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Species recognition and phylogeny of *Thelotrema* species in Australia (Ostropales, Ascomycota)

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This paper is dedicated to our colleague and friend Ana Crespo de las Casas (Madrid), on the occasion of her 60th birthday.

Abstract. Species circumscriptions based on morphological data are difficult in crustose lichens with limited characters as they often show remarkable variability. An example is the genus *Thelotrema* s.str., a speciose genus of mostly tropical lichens. Morphological studies on Australian *Thelotrema* spp. were accompanied by a phylogenetic analysis of mt SSU rDNA sequence data of 19 species, including 25 newly obtained sequences. We performed maximum parsimony and Bayesian phylogenetic analyses of 50 samples, representing 25 species. Our results indicate that more species need to be accepted in *Thelotrema* than previously thought. Subtle morphological differences were found to be associated with independent lineages in the phylogenetic trees. Furthermore, monophyly of *Thelotrema* s.str. is strongly supported. On the basis of the corroboration of morphological evidence by molecular data, the new species *Thelotrema capetribulense* Mangold, *T. crespoae* Mangold, Lumbsch & Elix, *T. oleosum* Mangold, and *T. pseudosubtile* Mangold are described. The new combinations *Chapsa phlyctidioides* (Müll.Arg.) Mangold and *Thelotrema defossum* (Müll.Arg.) Mangold are proposed.

Introduction

The recognition and circumscription of species as the fundamental evolutionary unit is one of the most important tasks for systematic biologists (Mayr 1963; Cracraft 1983; Wiens and Penkrot 2002). However, distinguishing informative characters for species recognition from intraspecific variability is a major challenge. This is especially true for groups of organisms such as fungi, where it is often difficult to obtain sufficient morphological data or where plasticity of simple characters sometimes makes it almost impossible to decide whether a difference in a character is due to environmental modifications or whether it is genetically based. As a consequence, it is notoriously difficult to circumscribe species on a morphological basis for some lichenised fungi. Since the middle of the last century, chemical differences were employed to distinguish morphologically indistinguishable species and this controversial practice has been the subject of much discussion (see reviews by Hawksworth 1976; Brodo 1978, 1986; Culberson 1986; Egan 1986; Rogers 1989; Lumbsch 1998*a*, 1998*b*). In some cases molecular data have shown that chemical differences may be better indicators for species recognition than morphology. However, there is no *a priori* way of deciding whether chemical characters are sufficient to distinguish morphologically identical or very similar species. In some genera, such as *Porpidia*, chemotypes did not form monophyletic groups (Buschbom and Mueller 2006), whereas the opposite was shown for *Parmeliopsis* (Tehler and Källersjö 2001), *Ramalina* (LaGreca 1999), *Letharia* (Kroken and Taylor 2001), *Haematomma* (Lumbsch *et al*. 2008) and *Heterodermia* (Lücking *et al*. 2008). Further, detailed morphological and anatomical studies revealed subtle, but distinct differences in taxa that were previously believed to represent single variable species. In these cases molecular data were helpful in corroborating morphological results or in stimulating re-investigations. This included cortical anatomy in *Physconia* (Cubero *et al*. 2004; Divakar *et al*. 2007), thallus surface ultrastructure in *Parmelina* (Arguello *et al*. 2007) and subtle characters in thallus morphology and anatomy in *Parmelia* (Molina *et al*. 2004; Divakar *et al*. 2005), *Sphaerophorus* (Högnabba and Wedin 2003) and *Usnea* (Wirtz *et al*. 2008).

In a continuation of our recent studies of thelotremoid Graphidaceae in Australia (Mangold *et al*. 2006, 2007*a*, 2007*b*, 2008; Lumbsch and Mangold 2007), we turned our attention to the genus *Thelotrema.* The genus was first described by Acharius (1803), with *T. lepadinum* as the type and most widespread and common species. After some earlier refinements, the genus was widely accepted to accommodate

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species with uncarbonised ascomata and lateral paraphyses (Salisbury 1972; Hale 1980, 1981). Subsequently, several aberrant species were excluded and placed in other genera, including *Topeliopsis* (Kantvilas and Vězda 2000), *Reimnitzia* (Kalb 2001) and *Chapsa* (Frisch *et al*. 2006). The latter authors (Frisch *et al*. 2006) only accepted species of the *T. lepadinum*-group (= *Thelotrema* s.str.) in this genus, species which are characterised by perithecioid to apothecioid ascomata, uncarbonised and ± free proper exciple, and lateral paraphyses.

When revising the Australian species of *Thelotrema*, it became evident that some taxa showed remarkable variability. Our morphological studies suggested that more species were involved that could be distinguished by a combination of minor morphological and anatomical characters. To address the issue of species circumscription based on a combination of minor morphological characters, we obtained partial DNA sequences of mt SSU of 19 *Thelotrema* species and performed maximum parsimony and Bayesian analyses of these data.

Materials and methods

Specimens of the herbaria B, BRI, CANB, F, GZU, NSW, S, UPS and US, and the private collection of K. Kalb (Neumarkt/Opf.) were examined.

Chemical methods

Secondary compounds were identified by thin-layer chromatography (TLC) according to the methods standardised for lichen products (Culberson 1972), and by high-performance liquid chromatography (HPLC) (Feige *et al*. 1993) coupled with a photodiode array detector.

Molecular methods

Data matrices of 43 sequences of 19 *Thelotrema* species were assembled by using sequences of mitochondrial small-subunit rDNA sequences (Table 1). We also included seven sequences of six *Chapsa* species as an out-group since the genus was sister-group to *Thelotrema* s.str. in a recent study (Mangold *et al*. 2008).

