




## Standard Paper

# A molecular-genetic reassessment of the circumscription of the lichen genus *Icmadophila*

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## Abstract

The circumscription of the lichenized ascomycete genus *Icmadophila* Trevis. in the family *Icmadophilaceae* Triebel was investigated. Sequences of the internal transcribed spacer (ITS) region and large subunit of nuclear ribosomal DNA (nuLSU) were generated for the five *Icmadophila* species and additional members of *Icmadophilaceae* from the genera *Dibaeis*, *Endocena*, *Knightiella*, *Siphula*, *Siphulella* and *Thamnolia*. Phylogenetic analyses indicate that three *Icmadophila* species are closely related: the type, *I. ericetorum* (L.) Zahlbr., which is widespread in Eurasia and North America, *I. aversa* (Nyl.) Rambold & Hertel from Central and South America, and *I. japonica* (Zahlbr.) Rambold & Hertel, which is restricted to Far East Russia and Japan. The genus *Knightiella* Müll. Arg. is reinstated to accommodate *I. splachnirima* (Hook.f. & Taylor) D. J. Galloway emend. L. Ludw., which occurs in New Zealand and Australia. Two further species of *Knightiella*, *K. eucalypti* (Kantvilas) Kantvilas and *K. queenslandica* Kantvilas, are found to be unrelated to *K. splachnirima*, and are accommodated in two newly described genera, *Knightiellastrum* and *Siphulopsis* respectively. *Knightiellastrum* L. Ludw. & Kantvilas is characterized by a squamulose, erhizinate, whitish to pale grey thallus with a green, coccoid photobiont and by containing thamnolic acid. *Siphulopsis* Kantvilas & A. R. Nilsen is similarly characterized by an erhizinate, whitish to pale grey thallus, with a green, coccoid photobiont and containing thamnolic acid, but is instead fruticose. This study reveals considerable diversity within Australasian *Icmadophilaceae*; ongoing work in the Southern Hemisphere and tropical regions may reveal additional species in this family and clarify the relationships of these newly described genera.

**Key words:** *Glossodium*, *Icmadophilaceae*, *Knightiella*, *Knightiellastrum eucalypti*, *Siphula decumbens* group, *Siphulopsis queenslandica*

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## Introduction

The lichen genus *Icmadophila* Trevis. has most recently included five species with a green-algal photobiont and a crustose or small-foliose thallus: *I. ericetorum* (L.) Zahlbr. (the type species), *I. aversa* (Nyl.) Rambold & Hertel, *I. japonica* (Zahlbr.) Rambold & Hertel, *I. splachnirima* (Hook.f. & Taylor) D. J. Galloway emend. L. Ludw. and *I. eucalypti* Kantvilas.

Together with the genera *Dibaeis* Clem., *Endocena* Cromb. including *Chirleja*, following Fryday *et al.* (2017), *Pseudobaeomyces* M. Satô, *Siphula* Fr., *Thamnolia* Ach. ex Schaer. and *Siphulella* Kantvilas *et al.*, *Icmadophila* is classified in the family *Icmadophilaceae* Triebel (Rambold *et al.* 1993; Tehler & Wedin 2008; Lendemer & Hodgkinson 2012; Jaklitsch *et al.* 2016).

Until 1993, *Icmadophila* contained the single species *I. ericetorum*, which has a Holarctic distribution but had also been reported from New Zealand (Galloway 1985, 2007), South

Africa (Drège 1843; Doidge 1950) and South America (Pereira *et al.* 2006). However, the presence of this species in New Zealand and South Africa is now considered highly doubtful (Ludwig 2015). It grows mainly in heathland and bogs on damp peaty soil, plant debris, bryophytes or rotting bark and wood, and is characterized by a pale green, granular-crustose thallus with pink, sessile to shortly stalked, biatorine apothecia. Rambold *et al.* (1993) synonymized *Glossodium* Nyl. with *Icmadophila*, and transferred *G. aversum* Nyl. and *G. japonicum* Zahlbr. into the genus on the basis of similarities in thallus morphology, secondary chemistry, substratum preference and ecology, as well as on spore and ascus characters. The main difference between *I. ericetorum* and *Glossodium* is the morphology of the ascomata, which are bisymmetrically tongue-shaped and distinctly stalked in the latter but discoid and usually sessile to subpedicillate in *I. ericetorum*. *Icmadophila aversa* is endemic to Central and South America (Wilk 2010) whereas *I. japonica* is known from Far East Russia and Japan (Ohmura 2011), where it is sympatric with *I. ericetorum*.

