




## Standard Paper

# *Caloplaca tephromelae* (Teloschistaceae), a new lichenicolous species from Tasmania

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### Abstract

*Caloplaca tephromelae* Kantvilas, Suija & Motiej., a lichenicolous species growing on saxicolous thalli of species of *Tephromela*, is described from Tasmania. The new species is characterized by lecanorine to zeorine apothecia with a whitish grey thalline margin devoid of anthraquinone pigments, a non-inspersed hymenium, paraphyses without oil vacuoles and ascospores 10–14 × 5–8 μm, with a septum 5–8 μm thick. It is compared with selected taxa of *Caloplaca* s. lat. that share these salient features. Molecular data support the distinctiveness of the new species but do not suggest any obvious close relatives.

**Key words:** Australia, biodiversity, lichens, *Tephromela*

(Accepted 7 March 2021)

### Introduction

The lichen genus *Caloplaca* Th. Fr. is a highly conspicuous and species-rich component of the temperate Australasian lichen flora. It is responsible for the vivid, eye-catching red, orange and yellow coloration of coastal rocks, man-made substrata such as tile roofs, stone walls and concrete, as well as tree bark and rock outcrops in natural habitats. Recent decades have seen considerable advances in the study of *Caloplaca* in Australia (including Tasmania), chiefly by the Ukrainian lichenologist Sergey Kondratyuk who, with collaborators, has described more than 75 species based on Australian types (Kärnefelt & Kondratyuk 2004; Kondratyuk *et al.* 2007a, b, 2009a, b, 2010, 2011, 2013a; Lumbsch *et al.* 2011; Kantvilas & Kondratyuk 2013), culminating in a key to the more than 120 species recorded for Australia (Kondratyuk *et al.* 2012). Other taxa have been added by Hafellner (1982), Kantvilas & Søchting (2013) and Kantvilas (2016), to the extent that the complement of species for the region today stands at 135 taxa, of which 45 are reported for Tasmania (McCarthy 2020).

Traditionally, *Caloplaca* has encompassed lichens with a trebouxioid photobiont, a subfruticose, placodioid, squamulose or crustose thallus, apothecial ascomata, *Teloschistes*-type asci, hyaline, usually polaribilocular ascospores and, in most species, orange or yellowish, K+ purple anthraquinone pigments in the thallus and/or apothecia (e.g. Fletcher & Laundon 2009; Kantvilas 2016). However, phylogenetic studies using DNA sequence data indicated that the genus is heterogeneous (Søchting & Lutzoni 2003; Gaya *et al.* 2012; Bungartz *et al.*

2020) and, as a result, a large number of smaller, more natural genera have been erected (Arup *et al.* 2013; Kondratyuk *et al.* 2013b, 2014a, b, 2015a, b, 2016, 2017, 2018a, b), many with representatives in Australia. This new classification has not been without controversy, and has also proved unwieldy to most taxonomists working with traditional morphological and anatomical characters. Consequently, it has not been generally taken up (e.g. see Gaya *et al.* 2015; Aptroot & Cáceres 2016; Kantvilas 2016; McCune 2017; McCarthy 2020) and it seems inevitable that, because of its easy recognition, *Caloplaca* in the broad sense is likely to remain in use for the foreseeable future.

Species of *Caloplaca* can occur on almost every conceivable lichen substratum (wood, bark, soil, humus, bryophytes, calcareous and non-calcareous rock, man-made surfaces) with the notable exception of living leaves. Almost 40 species, spanning several of the segregate genera (e.g. *Athallia*, *Catenarina*, *Flavoplaca*, *Gyalolechia*, *Pachypeltis*, *Variolaria*), are obligately or facultatively lichenicolous (Poelt & Hinteregger 1993; Nimis *et al.* 1994; Vondrák *et al.* 2016; Diederich *et al.* 2018). In general, many of these lichenicolous *Caloplaca* species have a comparatively wide range of hosts and their distribution appears to be determined as much by their substratum as by the taxonomy of their host lichen.

In the course of the Tasmanian Museum and Art Gallery's inaugural Expedition of Discovery, an initiative aimed at the collection and documentation of the flora and fauna from poorly studied areas of Tasmania (Baker *et al.* 2019), a lichenicolous *Caloplaca*, growing on the widespread, saxicolous species *Tephromela atra* (Huds.) Hafellner, was discovered. No lichenicolous species of the genus are known from Australia, nor does the species closely resemble any non-lichenicolous species known in the region. After comparison of our species against the worldwide literature on lichenicolous *Teloschistaceae*, and to morphologically similar species from Australia, we conclude that it is new to science and describe it below.