Total DNA was extracted from freshly collected material or herbarium specimens with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) or E.Z.N.A. Fungal MiniPrep Kit (Omega-Biotech, Victoria, Canada), following the instructions of the manufacturer. Dilutions (10^{-1} up to 10^{-2}) of DNA were used for PCR amplifications. Primers for amplification were mr SSU1 (Zoller *et al*. 1999) and MSU 7 (Zhou and Stanosz 2001). The 25-μL PCR reactions contained 2.5 μL buffer, 2.5 μL dNTP mix, 1 μL of each primer (10 μM), 5 μL BSA, 2 μL Taq, 2 μL genomic DNA extract and 9 μL distilled water. Thermal cycling parameters were as follows: initial denaturation for 3 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 52°C, 1 min at 73°C and a final elongation for 7 min at 73°C. Amplification products were viewed on 1% agarose gels stained with ethidium bromide and subsequently purified with the QIAquick PCR Purification Kit (Qiagen) or Nucleo Spin DNA purification kit (Macherey-Nagel, Düren, Germany). Fragments were sequenced by using the Big Dye Terminator reaction kit (ABI PRISM, Applied Biosystems, Foster City, CA, US). Sequencing and PCR amplifications were performed with the same sets of primers. Cycle sequencing was executed with the following program: 25 cycles of 95°C for 30 s, 48°C for 15 s and 60°C for 4 min. Sequenced products were precipitated with 10 μL of sterile dH_2O, 2 μL of 3 M Napa and 50 μL of 95% EtOH before they were loaded on an ABI 3100 (Applied Biosystems) automatic sequencer. Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR, Madison, WI, US) and manually adjusted.

Sequence alignments and phylogenetic analysis

Alignments were done with Clustal W (Thompson *et al*. 1994). Ambiguously aligned regions were delimited manually. The alignments were analysed by maximum parsimony (MP) and a Bayesian approach (B/MCMC).

Maximum parsimony analyses were performed by using the program PAUP* (Swofford 1993). Heuristic searches with 1000 random taxon-addition replicates were conducted with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping (Felsenstein 1985) was performed on the basis of 2000 replicates with random sequence additions. The B/MCMC analyses were conducted by using the MrBayes 3.1.1 program (Huelsenbeck and Ronquist 2001). The analyses were performed assuming the general time reversible model of nucleotide substitution (Rodriguez *et al*. 1990) including estimation of invariant sites, assuming a discrete gamma distribution with six rate categories. A run with 20 000 000 generations, starting with a random tree and employing four simultaneous chains, was executed. Every 100th tree was saved into a file. The first 1 000 000 generations (i.e. the first 10 000 trees) were deleted as the 'burn in' of the chain. We plotted the log-likelihood scores of sample points against generation time with TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) to ensure that stationarity was achieved after the first 1 000 000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist 2001). Of the remaining 380 000 trees (190 000 from each of the parallel runs), a majority-rule consensus tree with average branch lengths was calculated by using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal or above 75% under MP and posterior probabilities ≥ 0.95 were considered as strongly supported. Phylogenetic trees were visualised with the program Treeview (Page 1996).

Results and discussion

Twenty-five new sequences were generated in the present study and aligned with sequences obtained from GenBank as listed in Table 1. A matrix of 698 unambiguously aligned nucleotide position characters was produced; 484 characters in the alignment were constant. Maximum parsimony analysis yielded 288 most parsimonious trees (438 steps long). The strict consensus tree did not contradict the Bayesian tree topology. In the B/MCMC analysis, the likelihood parameters in the sample had the following mean (Variance): LnL = −3552.639 (0.32), base frequencies $\pi(A) = 0.334$ (0.00081), $\pi(C) = 0.157$ (0.00076), $\pi(G) = 0.202$ (0.00076), $\pi(T) = 0.308$ (0.0008), the gamma shape parameter $\alpha = 0.609$ (0.0017) and p(invar) = 0.371 (0.00052).

Table 1. Species and specimens used in the current study with GenBank accession numbers