The Australasian endemic lichen, *Knightiella splachnirima* (Hook.f. & Taylor) Gyelnik (see Ludwig (2016) for detailed distribution), was transferred to *Icmadophila* by Galloway (2000), as

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had been previously suggested by Galloway (1992), Gierl & Kalb (1993) and Rambold *et al.* (1993). This view, however, was rejected by Stenroos *et al.* (2002), whose analysis of nuclear SSU rDNA sequences placed *K. splachnirima* in a sister relationship with all remaining members of *Icmadophilaceae*, considerably removed from *I. ericetorum*. Despite this, the genus *Knightiella* Müll. Arg. has been absent from subsequent checklists of *Icmadophilaceae* genera (e.g. Eriksson *et al.* 2003, 2004; Eriksson 2005; Lumbsch & Huhndorf 2007, 2010; Tehler & Wedin 2008; Lumbsch *et al.* 2011; Jaklitsch *et al.* 2016; Lücking *et al.* 2017), and the combination *I. splachnirima* has remained in use throughout much of the Australasian lichenological literature (e.g. Galloway 2007, 2008; Ludwig 2011; De Lange *et al.* 2012; Knight 2014; McCarthy 2016), although Kantvilas (2018) retained *K. splachnirima* in *Knightiella*. A sorediate and usually sterile form of *K. splachnirima* is considered to be a result of environmental plasticity rather than a separate taxon (Ludwig 2011, 2015).

A further Australasian species, *Icmadophila eucalypti*, was described from Tasmania by Kantvilas (in Lumbsch *et al.* 2011) and transferred to *Knightiella* by Kantvilas (2018) based on geographical and ecological factors, and on the small-squamulose thallus with an ecorticate, white lower surface that bears some resemblance to *K. splachnirima*. A third *Knightiella* species, *K. queenslandica* Kantvilas, has recently been described from Queensland, Australia, based on morphological similarities to *K. eucalypti* (Kantvilas) Kantvilas (Kantvilas 2018).

The present study explores the relationships between the five species of *Icmadophila* using a molecular phylogenetic approach. We aim to re-evaluate the circumscription of *Icmadophila* in relation to the genus *Glossodium* and the apparently Southern Hemisphere genus *Knightiella*. In addition, we aim to ascertain whether the rare, newly described *K. queenslandica* shows genetic affinities with other *Knightiella* species.

## Materials and Methods

### Specimens examined

Specimens of all five described *Icmadophila* species were used in the analysis, along with *K. queenslandica* and additional species of *Icmadophilaceae* representing all currently recognized genera. The only exception was the monotypic genus *Pseudobaeomyces* which was not represented in online nucleotide databases, and specimens were not available to us at the time of the study. Voucher details of study specimens are given in Table 1.

### DNA extraction, PCR and sequencing

DNA extraction was performed as described in Ludwig (2015) and Ludwig *et al.* (2017). Initially, a genomic DNA extract was obtained using a CTAB (cetyltrimethylammonium bromide) extraction protocol modified from Cubero *et al.* (1999) and Summerfield (2003). This method involved cell disruption through grinding with liquid N<sub>2</sub>, and cell lysis in a CTAB extraction buffer followed by one chloroform extraction. Based on the approach of Ye *et al.* (2004), the genomic DNA was purified using silica membrane spin columns (EconoSpin® All-In-One Mini Spin Columns, Epoch Life Sciences Inc., Missouri City, TX, USA).

We amplified the nuclear internal transcribed spacer (ITS) region (comprising ITS1, 5.8S rDNA and ITS2; c. 610 bp excluding insertions) as well as portions of the nuclear ribosomal large subunit (nuLSU; c. 890 bp excluding introns). Primer

combinations used in this study were: ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) or ITS4A (D. L. Taylor in Kroken & Taylor (2001)) for the ITS region; LR0R (Cubeta *et al.* 1991) and LR5 (modified from Vilgalys & Hester (1990), following Vilgalys Lab website: [https://sites.duke.edu/vilgalyslab/rdna\\_primers\\_for\\_fungi/](https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/)) or LR16 (Moncalvo *et al.* 1993) for nuLSU.