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**Cite this article:** Kantvilas G, Suija A and Motiejūnaitė J (2021) *Caloplaca tephromelae* (Teloschistaceae), a new lichenicolous species from Tasmania. *Lichenologist* 53, 317–325. <https://doi.org/10.1017/S0024282921000207>

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**Table 1.** Taxon sampling and GenBank accession numbers of sequences of *Teloschistales* used in the molecular phylogenetic analyses. Newly generated sequences are in bold. Lichenicolous taxa are marked with <sup>L</sup>.

Taxon	nuITS	nuLSU	mtSSU
<i>Amundsenia approximata</i>	KJ789963	KJ789972	KJ789974
<i>A. austrocontinentalis</i>	KJ789961	–	KJ789975
<i>Athallia cerinella</i>	HM582148	–	–
<i>A. holocarpa</i>	HM582157	AJ535268	–
<i>Austroplaca cirrochrooides</i>	KC179082	KC179152	KC179482
<i>A. lucens</i>	KC179087	KC179155	KC179485
<i>Blastenia circumpolaris</i>	MF114845	–	–
<i>B. ferruginea</i>	MN989252	KC179163	KC179493
<i>Brownliella aequata</i>	KF264627	KF264662	KF264688
<i>Bryoplaca sinapisperma</i>	MN483095	–	KC179495
<i>B. tetraspora</i>	KP314331	–	–
<i>Calicium viride</i>	–	AY340538	AY143402
<i>Calogaya altynis</i>	KY748973	–	–
<i>C. saxicola</i>	HM800887	–	–
<i>Caloplaca cerina</i>	HM538547	JQ301549	JQ301483
<i>C. cerina</i>	AF279885	–	–
<i>C. chlorina</i>	MK811786	KC179169	JQ301486
<i>C. epithallina</i> <sup>L</sup>	MH155284	–	–
<i>C. epithallina</i> <sup>L</sup>	MH155283	–	–
<i>C. gloriae</i>	EU63958	JQ301555	JQ301491
<i>C. inconnexa</i> <sup>L</sup>	EU639647	KT291547	KT291494
<i>C. irrubescens</i>	EU639650	–	–
<i>C. isidiigera</i>	KT804956	JQ301556	JQ301492
<i>C. lactea</i>	MN512252	–	–
<b><i>C. tephromelae</i><sup>L</sup></b>	<b>MW485494</b>	<b>MW483077</b>	<b>MW483076</b>
<i>C. wetmorei</i> <sup>L</sup>	HQ317923	–	–
<i>Catenarina desolata</i>	KY983103	–	KF657319
<i>C. vivasiana</i>	KF657311	–	–
<i>Cerothallia subluteoalba</i>	MG820705	MH216681	KC179512
<i>C. yarraensis</i>	JF826399	KJ133493	KJ133513
<i>C. yorkensis</i>	KC179101	KC179178	KC179513
<i>Charcotiana antarctica</i>	KJ789968	KJ789973	KJ789976
<i>Dijigiella kaernefeltiana</i>	KY614397	KY614445	KY614476
<i>D. subaggregata</i>	KY614398	KY614446	KY614477
<i>Dufourea alexanderbaai</i>	KC179350	KC179179	KC179514
<i>D. flammea</i>	EU681316	–	EU680898

(Continued)