Species	Sample	mt SSU
Chapsa astroidea		AY300917
Chapsa indica		DQ384911
Chapsa leprocarpa	Australia, Queensland, *Lumbsch 19125 k & Mangold* (F)	EU675274
Chapsa niveocarpa		EU075567
Chapsa phlyctidioides 1		EU075569
Chapsa phlyctidioides 2	Australia, Queensland, *Mangold 39 ze* (F)	EU675275
Chapsa pulchra		EU075571
Thelotrema capetribulense 1	Australia, Queensland, *Lumbsch 19160 q & Mangold* (F)	EU675276
Thelotrema capetribulense 2	Australia, Queensland, *Lumbsch 19161 xA & Mangold* (F)	EU675277
Thelotrema capetribulense 3	Australia, Queensland, *Lumbsch 19162 I & Mangold* (F)	EU675278
Thelotrema conveniens	Australia, Queensland, *Mangold 29af* (F)	EU675279
Thelotrema crespoae		EU075606
Thelotrema defossum	Australia, Queensland, *Lumbsch 19161 v & Mangold* (F)	EU675280
Thelotrema diplotrema 1		EU075599
Thelotrema diplotrema 2	Australia, Queensland, *Lumbsch 19161 r & Mangold* (F)	EU675281
Thelotrema diplotrema 3	Australia, Queensland, *Lumbsch 19139 r & Mangold* (F)	EU675282
Thelotrema gallowayanum		EU075600
Thelotrema lepadinum 1		DQ431957
Thelotrema lepadinum 2		DQ384921
Thelotrema lepadinum 3		DQ384922
Thelotrema lepadinum 4		DQ972997
Thelotrema lepadinum 5	Australia, Victoria, *Mangold 1 a* (F)	EU675283
Thelotrema lepadinum 6		AY300916
Thelotrema lepadodes	Australia, Queensland, *Lumbsch 19158 k & Mangold* (F)	EU675284
Thelotrema monosporum 1		EU075601
Thelotrema monosporum 2	Australia, Queensland, *Lumbsch 19158 v & Mangold* (F)	EU675285
Thelotrema monosporum 3		EU075596
Thelotrema monosporum 4		DQ384919
Thelotrema nureliyum 1	Australia, Queensland, *Lumbsch 19174 x & Mangold* (F)	EU675286
Thelotrema nureliyum 2		EU075604
Thelotrema nureliyum 3	Australia, Queensland, *Lumbsch 19174 I & Mangold* (F)	EU675287
Thelotrema nureliyum 4		EU075597
Thelotrema nureliyum 5	Australia, Queensland, *Lumbsch 19100 k & Mangold* (F)	EU675288
Thelotrema nureliyum 6		EU075603
Thelotrema oleosum	Australia, New South Wales, *Mangold 25 m* (F)	EU675289
Thelotrema pachysporum 1		DQ384918
Thelotrema pachysporum 2	Australia, Queensland, *Lumbsch 19162 j & Mangold* (F)	EU675290
Thelotrema porinaceum	Australia, Queensland, *Lumbsch 19156 days & Mangold* (F)	EU675291
Thelotrema porinoides 1		DQ384920
Thelotrema porinoides 2	Australia, Queensland, *Lumbsch 19137 i & Mangold* (F)	EU675292
Thelotrema pseudosubtile	Australia, Queensland, *Lumbsch 19117 k & Mangold* (F)	EU675293
Thelotrema rugatulum		EU075605
Thelotrema saxatile 1	Australia, Queensland, *Lumbsch 19104 a & Mangold* (F)	EU675294
Thelotrema saxatile 2	USA, Lendemer 2149 (PH)	EU675295
Thelotrema saxatile 3	Australia, Queensland, *Mangold 32 v* (F)	EU675296
Thelotrema subtile 1		DQ871020
Thelotrema subtile 2		EU075607
Thelotrema subtile 3	Australia, Victoria, *Mangold 3 e* (F)	EU675297
Thelotrema suecicum 1		AY300917
Thelotrema suecicum 2	Australia, Victoria, *Mangold 5 f* (F)	EU675298

Since the topologies of the MP and B/MCMC analyses did not show any strongly supported conflicts, only the 50% majority-rule consensus tree of Bayesian tree sampling is shown. Those nodes that received strong support (i.e. PP $\geq$ 0.95 in B/MCMC analysis and MP bootstrap $\geq$70%) in both the MP and Bayesian analyses are in bold as shown in Fig. 1. In the majority-rule consensus tree, *Thelotrema* s.str. is monophyletic as in Mangold *et al*. (2008). The backbone of the phylogeny within *Thelotrema* is not resolved with confidence, with the single exception of a placement of a group of five species (*T. conveniens*, *T. crespoae*, *T. gallowayanum*, *T. oleosum*, *T. porinaceum*) at the base. All these species have perithecioid—at least in younger stages—to urceolate apothecia and large ascospores. They

Fig. 1. Phylogeny of *Thelotrema* based on partial mt SSU rDNA sequences. This is a majority-rule consensus tree based on a B/MCMC tree sampling procedure. Branches with posterior probabilities equal or above 0.95 and MP-bootstrap values equal or above 70% are in bold.

either lack secondary metabolites or contain the norstictic acid chemosyndrome (Mangold *et al*. 2007*a*). The relationships among these basal species are not clear, with the exception of a strongly supported sister-group relationship between *T. oleosum* and *T. porinaceum* (Clade A). Within the remaining group of *Thelotrema* species, six well supported clades (Clades B–G) can be distinguished; the relationships among these clades do not receive strong support and hence are uncertain. The new species, *T. capetribulense*, forms a well supported sister-group with *T. porinoides* (Clade B). These two species have emergent ascomata with a free proper exciple, transversely septate, hyaline, thick-walled, amyloid ascospores and contain the stictic acid chemosyndrome. Three samples of *T. diplotrema* cluster together (Clade C); however, the relationships of this species remain unresolved, which is also true for the relationships of *T. nureliyum* (Clade D) and *T. subtile* (Clade F). These species belong to a group of morphologically similar species that are difficult to separate. *T. diplotrema* and *T. defossum*, *T. lepadinum*, *T. nureliyum*, *T. pseudosubtile*, *T. rugatulum*, *T. subtile* and *T. suecicum* share a similar thallus morphology, lack secondary metabolites and have hyaline, thick-walled ascospores. *T. lepadinum* and *T. rugatulum* differ in having muriform ascospores, whereas all the other species have transversely septate ascospores and can be distinguished by a combination of morphological characters (Table 2). Within Clade E, five species cluster together. *T. lepadinum* has a well supported sister-group relationship with *T. suecicum* and these two species are strongly supported as sister to *T. rugatulum.* These three species are related to an unsupported sister-group, comprising *T. defossum* and *T. pseudosubtile.* The distinction of *T. subtile* and *T. suecicum*, which were treated as synonyms (Salisbury 1972) and discussed by Purvis *et al*. (1995), is supported in our phylogenetic study. Clade G includes four species that lack secondary metabolites and have brown ascospores (at least when mature). One species has transversely septate ascospores, although eumuriform ascospores are more common in this clade. This group includes some species (e.g. *T. monosporum* and *T. saxatile*) that are morphologically similar and, because of different concepts used in their circumscription, there has been considerable confusion in the application of these names (Frisch *et al*. 2006, see discussion under taxonomic consequences). *T. monosporum* forms a well supported sister-group with *T. lepadodes*; however, with a single sequence of *T. monosporum* nested within *T. pachysporum*, this position lacks support. The *T. monosporum* 4 sequence was downloaded from GenBank and may come from a sample that included *T. pachysporum* according to our species circumscription. Three samples of *T. saxatile* cluster together, but the relationships of this species within clade G remain uncertain.