Two kinds of commercial ready-to-use PCR master mix were used: 1.1 × ReddyMix PCR Master Mix (1.5 mM MgCl<sub>2</sub>) (Thermo Fisher Scientific) and 1.1 × ReddyMix PCR Master Mix (2.0 mM MgCl<sub>2</sub>) (Thermo Fisher Scientific). The PCR reaction contained 1 × ReddyMix PCR Master Mix, 5 pmol of each primer and 10–200 ng genomic DNA. For specimens older than three years, failed amplifications were successfully repeated with the addition of 0.04 units/μl of 'FailSafe™ PCR Enzyme Mix' (Epicentre®) to the reaction. All reactions were prepared on ice and PCR was performed using the Eppendorf Mastercycler® or Eppendorf Mastercycler® gradient.

For amplification of the ITS region, the following cycling conditions were used: initial denaturation at 95 °C for 5 min; followed by 36 cycles consisting of denaturation at 94 °C for 1 min (or 45 s), annealing at 45 °C for 1 min (or 45 s) and extension at 72 °C for 1 min (or 45 s or 90 s); followed by a final extension at 72 °C for 10 min (or 5 min). Cycling conditions for nuLSU were: 95 °C for 5 min; followed by 36 cycles consisting of 94 °C for 1 min, 42 °C for 1 min and 72 °C for 80 s; followed by a final extension at 72 °C for 5 min.

PCR products were initially purified using the PureLink® PCR Purification Kit (Invitrogen, Life Technologies™, Carlsbad, CA, USA) and later using EconoSpin® columns. Sanger sequencing of purified PCR products was performed by the University of Otago Genetic Analysis Service using BigDye® Terminator v.3.1 Ready Reaction Cycle Sequencing Kit followed by capillary separation using the 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

### Sequence alignments and phylogenetic analyses

Electropherograms were assembled and edited using Geneious R7-11.1 (Biomatters Ltd, <http://www.geneious.com>). Sequence quality was assessed according to the guidelines of Nilsson *et al.* (2012). The newly generated rDNA sequences listed in Table 1 have been deposited in GenBank. Additional sequences from *Icmadophilaceae* were retrieved from GenBank. *Ochrolechia balcanica* Verseghe was used as the outgroup because *Ochrolechiaceae* R. C. Harris ex Lumbsch & I. Schmitt and *Icmadophilaceae* are both placed in the order *Pertusariales* M. Choisy ex D. Hawksw. & O. E. Erikss. (Tehler & Wedin 2008), based on earlier molecular phylogenetic analyses (Miadlikowska & Lutzoni 2004; Wedin *et al.* 2005; Miadlikowska *et al.* 2006; Lumbsch *et al.* 2007). Sequences from each locus were aligned using MAFFT v.7.388 (Kato & Standley 2013) and Geneious R7-11.1 and obvious misalignments were manually corrected. Large insertions between the ITS1F primer binding site and the end of the 18S subunit were removed before alignment from the following taxa: *Icmadophila ericetorum*, *Siphula decumbens* Nyl., *S. dissoluta* Nyl., *S. fastigata* (Nyl.) Nyl., *S. ceratites* (Wahlenb.) Fr. and *Thamnolia vermicularis* (Sw.) Schaer. The ITS and nuLSU alignments were concatenated yielding a length of 2134 bp.

The concatenated alignment was partitioned into the ITS and nuLSU regions. DNA substitution models for each region were determined by PartitionFinder2 using the 'greedy' algorithm

**Table 1.** Species information and sequences used in the current study. GenBank Accession numbers of newly generated sequences are in bold. Voucher information is given as herbarium code and herbarium voucher number if used, followed by (in quotes) collector and collector's reference if used, and in some cases the strain number.