**Table 1.** (Continued)

Taxon	nuITS	nuLSU	mtSSU
<i>Eilifdahlia dahlii</i>	KJ021318	KJ021253	KJ021279
<i>Fauriea chujaensis</i>	KX793095	KX793098	KX793101
<i>F. orientochinensis</i>	KX793097	KX793100	KX793103
<i>Filsoniana kiamae</i>	KF264634	KF264667	–
<i>F. kiamae</i>	KC179123	–	–
<i>F. scarlatina</i>	KF264641	–	–
<i>Flavoplaca citrina</i>	DQ173226	KC179186	KC179521
<i>F. mereschkowskiana</i>	KC179367	–	–
<i>Follmannia orthoclada</i>	KC179291	KC179191	–
<i>Franwilsia bastowii</i>	KJ021325	KJ021258	KJ021285
<i>F. kilcundaensis</i>	KJ021328	KJ021261	KJ021288
<i>Gondwania cribrosa</i>	KC179102	KC179192	KC179526
<i>G. sublobulata</i>	DQ534455	EF489950	–
<i>Gyalolechia flavorubescens</i>	AF279887	AY300831	AY143403
<i>G. flavovirescens</i>	AF353966	KC179198	KC179532
<i>G. fulgens</i>	AF278773	JQ301567	JQ301503
<i>Haloplaca sorediella</i>	MN586955	–	–
<i>H. suaedae</i>	HM582200	–	KC179538
<i>Hosseusiella gallowayana</i>	MG811848	–	–
<i>H. pergracilis</i>	MG811850	–	–
<i>Huneckia pollinii</i>	KJ021338	KJ021265	KJ021296
<i>H. rheinigeri</i>	KJ021222	–	–
<i>Ioplaca pindarensis</i>	JQ301672	–	–
<i>Jasonhuria bogilana</i>	KT220199	KT220208	KT220217
<i>Josefpoeltia parva</i>	KC179296	KC179204	KC179539
<i>Kaernefia gilfillaniorum</i>	KF264649	KF264679	KF264700
<i>K. kaernefeltii</i>	KF264651	KF264677	KF264702
<i>Leproplaca cirrochroa</i>	EU639610	–	–
<i>L. obliterans</i>	MK812503	KC179207	KC179541
<i>Loekoesia austrocoreana</i>	KT220202	–	KT220220
<i>Marchantiana occidentalis</i>	KJ021228	KJ021269	KJ021303
<i>M. seppeltii</i>	KJ021229	KJ023186	KJ021305
<i>Nevilleiella lateritia</i>	KY614426	KY614463	KY614501
<i>N. marchantii</i>	KY614425	KY614462	KY614500
<i>Orientophila loekoesii</i>	KC179374	–	KJ133537
<i>Orientophila</i> sp.	KC179372	KC179210	KC179544
<i>Pachypeltis castellana</i> <sup>L</sup>	KC179105	–	KC179547
<i>P. insularis</i> <sup>L</sup>	MG954169	–	–

(Continued)

Table 1. (Continued)

Taxon	nuITS	nuLSU	mtSSU
<i>P. invadens</i> <sup>L</sup>	KC179108	KC179212	KC179548
<i>P. phoenicopta</i> <sup>L</sup>	MG954170	–	–
<i>Parvoplaca chelyae</i>	KT162000	–	–
<i>P. nigroblastidiata</i>	KT161983	–	–
<i>Physcia dubia</i>	JQ301695	JQ301596	EF582796
<i>P. stellaris</i>	MK812381	AY860584	–
<i>Polycauliona candalaria</i>	MN630378	JQ301587	JQ301528
<i>Rufoplaca</i> sp.	MG954203	–	–
<i>Rusavskia elegans</i>	MG954156	DQ912352	DQ912304
<i>Shackletonia buelliae</i> <sup>L</sup>	KC179117	–	KC179578
<i>Sirenophila bermaguiana</i>	KC179299	KC179245	KC179584
<i>S. gintarasii</i>	KY614437	KY614470	–
<i>Solitaria chrysophthalma</i>	MN592665	KT291537	KT291484
<i>Stellarangia elegantissima</i>	KT291454	KT291541	KT291488
<i>S. namibensis</i>	KC179311	–	–
<i>Tassiloa digitaurea</i>	KP096223	–	KP096225
<i>Teloschistes hosseusianus</i>	JQ301686	JQ301579	–
<i>Teloschistopsis chrysocarpoides</i>	KC179323	–	–
<i>T. eudoxa</i>	KC179324	KC179258	KC179597
<i>Teuvoahitia rugulosa</i>	KY614442	KY614473	KY614517
Uncultured fungus	KC965535	–	–
<i>Upretia squamulosa</i>	MH497058	MH497052	–
<i>Usnochroma carphineum</i>	KC179468	JQ301560	JQ301482
<i>U. scoriophilum</i>	KC179469	JQ301560	JQ301496
<i>Variospora australis</i>	AF277663	–	–
<i>V. dolomiticola</i> <sup>L</sup>	MG954125	KC179262	KC179601
<i>V. sororicida</i> <sup>L</sup>	MG954123	–	–
<i>Villophora</i> sp.	KC179330	KC179267	KC179607
<i>Villophora</i> sp.	KC179328	KC179268	–
<i>Wetmoreana appressa</i>	KC179332	–	–
<i>Xanthocarpia</i> cf. 'jerramungupensis'	KJ133486	KJ133505	KJ133545
<i>Xanthocarpia</i> sp.	KJ133485	KJ133504	KJ133543
<i>Xanthopeltis rupicola</i>	KC179146	–	KC179626
<i>Xanthoria parietina</i>	KY198388	KJ766678	KJ766515
<i>Yoshimuria cerussata</i>	KJ021248	–	KT291519
<i>Y. galbina</i>	KJ021251	–	KJ023197
<i>Y. spodoplaca</i>	LC490370	–	KJ023194

## Materials and Methods

The study is based on the collections of the new species from Tasmania and housed in the Tasmanian Herbarium (HO). For comparison with other *Caloplaca* taxa, we consulted reference herbarium specimens or, where these were unavailable, published species descriptions.