The present study provides a further example where molecular data have assisted in identifying diagnostic morphological characters for distinguishing similar species. The circumscription of two of the species groups within *Thelotrema* included in this study (*T. monosporum* and *T. subtile* and related taxa) were previously very unclear. Different opinions have been expressed regarding the number of species in these groups in the literature. In both cases, molecular data supported the distinction of species on the basis of a combination of subtle morphological and anatomical characters, indicating that cryptic species (previously reported from foliose and fruticose lichens (Kroken and Taylor 2001; Högnabba and Wedin 2003; Cubero *et al*. 2004; Molina *et al*. 2004; Divakar *et al*. 2005, 2007; Arguello *et al*. 2007; Wirtz *et al*. 2008)) also occur in groups of crustose lichens.

Taxonomic consequences

Full descriptions of all the species treated here and additional *Thelotrema* spp. occurring in Australia will be published elsewhere. Here we focus on describing the four new species of *Thelotrema* which were shown to be independent by our molecular and morphological studies. In addition, one species of *Chapsa* and one of *Thelotrema* included in the present study are formally transferred to the genus. The *Chapsa* species has formerly been treated in *Thelotrema*, but according to the new generic concept of Frisch *et al*. (2006), had to be transferred to *Chapsa* because of its chroodiscoid apothecia. The *Thelotrema* species was previously included in *Ocellularia* because of its transversely septate ascospores; however, its excipular characters are consistent with those of *Thelotrema* so it is combined into this genus here.

In addition to the description of four new species, four additional *Thelotrema* species are reported from Australia for the first time, including *T. conveniens*, *T. defossum*, *T. nureliyum* and *T. rugatulum.*

Since there has been considerable confusion in the application of the names *T. lepadodes*, *T. monosporum* and *T. saxatile*, we re-exmined the type material of these taxa and consider them to represent three distinct species. Within this species complex, *T. lepadodes* is characterised by moderately large, muriform, indistinctly brownish, thick-walled, non- to faintly amyloid ascospores and 8-spored asci, whereas *T. monosporum* has moderately large, densely muriform, brown, thin-walled, non- or faintly amyloid ascospores and 1–4-spored asci. *T. saxatile* is characterised by large, densely muriform, brown, thick-walled (when immature) to thin-walled (when mature), non-amyloid ascospores and 1–(2)-spored asci. A more detailed discussion of these species will be presented elsewhere.

Chapsa phlyctidioides (Müll.Arg.) Mangold, *comb. nov.*

Bas.: *Ocellularia phlyctidioides* Müll.Arg., Hedwigia 32: 130 (1893). *Thelotrema phlyctidioides* (Müll.Arg.) Hale, Mycotaxon 11: 132 (1980).

Type: Australia, Queensland, Brisbane, *Bailey 354* (*holo:* G!).
Mycobank no. MB 511756.

Thelotrema defossum (Müll.Arg.) Mangold, *comb. nov.*

Bas.: *Ocellularia defossa* Müll. Arg. in Engler, Botan. Jahrbücher 5: 138 (1884).

Type: Indonesia, Timor, Mount Taimanani, 1883, *Naumann 386* pr. p. (*holo:* G!).
Mycobank no. MB 511757.

Thelotrema capetribulense Mangold, *sp. nov.* (Fig. 2*A*, *E*)

A *Thelotrema porinoides* thallis crassisibus et ascosporis parvibus differt.

Table 2. Characteristics and distribution of *Thelotrema* species morphologically similar to *T. diplotrema* and *T. subtile*

Characteristics and distribution	*T. suecicum*	*T. subtile*	*T. defossum*	*T. pseudosubtile*	*T. diplotrema*	*T. nureliyum*
Distribution in Australia	Subtropical to temperate (NSW, Vic., Tas.)	Cool-temperate (Vic., Tas.)	Tropical and subtropical (Qld, NSW)	Tropical and subtropical (Qld, NSW)	Tropical and subtropical (Qld, NSW)	Tropical and subtropical (Qld, NSW)
Ascomata position and size	±Emergent, up to 0.7 mm in diam.	±Emergent, up to 0.6 mm in diam.	Immersed, up to 0.3 mm in diam.	Immersed to slightly emergent, up to 0.3 (0.6) mm in diam.	Immersed to emergent, up to 0.4–(0.7) mm in diam.	Emergent, up to 1.2 mm in diam.
Ascospores						
Cell wall	Very thick	Moderately thick	Thin	Moderately thick to thick	Thick	Thick to very thick
Halo	Present, thin to thick, smooth	Present, thin, smooth to often crenate	Present, thick, smooth to crenate	Present, thin, smooth to often crenate	Present, thin, smooth	Absent
Amyloid reaction	Weak or none	Weak to strong	Weak	Weak to strong	Strong	Weak to strong
Pigmentation	Hyaline	Hyaline, brown in over-mature stages	Hyaline	Hyaline	Hyaline	Hyaline, yellowish in over-mature stages
Form	Ellipsoid to broadly fusiform (broadly clavate)	Clavate (oblong to fusiform)	Clavate (oblong to fusiform)	Clavate (oblong to fusiform)	Fusiform (clavate)	Cylindrical to fusiform
Loci	Irregular, roundish to angular, subglobose to cuboid	Regular, roundish to angular, lentiform (subglobose)	Regular, angular, lentiform to cuboid	Irregular, roundish to angular, lentiform to subglobose	Regular roundish, subglobose to lentiform	Regular, roundish, subglobose to lentiform (biconcave)
Spores/ascus	8	4–8	4–8	2–8	4–8	(2)–4–8
Size	20–40–(60) × 8–15 µm, with 6–12–(14) loci	30–50 × 7–10 µm, with 8–16 loci	10–30–(40) × 5–8µm, with 4–11–(12) loci	25–60 × 6–9 µm, with 7–16 (×2) loci	50–90–(110) × 8–12 µm, with 14–20–(22) (×2) loci	60–220 × 10–20 µm, with 12–35 (×2) loci

Type: Australia, Queensland, Cape Tribulation Area, Myall Beach, *Lumbsch & Mangold 19161 x* (*holo:* CANB; *iso:* BRI, F).