Species	Locality and voucher information	References	GenBank Accession no.	
			ITS	nuLSU
<i>Dibaeis absoluta</i>	China, Hainan, HMAS-L 118073	Cao <i>et al.</i> (2013)	KC414626	–
<i>D. absoluta</i>	China, Hainan, HMAS-L 118071	Cao <i>et al.</i> (2013)	KC414625	–
<i>D. absoluta</i>	New Zealand, OTA 063977, 'Ludwig'	current publication	<b>KP759327</b>	–
<i>D. arcuata</i>	New Zealand, OTA 063978, 'Ludwig'	current publication	<b>KP759335</b>	<b>KP759338</b>
<i>D. baeomyces</i>	USA, Alaska, GZU 'Spribille 38948'	Spribille <i>et al.</i> (2014)	KJ462265	KJ462342
<i>D. baeomyces</i>	Canada, Quebec, DUKE 47587, AFTOL-ID 358	James <i>et al.</i> (2006)	DQ782844	–
<i>D. soreliata</i>	China, Hainan, HMAS-L 118090	Cao <i>et al.</i> (2013)	KC414627	–
<i>Endocena buckii</i>	Chile, NY, 'Buck 57033'	Lendemmer & Hodkinson (2012)	JX673914	–
<i>E. informis</i> var. <i>informis</i>	Falkland Islands, East Falkland, MSC, 'Fryday 10728'	Fryday <i>et al.</i> (2017)	KY495226	–
<i>E. informis</i> var. <i>falklandica</i>	Falkland Islands, Weddell Island, MSC, 'Fryday 10857'	Fryday <i>et al.</i> (2017)	KY495225	–
<i>Icmadophila aversa</i>	Bolivia, KRAM, 'Wilk 7685'	current publication	<b>KP759332</b>	<b>KP759343</b>
<i>I. ericetorum</i>	USA, Alaska, GZU, 'Spribille 36042'	Spribille <i>et al.</i> (2014)	KJ462267	KJ462344
<i>I. ericetorum</i>	Austria, OTA 061856, 'Bilovitz & Mayrhofer 19222'	current publication	<b>KP759337</b>	<b>KP759340</b>
<i>I. ericetorum</i>	Germany, OTA 063991, 'Ludwig'	current publication	–	<b>KP759320</b>
<i>I. japonica</i>	Japan, TNS YO 6762, 'Ohmura'	current publication	<b>KP759324</b>	<b>KP759321</b>
<i>Knightiella splachnirima</i>	New Zealand, OTA 062507, 'Ludwig'	current publication	<b>KP759346</b>	–
<i>K. splachnirima</i>	New Zealand, OTA 064242, 'Ludwig'	current publication	<b>MK408636</b>	<b>MK408633</b>
<i>K. splachnirima</i>	New Zealand, OTA 064245, 'Ludwig'	current publication	<b>MK408637</b>	<b>MK408634</b>
<i>Knightiellastrum eucalypti</i>	Tasmania, HO 568315, 'Kantvilas 6/13'	current publication	<b>MK408638</b>	<b>KP759344</b>
<i>K. eucalypti</i>	Tasmania, HO 559275, 'Kantvilas 270/10'	current publication	<b>KP759342</b>	–
<i>Ochrolechia balcanica</i>	Greece, ESS 20968, 'Schmitt'	Schmitt <i>et al.</i> (2001), Schmitt & Lumbsch (2004)	AF329172	AF329171
<i>Siphula ceratites</i>	Costa Rica, DUKE 47639, AFTOL-ID 849	Schmull <i>et al.</i> (2011), Miadlikowska <i>et al.</i> (2006)	HQ650642	DQ986775
<i>S. ceratites</i>	USA, Alaska, GZU, 'Spribille 38923'	Resl <i>et al.</i> (2015)	KR017095	KR017179
<i>S. decumbens</i>	New Zealand, OTA 062495, 'Ludwig'	current publication	<b>KP759323</b>	–
<i>S. decumbens</i>	New Zealand, OTA 063984, 'Ludwig'	current publication	<b>KP984796</b>	<b>KP759331</b>
<i>S. dissoluta</i>	New Zealand, OTA 062502, 'Webb & Lyttle'	current publication	<b>KP759334</b>	<b>KP984798</b>
<i>S. fastigiata</i>	New Zealand, OTA 062499, 'Knight'	current publication	<b>KP984797</b>	<b>KP984800</b>
<i>S. fastigiata</i>	Tasmania, HO 564725, 'Kantvilas 88/12'	current publication	<b>KP984795</b>	<b>KP759322</b>
<i>S. pickeringii</i>	Unknown origin, OSC F2F674BF	Platt & Spatafora (2000)	–	AF113727
<i>S. polyschides</i>	Unknown origin, OSC 39EC4EDE	Platt & Spatafora (2000)	–	AF113726
<i>Siphulella coralloidea</i>	Tasmania, HO 569181, 'Kantvilas 93/13'	current publication	<b>KP759330</b>	<b>KP759328</b>
<i>Siphulopsis queenslandica</i>	Australia, HO 575959, 'Kantvilas'	current publication	<b>MK408635</b>	<b>MK408632</b>
<i>Thamnolia subuliformis</i>	USA, Colorado/Alaska, DUKE 47604, AFTOL-ID 401	Schmull <i>et al.</i> (2011)	HQ650718	–
<i>T. subuliformis</i>	New Zealand, OTA 59464, 'Knight'	Lord <i>et al.</i> (2013)	AY961605	–
<i>T. vermicularis</i>	Peru, OTA 58855, 'Halloy'	Lord <i>et al.</i> (2013)	JQ409343	–
<i>T. vermicularis</i>	Russia, Altai Republic, GZU, 'Resl 1136'	Resl <i>et al.</i> (2015)	KR017094	–

