopezizella* (Table 2). Morphologically, apothecia of the genera in *Solenopezaceae* differ from *Tricladiaceae* in having conspicuous, smooth-walled hairs and a calycina-like ascus apex, and morphologically complex asexual spores are not known for them (Baral in Jaklitsch *et al.* 2016). Note that the phylogeny that places *Solenopezia* with *Trichopeziza* and its relatives is based on ITS sequences only (Johnston *et al.* 2019) and that the type species *S. solenia* has hairs smooth to granulate (Seaver 1930, Raitviir *et al.* 1991).

Based on morphology, Raitviir *et al.* (1991) accept *Solenopezia* as a member of the subfamily *Trichopezizelloideae*, along with *Trichopeziza* and *Albotricha*. The species of *Albotricha* treated by Johnston *et al.* (2019) belong in the core *Lachnaceae* clade, sister to *Solenopezaceae* as accepted here, but the type species of the genus was not treated. Raitviir (1970) describes the hairs of *Albotricha* as smooth with loosely attached granules. The morphological limits of *Solenopezaceae* versus *Lachnaceae* remain to be definitively resolved.

The current generic taxonomy of “aquatic hyphomycetes” is strongly influenced by the conspicuous morphology of the asexual spores. It is now known that several of those spore types have evolved independently, not only across the *Leotiomycetes*, but across several different classes of *Ascomycota* and *Basidiomycota* (Belliveau & Bärlocher 2005, Baschien *et al.* 2006, Campbell *et al.* 2009, Tsui *et al.* 2016). Based on the DNA sequencing data available for these fungi within *Leotiomycetes*, there seems to be limited congruence between the asexual morphology and phylogeny. More intensive sampling of both taxa and genes is needed before a phylogenetically robust generic classification can be resolved for *Tricladiaceae*. For now, we take a conservative approach, genera with type species in *Tricladiaceae* are accepted as good genera, and species in large paraphyletic or polyphyletic genera such as *Tricladium*, *Helicodendron* and *Anguillospora* are retained in their existing genera.

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