Mycobank no. MB 511758.

Etymology: the epithet refers to Cape Tribulation in northern Queensland where most specimens of this species were collected.

Thallus epi- to hypophloedal, up to ~400 µm high, grey to pale greyish-green. Surface slightly shiny, smooth, verrucose, unfissured to slightly fissured. Protocortex ± discontinuous, up to ~30 µm thick, in some parts becoming conglutinated forming a true cortex of irregular to periclinal hyphae. Algal layer discontinuous, crystals abundant, large or small, scattered or clustered. Vegetative propagules not seen. Ascomata conspicuous, up to ~700 µm in diam., roundish, apothecioid, solitary to slightly fused marginally, emergent, subglobose to broad-cylindrical with ± verrucose surface. Disc not visible from surface to rarely becoming visible, then pale brownish-grey, slightly pruinose. Pores small to moderately wide, up to ~300 µm in diam., roundish, entire to split, proper exciple visible from surface, becoming free, apically bright, darker towards the base, incurved. Thalline rim margin entire to split to slightly lacerate or eroded, ± roundish, whitish or brighter than thallus, sometimes slightly pruinose, incurved to rarely erect. Proper exciple becoming free, hyaline internally to pale yellowish-brown marginally, amyloid at the base. Hymenium up to ~150 µm high, non-inspersed, moderately conglutinated, paraphyses interwoven, unbranched, tips slightly thickened, lateral paraphyses present, up to ~30 µm long, columellar structures absent. Epihymenium hyaline, granulose. Asci 8-spored, tholus thick to moderately thin at maturity. Ascospores transversely septate, cell walls thick, with thin, sometimes irregular halo, hyaline, amyloid, claviform to fusiform, ends narrowed-roundish to subacute, loci irregular and variable, end cells hemispherical to conical, septa thick, regular to ± irregular, 25–50 × 5–10 µm with 6–14 loci.

Pycnidia not seen.

Chemistry: thallus K+ yellowish becoming brown, C-, PD+ orange; containing constictic, stictic, hypostictic (majors), hypoconstictic and cryptostictic acids (traces).

This taxon is characterised by a thick thallus, emergent ascomata, amyloid ascospores with thick cell walls, and the presence of the stictic acid chemosyndrome. It is similar to *T. porinoides*, but this species differs in having a thinner thallus and larger ascospores with more septa. Even so, the size of the ascospores in *T. capetribulense* lies within the lower limit of those of *T. porinoides.* The distinction of the two species is supported by the present phylogenetic study which reveals that the two taxa are strongly supported as monophyletic sister-groups. The ascospores of *T. capetribulense* are also similar to those of *T. suecicum*. This species, however, has smaller ascospores and lacks secondary compounds, and according to our phylogenetic study, the two species are not closely related. *T. bicinctulum* can be readily distinguished by smaller (up to 400 µm in diam.), immersed ascomata and smaller ascospores (up to 35 µm long with up to 11 loci).

Ecology and distribution: *T. capetribulense* grows on tree bark in tropical rainforests and mangroves, at elevations ranging from sea level up to 700 m. It is known only from northern Queensland.

Specimens examined

QUEENSLAND: Cape Tribulation Area, Myall Beach, *Lumbsch & Mangold 19158 u, 19160 q, y, 19162 i* (F). Atherton Tablelands, Lamb Range, N of Gillies Rd, 22 km ENE of Atherton, *Thor 5738* pr.p. (S).

Thelotrema crespoae Mangold, Elix & Lumbsch, *sp. nov.* (Fig. 2*B, F*)

A *Thelotrema lacteum* ascis monosporibus et ascosporis maioribus differt.

Type: Australia, Queensland, Wooroi State Forest Park, W of Tewantin, *Hale 832786* (*holo:* US).

Mycobank no. MB 511759.

Etymology: this new species is named in honour of the Spanish lichenologist, Ana Crespo (Madrid).

Thallus epi- to hypophloedal, up to ~150 µm high, pale greyish-green to grey. Surface dull, roughened, continuous, unfissured. Cortex absent. Algal layer discontinuous, crystals abundant, small, scattered to rarely clustered. Vegetative propagules not seen. Ascomata inconspicuous, up to ~800 µm in diam., roundish, perithecioid in younger stages, becoming apothecioid with age, solitary, emergent, hemispherical. Disc usually not visible from surface, when visible, greyish-pruinose. Pores small to moderately wide, up to ~400 µm in diam., roundish to slightly irregular, entire to slightly split, off-white to pale brown. Thalline rim margin narrow, becoming moderately wide to gaping with age, split, incurved to slightly erect, concolorous with the thallus. Proper exciple becoming entirely free, hyaline within, yellowish-brown at margin, non-amyloid. Hymenium up to ~300 µm high, non-inspersed, moderately conglutinated, paraphyses parallel to somewhat interwoven, unbranched, tips slightly to distinctly thickened, lateral paraphyses present, inconspicuous, up to ~20 µm long, columellar structures absent. Epihymenium hyaline, granulose. Asci 1-spored, tholus moderately thick, not visible at maturity. Ascospores transversely septate, cell walls thick, non-halonate, hyaline to ± distinctly brown in latter stages, non-amyloid to amyloid before pigmented, oblong-fusiform, ends distinctly acute, appendiculate, loci roundish to acute, oblong to lentiform, loci in tapered areas ± rectangular to irregular, end cells conical, septa thin to thickened in late maturity, distinctly regular, 150–280 × 25–35 µm with multiple loci.