(Guindon *et al.* 2010; Lanfear *et al.* 2017). Selected models were: SYM + G for the ITS region and GTR + I + G for nuLSU. Bayesian inference (BI) was carried out using Markov chain Monte Carlo (MCMC) sampling in MrBayes 3.2.1 (Ronquist & Huelsenbeck 2003). Four independent Markov chains were run for ten million

generations, sampling every 100 generations. The runs were determined to have converged when the average standard deviation of the split frequencies was less than 0.01. A Bayesian inference tree and the posterior probabilities (PP) were estimated from the samples after the first 25% of trees was discarded.

Maximum parsimony (MP) analysis was conducted using PAUP\* 4.0a142 (Swofford 1991). Of the 2134 characters in the alignment, 432 were informative and 1702 were uninformative and excluded from the analysis. The most parsimonious trees were found by 1000 replicate heuristic searches using the tree bisection-reconnections algorithm, saving 100 trees per replicate with a maximum of 10 000 trees. Branch support was determined by 1000 bootstrap replicates, each replicate comprising five heuristic searches, saving ten trees per replicate. The resulting bootstrap support (BS) values were transferred onto the Bayesian inference phylogeny. Strongly supported nodes were defined as those with BS  $\geq$  70% in the MP analysis and  $\geq$  0.95 PP support in the BI analysis.

Phylograms were visualized and rooted on the branch leading to the outgroup species *Ochrolechia balcanica* in FigTree v.1.4.2 (Rambaut 2014). The alignment and phylogenies are available on TreeBASE. Study Accession: <http://purl.org/phylo/treebase/phylogenies/study/TB2:S24007>.

## Results and Discussion

### Phylogenetic analyses

Bayesian inference and maximum parsimony analyses produced congruent topologies for the combined ITS + nuLSU phylogenetic analysis (Fig. 1) and indicated that *Icmadophila* as currently circumscribed is not monophyletic. The analyses provided consistent support for a core group that includes the type, *I. ericetorum*, together with *I. aversa* and *I. japonica* (PP = 1, BS = 99%). The sister relationship between *I. aversa* and *I. japonica* is strongly supported only by the Bayesian analysis (PP = 0.99). *Icmadophila* (hereafter *Knightiellastrum eucalypti*) and *Icmadophila* (hereafter *Knightiella splachnirima*) are distantly related to each other and to members of *Icmadophila* s. str. The new genus *Knightiellastrum* L. Ludw. & Kantvilas is therefore introduced below to accommodate *K. eucalypti*. *Knightiella splachnirima* has a well-supported sister relationship to all the remaining members of *Icmadophilaceae* (PP = 1, BS = 100%). *Knightiellastrum eucalypti* (Kantvilas) L. Ludw. & Kantvilas is sister to the large clade comprising *Icmadophila* s. str., *Endocena*, *Dibaeis*, *Siphulella* and *Siphula* s. lat. The latter is split into the *S. ceratites* group (*S. ceratites*, *S. pickeringii* Tuck. and *S. polyschides* Kremp.), which is closely related to *Icmadophila* s. str., and the *S. decumbens* group (*S. decumbens*, *S. dissoluta* and *S. fastigiata*), which is sister to *Siphulella coralloidea* Kantvilas. There is no support for a close relationship between *Knightiellastrum eucalypti* and the rare Australian species *Knightiella* (hereafter *Siphulopsis*) *queenslandica*. Consequently, the new genus *Siphulopsis* Kantvilas & A. R. Nilsen is introduced for the latter.

### The circumscription of *Icmadophila*

The main aim of our study was to re-evaluate the circumscription of the genus *Icmadophila*. Our results indicate that, of the five species that have been placed in *Icmadophila* at various times, only three are closely related: *I. ericetorum* (the type), which is widely distributed in Eurasia and North America, *I. aversa*, which occurs in high tropical mountain areas in Central and South America (Wilk 2010), and *I. japonica*, which is restricted to Far East Russia and Japan (Ohmura 2011). The last two species had been placed in the separate genus *Glossodium*. Although their apothecial morphology is distinctive, we found only partial

support for a sister relationship between these two species, so the degree to which *Glossodium* and *Icmadophila* are distinct remains unclear. In contrast, the clade consisting of *I. ericetorum*, *I. aversa* and *I. japonica* is strongly supported phylogenetically and provides a better basis for delimiting the genus *Icmadophila* as it also accommodates the morphological similarities between the three species noted by Rambold et al. (1993).

The distinctive Australasian species *Knightiella splachnirima* is distantly related to *I. ericetorum*, having a sister relationship to all other *Icmadophilaceae*, as previously indicated by the nuclear SSU phylogeny of Stenroos et al. (2002) and by Kantvilas (2018). Therefore, the monophyletic genus *Knightiella* based on *K. splachnirima* is supported for this taxon, as previously suggested.

The Tasmanian endemic *Knightiellastrum eucalypti*, which was provisionally ascribed to *Icmadophila* by Kantvilas (in Lumbsch et al. 2011: 73) and then to *Knightiella* by Kantvilas (2018), is neither closely related to *I. ericetorum* nor to any other species studied here. In our analysis this taxon is distinctly different and sister to all other genera in the family apart from *Thamnia* and *Knightiella*. The new genus *Knightiellastrum* is described below for this taxon.

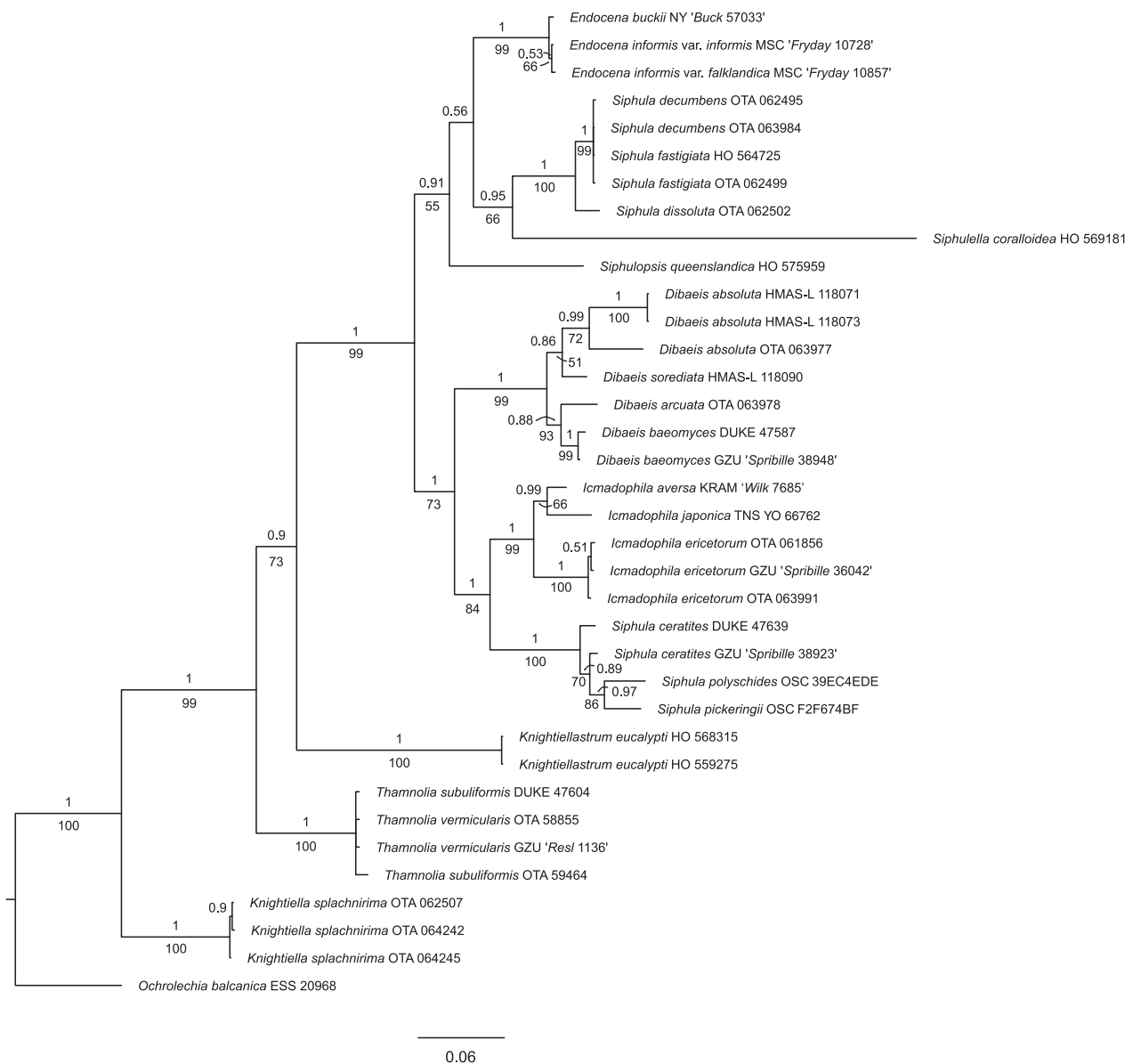
### Relationships among the remaining genera of *Icmadophilaceae*