Pycnidia not seen.

Chemistry: thallus K-, C-, PD-; no secondary compounds detected.

This new species is characterised by its large, transversely septate, appendiculate ascospores. It is unique within *Thelotrema*, since in other species with long ascospores, the ascospores are usually muriform. *T. lacteum* is somewhat similar but can readily be distinguished by the smaller (up to 130 µm) ascospores and 4–8-spored asci. The taxon is well characterised morphologically and belongs to the basal group of *Thelotrema* species.

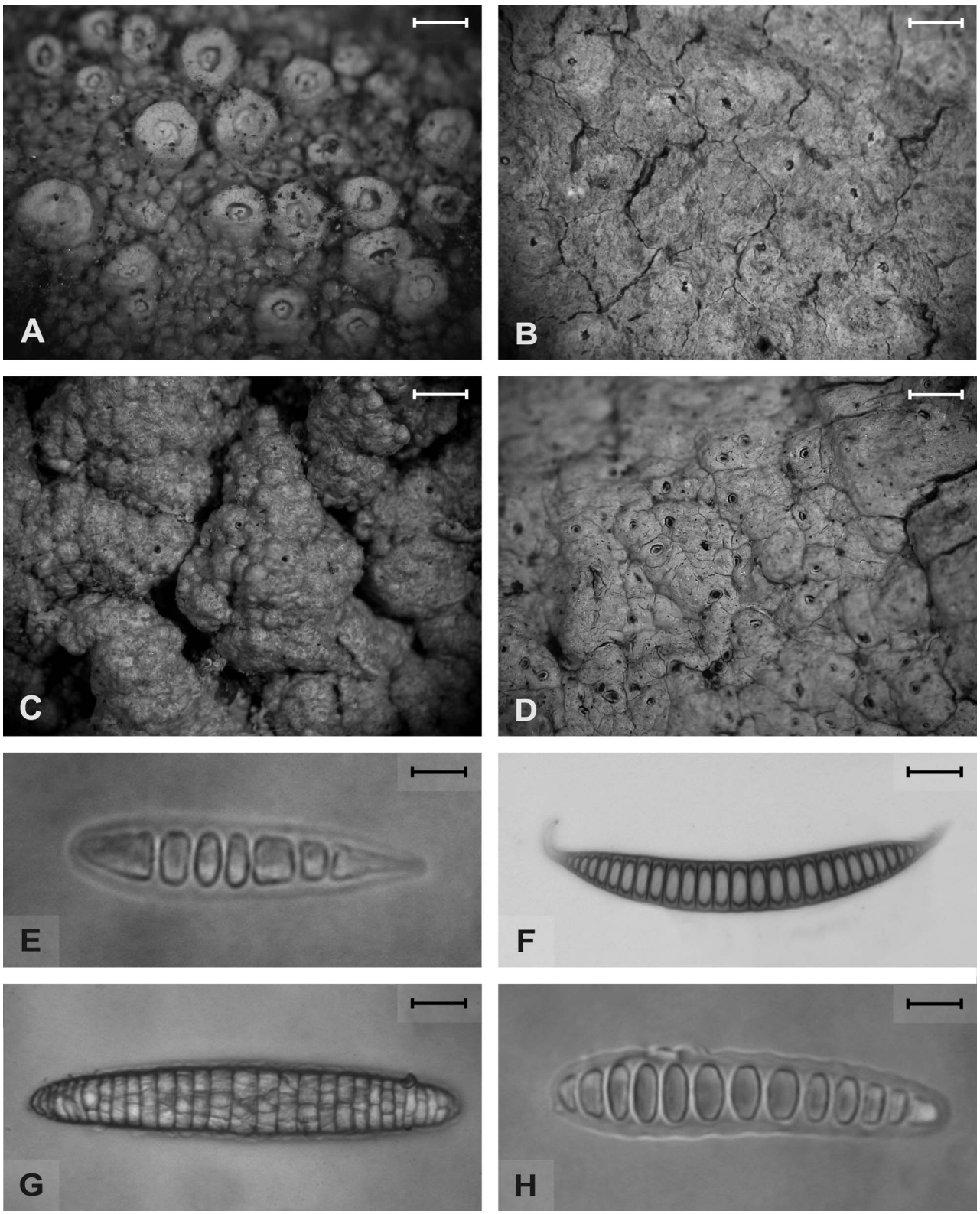

Fig. 2. Morphology and ascospores of four new *Thelotrema* spp. (*A*–*D*) Habit. (*A*) *T. capetribulense* (holotype). (*B*) *T. crespoae* (holotype). (*C*) *T. oleosum* (holotype). (*D*) *T. pseudosubtile* (holotype). (*E*–*H*) Ascospores. (*E*) *T. capetribulense* (holotype). (*F*) *T. crespoae* (holotype). (*G*) *T. oleosum* (holotype). (*H*) *T. pseudosubtile* (holotype). Scale bar = 500 µm (*A*), 700 µm (*B*), 1.5 mm (*C*), 400 µm (*D*), 5 µm (*E*, *H*), 35 µm (*F*) and 30 µm (*G*).

Ecology and distribution: *T. crespoae* occurs on tree bark in subtropical to warm-temperate rainforests at low altitude (10–50 m). It is currently known only from southern Queensland and central New South Wales.

Additional specimen examined

NEW SOUTH WALES: Myall Lakes NP, Mungo Brush Camping Area, *Mangold 27 v* (F).

Thelotrema oleosum Mangold, *sp. nov.* (Fig. 2*C, G*)

A *Thelotrema porinaceum* ascosporis fuscibus et acidis norstictibus deficiens differt.