Although our study was focused on *Icmadophila*, our analyses add to a growing understanding of relationships among genera within the family *Icmadophilaceae*, as well as its diversity in the Southern Hemisphere. Consistent with the topologies of the nuLSU phylogeny by Grube & Kantvilas (2006) and the nuSSU analysis of Stenroos et al. (2002), our analyses recovered two distinct clades of *Siphula*, with the *S. decumbens* group being separate from the type species of *Siphula*, *S. ceratites*. This, together with reports of ascomata in *S. decumbens* and *S. fastigiata*, indicates that the *S. decumbens* group might warrant a genus of its own (Stenroos et al. 2002; Ludwig 2015; Ludwig et al. 2016). Such a decision cannot be made, however, without studies of further species of *Siphula* s. lat., particularly those from the Neotropics (Kantvilas & Elix 2002).

We also found a sister relationship between *Endocena* and the *S. decumbens* group, as previously suggested by the nuSSU analyses of Stenroos et al. (2002) and Fryday et al. (2017), and a distinct *Dibaeis* clade, concurring with Kantvilas (2018) and references therein.

The placement of *Siphulella coralloidea* in *Icmadophilaceae* by Rambold et al. (1993) is supported here, with partial support (PP = 0.95) for a sister relationship with the *Siphula decumbens* group. However, even if this sister relationship receives further support in the future, this would not necessarily justify an inclusion of *S. coralloidea* in the *S. decumbens* group. *Siphulella coralloidea* has a highly distinctive chemistry (Kantvilas et al. 1992) and our analyses also indicate it is highly distinctive genetically, as evidenced by its terminal branch length.

*Siphulopsis queenslandica* (Kantvilas) Kantvilas & A. R. Nilsen, previously placed in *Knightiella* by Kantvilas (2018), is another clearly distinct Australian entity, with partial support (PP = 0.91) in our analysis for a sister relationship with a clade containing the *Siphula decumbens* group, *Siphulella* and *Endocena* but distant from *Knightiella* and *Knightiellastrum*. To incorporate this species into a much more broadly circumscribed genus including *Endocena*, *Siphula pro parte* and *Siphulella* would be at odds with the genetic and morphological distinctiveness of these genera. Therefore, the new genus *Siphulopsis*, described



**Fig. 1.** *Icmadophilaceae* phylogeny showing the positions of *Icmadophila*, *Knightiella*, *Knightiellastrum* and *Siphulopsis* species based on Bayesian inference analysis of the concatenated alignment (ITS + nuLSU). Bayesian posterior probability (PP) branch support values are given above the line and bootstrap (BS) values based on maximum parsimony are below the line. Collections are labelled with herbarium code and voucher number. When the herbarium voucher was unavailable, the collector's voucher was used instead (in quotation marks). Information for sequences used is in Table 1. Scale bar = nucleotide substitutions per site.

below, is proposed for this distinctive species. Additional, still unidentified, recent collections from tropical Queensland and the Caribbean may prove to represent related taxa.

Lichen diversity is significantly underestimated worldwide, in part due to a paucity of taxonomists (Lücking *et al.* 2014), but also due to taxonomic classifications that mask local and regional diversification (Lumbsch *et al.* 2011). Our analyses, which have led us to propose two new monotypic genera within the family, suggest that previous taxonomic arrangements have underestimated the diversity and distinctiveness of *Icmadophilaceae* in Australasia. While the taxonomic redundancy inherent in monotypic genera is undesirable, we believe that the alternative, amalgamating several distinctive genera within the family, is not helpful. Ongoing work, particularly in the Southern Hemisphere and tropical regions, may reveal additional species in the family

*Icmadophilaceae*, enabling clarification of relationships among the genera in our phylogenetic framework.

### Taxonomic Treatment

#### *Knightiellastrum* L. Ludw. & Kantvilas gen. nov.

Mycobank No.: MB 833780

Thallum parvum squamulosum sterilemque, rhizinas destitutum, acidum thamnolicum continentem praebens, ergo characteres aliquam *Knightiellae Icmadophilaeque* ostendens, sed his generibus genetice non affinis demonstratum.