Type: Australia, New South Wales, Dorrigo NP, Sassafras Creek Track, *Mangold 25 m* (*holo:* CANB; *iso:* NSW).

Mycobank no. MB 511760.

Etymology: the epithet refers to the inspersed hymenium of the species (from the Latin *oleosus* = oily).

Thallus epi- to hypophloedal, up to ~250 µm high, greenish-grey to olive, with a distinct reticulate pattern. Surface dull to shiny, smooth, verrucose to verruculose, ± fissured. True cortex discontinuous or continuous, consisting of periclinal hyphae up to ~30 µm thick, sometimes only a discontinuous protocortex present. Algal layer discontinuous, crystals abundant, small to large, clustered or rarely scattered. Vegetative propagules not seen. Ascomata inconspicuous, up to 1.5 mm in diam., roundish to irregular in fused ascomata, perithecioid, solitary to marginally fused, immersed to emergent, then hemispherical to somewhat subglobose. Disc not visible from above. Pores small, up to ~150 µm, roundish to roundish-irregular, entire, upper parts of proper exciple visible from surface, fused to free, whitish to greyish, incurved. Thalline rim margin roundish to roundish-irregular, small, entire, thin to thick, incurved, concolorous with thallus. Proper exciple fused, becoming apically detached in older stages, moderately thin, hyaline internally, pale yellowish marginally, apically rarely carbonised, non-amyloid. Hymenium up to ~400 µm high, inspersed, strongly conglutinated, paraphyses ± bent and wavy, interwoven, unbranched, tips not thickened to slightly thickened, lateral paraphyses present, up to ~30 µm long, columellar structures absent. Epihymenium hyaline, egranulose. Asci 1-spored, tholus absent, often with somewhat thickened lateral ascus walls in younger stages. Ascospores eumuriform, cell walls and endospore thin, often with a thin, indistinct halo, becoming greyish to brownish at maturity, dark brown when depauperate, ± distinctly amyloid, oblong to fusiform, with roundish to narrow-roundish ends, loci small, roundish to somewhat angular, irregular, transverse septa thin, ± distinct, regular to irregular, 120–20 × 30–50 µm with multiple loci.

Pycnidia not seen.

Chemistry: thallus K-, C-, PD-; no secondary compounds detected.

This species is readily identified by the perithecioid ascomata with a fused to apically free proper exciple, inspersed hymenium, monospored asci with muriform, amyloid, thin-walled ascospores, and the absence of secondary compounds. Similar species include *T. porinaceum* and *T. saxicola*. The former differs in having non-amyloid ascospores and the presence of the norstictic acid chemosyndrome, whereas *T. saxicola* contains the psoromic acid chemosyndrome. The thallus structure of *T. oleosum* is somewhat reminiscent of a *Leucodecton* spp. This genus, however, is distinguished from *Thelotrema* by the absence of lateral paraphyses and is more closely related to *Chapsa* (Mangold *et al*. 2008).

Ecology and distribution: *Thelotrema oleosum* grows on tree bark in (sub)tropical rainforests at elevations of 50–1130 m. It is known from Queensland and northern New South Wales.

Specimens examined

QUEENSLAND: Atherton Tablelands, Tumoulin Rd, 5 km from turnoff to Ravenshoe, *Lumbsch & Mangold 19151 x* (F). Culpa logging area, 13 km from Koombooloomba Rd turnoff, SE of Tully Falls, *Hale 830718* (US). Mt Spec NP, ridge on the Loop, on the Paluma Rd, WNW of Townsville, *Hale 831919, 832396* (US). Wooroi State Forest Park, W of Tewantin, *Hale 831957* (US). 13 km N of O'Reillys on road to Lamington NP, S of Brisbane, *Hale 830656, 831928* (US). Lamington NP, Main Border Track out of O'Reillys, *Hale 831509* (US). NEW SOUTH WALES: Dorrigo NP, Never Never Picnic Area and Rosewood Creek Track, *Mangold 24 a* (F).

Thelotrema pseudosubtile Mangold, *sp. nov.* (Fig. 2*D, H*)

A *Thelotrema subtile* ascomatis minus emergentibus et ascosporis haud fuscibus differt.

Type: Australia, Queensland, Crystal Cascades, 5 km W of Cairns, *Lumbsch & Mangold 19117 k* (*holo:* CANB; *iso:* BRI).

Mycobank no. MB 511761.

Etymology: the epithet refers to the similarities with *T. subtile*.

Thallus epi- to hypophloedal, up to ~200 µm high, pale yellowish-grey to pale olive. Thallus dull to shiny, smooth, continuous to verrucose or verruculose, fissured. Protocortex discontinuous to continuous, up to ~20 µm thick, sometimes becoming conglutinated and forming a true cortex of periclinal hyphae up to 40 µm thick. Algal layer continuous, crystals abundant to sparse, small to rarely large, often clustered. Vegetative propagules not seen. Ascomata inconspicuous, up to ~300–(600) µm in diam., roundish, sessile, apothecioid, solitary to marginally or entirely fused, immersed to slightly emergent, flattened-hemispherical. Disc often becoming partly visible, greyish to pale flesh-coloured, epruinose to slightly pruinose. Pores small, up to ~150–(250) µm in diam., roundish to slightly irregular, entire to slightly split, proper exciple apically to rarely entirely visible from surface, whitish to off-white, pale brownish towards the base, incurved. Thalline rim margin thin, becoming wide to rarely gaping with age, roundish to irregular-roundish, entire to slightly split, incurved to rarely erect, concolorous with thallus. Proper exciple becoming free, at least partly, hyaline to pale yellowish internally, brown or greyish-brown marginally, amyloid at the base. Hymenium up to ~150 µm high, non-inspersed, conglutinated, paraphyses moderately interwoven, unbranched to slightly branched, tips slightly thickened, lateral paraphyses present, inconspicuous, up to ~20 µm long, columellar structures absent. Epihymenium hyaline to brown, granulose. Asci 2–8-spored, tholus thick narrowing at maturity. Ascospores transversely septate, rarely

with a single longitudinal septum, cell walls thick, often crenulate, with a thin halo, hyaline, amyloid, clavate to oblong or fusiform, ends roundish to acute, loci roundish to angular, lentiform to subglobose, end cells hemispherical to conical, septa thick to thin, regular or irregular, 25–60 × 6–9 µm, with 7–16 (x 2) loci.

Pycnidia not seen.

Chemistry: thallus K-, C-, PD-; no secondary compounds detected.

Thelotrema pseudosubtile is characterised by the immersed or indistinctly emergent apothecia with a free proper exciple, transversely septate, amyloid ascospores that are hyaline throughout their entire development, a crenulate surface and the absence of secondary compounds. It is morphologically close to *T. subtile*, which differs in having more emergent apothecia and ascospores that become brown with age. Further, the distribution of these two species differs (Table 2). Another similar species is *T. diplotrema*, and this species exhibits a similar distribution to *T. pseudosubtile. T. diplotrema* differs in having a less compact, sometimes roughened thallus surface, more distinctly emergent ascomata, usually with a less distinctly free proper exciple and larger ascospores (up to 100 µm long with up to 22 loci), with thicker cell walls, and the absence of a crenulate surface.

Ecology and distribution: *T. pseudosubtile* occurs on bark in (sub)tropical to warm-temperate rainforests, rarely in wet sclerophyll forests from sea level to an altitude of 950 m. It is common and widespread in Queensland, New South Wales and Lord Howe Island. It is currently known only from Australia but may have been overlooked elsewhere since it is similar to other taxa in the *T. subtile*-group.

Specimens examined

QUEENSLAND: Cape Tribulation Area: Myall Beach, *Mangold 31 u* (F); track to Cape Tribulation Beach, *Mangold 32 t* (F); 4.5 km on Buchanan [Creek] Rd, *Hale 831723* (US). 45 km on Mt Windsor Rd, NW of Mossman, *Hale 831722* (US). Mt Lewis Rd, 4 km N of Kennedy Hwy, W of Mossman, *Hale 832737* (US). Manchans Beach, few kilometres N of Cairns, *K. & A. Kalb 20046* (hb. Kalb). W of Palm Cove, ~25 km N of Cairns, *K. & A. Kalb 19949* (hb. Kalb). Crystal Cascades, 5 km W of Cairns, *Lumbsch & Mangold 19117 k* (F). Atherton Tablelands: Lake Tinaroo, Downfall Creek Camping Area, *Lumbsch & Mangold 19125 j*, *h* (F); 10 km S of Ravenshoe on Tully Falls Rd, *Hale 830731* (US); 1–2 km N of Murray Falls, W of Kennedy, *Hale 831342*, *831648, 832533* (US). Eungella NP, along Broken River, *Lumbsch & Mangold 19100 y* (F). Cape Hillsborough NP, NW of Mackay, *Hale 831356*, *831701* (US). Wooroi State Forest Park, W of Tewantin, *Hale 830999*, *832080* (US). Booloumba Creek State Forest, SW of Kenilworth, *Hale 831367* (US). 6 km N of Jimna, *Tibell 12797* (UPS). Bunya Mountains: on the road from the ridge to Maidenwell, 1.3 km NE of the intersection, *Hafellner 19349* (GZU); Mt Bunya, Shire picnic area on southern NP boundary, S of Park, *Hale 831411*, *831419*, *832472*, *832500* (US); Mt Mowbullan, *K. & A. Kalb 20254*, *20268* (hb. Kalb). D'Anguilar Range NW of Brisbane, W of Mt Glorious township, *Hafellner 16955*, *16957* (GZU). Mt Mee State Forest, near Mt Mee, N of Brisbane, *Hale 58604* (US). Bennet Rd, Mt Glorious, *Rogers 8449* (BRI). Sankeys Scrub, Brisbane, *Wilson s.n.* (NSW-539342). Goodna, *Wilson s.n.* (NSW-539346). Lamington NP: Python Rock Track, *Hale 832375* (US); Beechmont Range, Binna Burra, *K. & A. Kalb 19896* (hb. Kalb). NEW SOUTH WALES: Nightcap Forest Drive, 1 km W of Minyon Falls, N of Lismore, *Hale 832087*, *832180*, *832543* (US). Dorrigo NP, Never Never Picnic Area and Rosewood Creek Track, *Mangold 24 b*, *d*, *f* (F). Saltwater, E of Taree, *Elix 3997* (CANB). Sugar Creek Flora Reserve, Wallingat State Forest, 16 km SW of Forster, *Streimann 44230 a* (B, CANB). Bulahdelah District, Myall River State Forest, E of Stroud, Jarrah Rd, *Kalb & Filson 18044*, *18046*, *18049* (hb. Kalb). Trail along bank of Mill Creek, 50 km NW of Sydney, *K. & A. Kalb 34272* (hb. Kalb). Below Katoomba Falls, trail to Giant Stairway, Katoomba, *Hale 58731* (US). Royal NP, S of Sydney, Bola Creek, E of Waterfall, *K. & A. Kalb 21692*, *21695*, *21709* (hb. Kalb). Lord Howe Island: Goat House Cave, *Elix 42175* (CANB), *42265* (B, CANB); Smoking Tree Ridge, *Elix 42149* (B, CANB); track from Smoking Tree Ridge to Rocky Run, *Elix 42429* (CANB); Track to Kims Lookout, *Elix 42393* (CANB).

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