Typus generis: *Knightiellastrum eucalypti* (Kantvilas) L. Ludw. & Kantvilas.

***Knightsiellastrum eucalypti* (Kantvilas) L. Ludw. & Kantvilas comb. nov.**

Mycobank No.: MB 833781

*Knightsiella eucalypti* (Kantvilas) Kantvilas, *Herzogia* **31**, 567 (2018).—*Icmadophila eucalypti* Kantvilas, *Phytotaxa* **18**, 72 (2011); type: Australia, Tasmania, Hartz Road near the entrance to the National Park, 43°12'S, 146°47'E, 570 m, on moist trunks of old *Eucalyptus obliqua* in mixed forest, 25 July 2007, G. Kantvilas 285/07 (HO—holotypus!; BM!— isotypus).

*Thallus* squamulose, whitish to pale grey, erhizinate, in section with a pseudocortex 20–30 µm thick comprising randomly orientated, short-celled hyphae 3–5 µm wide, interspersed with occasional dead algal cells; lower surface white, ecorticate. *Photobiont* a unicellular green alga with globose cells 5–11 µm diam.

*Ascomata* and *pycnidia* not seen.

**Secondary chemistry.** Thamnolic acid.

**Etymology.** From *Knightsiella* and the Latin suffix ‘-astrum’ indicating incomplete resemblance, because the thallus morphology of the type species is reminiscent of a small and infertile individual of *Knightsiella splachnirima*.

**Notes.** The new genus comprises a single species that occurs on the soft, rotting wood or bark of mature trees in the wet forests of Tasmania. In the absence of reproductive or molecular characters, the initial placement of this lichen in *Icmadophila* was based entirely on morphological, anatomical, ecological and chemical evidence (Lumbsch et al. 2011). A further character was the occasional occurrence of pinkish gall-like thickenings of unknown origin that resemble apothecial initials of *Icmadophilaceae* taxa such as *Dibaeis* and *Siphulella*. Subsequently, Kantvilas (2018) transferred it to *Knightsiella*, pending supporting molecular data but recognizing that it displayed closer morphological affinities to *Knightsiella* than to *Icmadophila*. The new molecular data confirm the original family classification of this species, but also highlight that its differences from the other genera are sufficient to warrant generic status. An image of *Knightsiella eucalypti* was published in Kantvilas (2018).

***Siphulopsis Kantvilas & A. R. Nilsen gen. nov.***

Mycobank No.: MB 833782

*Siphulae* Fr. thallo fruticoso, acidum thamnolicum continenti aliquam similis sed rhizinas destituto et huic generi genetice non affino differt.

Typus generis: *Siphulopsis queenslandica* (Kantvilas) Kantvilas & A. R. Nilsen.

***Siphulopsis queenslandica* (Kantvilas) Kantvilas & A. R. Nilsen comb. nov.**

Mycobank No.: MB 833795

*Knightsiella queenslandica* Kantvilas, *Herzogia* **31**, 567 (2018); type: Australia, Queensland, D’Aguilar Range, Westridge outlook, 27°21’48’’S, 152°45’35’’E, 510 m, on the butt of an old, partially charred eucalypt in open forest, 13 November 2014, G. Kantvilas 460/14 (HO—holotypus!; BM!, BRI!— isotypi).

*Thallus* at first squamulose, soon becoming fruticose and forming pulvinate clumps, whitish to pale ashen grey, erhizinate, in section

with a pseudocortex 20–30 µm thick comprising poorly differentiated, short-celled hyphae c. 5 µm wide, interspersed with occasional dead algal cells. *Photobiont* a unicellular green alga with globose cells 6–10 µm diam.

*Ascomata* not seen.

*Pycnidia* immersed; *conidia* bacilliform.

**Secondary chemistry.** Thamnolic acid.

**Etymology.** From *Siphula* and the Greek suffix ‘-opsis’ indicating resemblance, because the thallus morphology of the type species is reminiscent of a species of that genus.

**Notes.** The new genus comprises a single species, *Siphulopsis queenslandica*, which is described, discussed and illustrated by Kantvilas (2018). At first glance, it resembles a species of *Siphula* in its whitish, fruticose lobes that contain thamnolic acid, a commonly occurring metabolite in that genus. However, it differs from *Siphula* chiefly by lacking the basal rhizines characteristic of the genus. Consequently it was described, with some hesitation, as a species of *Knightsiella*, seen at the time as a classification of ‘best fit’ and on account of some morphological similarities with *Knightsiella* (now *Knightsiellastrum eucalypti*).

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