## Anatomy and morphology

Observations of specimens were made using low-power and high-power microscopy. Thin, hand-cut sections of the apothecia were examined in a range of mounting media, including water, 10% KOH (K), Lugol's solution (I) and lactophenol cotton blue (LCB). Following the protocol described in Kantvilas (2016), all measurements were undertaken exclusively in sections hydrated in water and then mounted in LCB. Likewise, observations of paraphyses and oil vacuoles were undertaken in LCB.

## DNA extraction, PCR amplification and DNA sequencing

DNA extraction and amplification were carried out in the Mycology Laboratory of the University of Tartu (TU). Genomic DNA was extracted from ascomata using the High Pure PCR Template Preparation Kit (Roche Applied Science®), following the protocol provided by the manufacturer. We amplified three gene loci: internal transcribed spacer (nuITS) using primer pair ITS0F and LA-W (Tedersoo *et al.* 2008); large subunit nuclear ribosomal RNA gene (nuLSU) with LROR and LR7 (Vilgalys & Hester 1990); and mitochondrial small subunit ribosomal RNA gene (mtSSU) with mrSSU1 and mrSSU3R (Zoller *et al.* 1999). The PCR reaction mix (25 µl) consisted of 5 µl 5× HOT FIREPol Blend Master Mix (with 10 mM MgCl<sub>2</sub>; Solis BioDyne, Tartu, Estonia), 0.5 µl of both primers (all 20 µM) and 3–8 µl of target-DNA, with the remainder being distilled water. The temperatures and time for each cycle of the polymerase chain reaction (PCR) were as follows: denaturation was set at 95 °C for 30 s; annealing at 57 °C (nuITS) or 55 °C (nuLSU, mtSSU) for 30 s; and extension at 72 °C for 60 s. A total of 36 (nuITS) and 35 (nuLSU, mtSSU) cycles were run. The PCR products were visualized on a 1% agarose gel stained with ethidium bromide, and for the purification of PCR products, 1 µl of FastAP and 0.5 µl of Exonuclease I (Thermo Scientific, Waltham, Massachusetts, USA) were added to each tube per 20 µl of the product. Both complementary strands were sequenced by MacroGen Inc. (Amsterdam, The Netherlands). The nuITS sequences were sequenced with primer pair ITS4 and ITS5 (White *et al.* 1990), nuLSU with CTB6 (Garbelotto *et al.* 1997) and LR7, and for the mtSSU the same primers were used as for the amplification. Sequencher v.4.10.1. (Gene Codes Corp., Ann Arbor, Michigan, USA) was used to check, assemble and manually adjust the resulting sequence fragments. The consensus sequences were compared with those publicly available in GenBank using the 'blastn' algorithm (Altschul *et al.* 1990). The newly generated DNA sequences are deposited in the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>) and UNITE (Nilsson *et al.* 2019) databases.

## Phylogenetic analyses

We compiled DNA alignments for each gene, using taxon sampling that encompassed as many of the segregate caloplacoid

**Table 2.** Basic statistics for nuITS, nuLSU and mtSSU alignments of *Teloschistales* species in this study: number of sequences, number of nucleotide positions in original and curated (after implementation of Gblocks (Talavera & Castresana 2007)) alignments, and number of variable and informative sites in curated alignment.

Sequence	No. of taxa	Original	Curated	Variable	Informative
nuITS	110	868	345	120	92
nuLSU	67	2642	687	32	24
mtSSU	71	1274	700	237	171

genera as possible (Table 1). *Physcia dubia* and *P. stellaris* (Physciaceae) or *Calicium viride* (Caliciaceae) were included to root the phylogenies. The DNA sequences were aligned with the on-line version of MAFFT v.7 (Kato *et al.* 2019) using default options and corrected manually with SeaView v.4.6 (Gouy *et al.* 2010). The online version of Gblocks v.0.91b (Talavera & Castresana 2007) was used to eliminate poorly aligned positions and divergent regions, but allowing smaller final blocks and gap positions within the final blocks. The basic statistics for all three alignments are given in Table 2.

Alignments were analyzed using the Markov chain Monte Carlo (MCMC) and maximum likelihood (ML) approaches. The best-fit nucleotide substitution models (TIM+I+G for nuITS and GTR+I+G for nuLSU and mtSSU) were calculated over 56 models and selected based on the lowest value AIC criterion (Akaike 1974) with jModelTest v.2.1.6. (Darriba *et al.* 2012). The Bayesian analysis was performed with MrBayes v.3.2.1. (Ronquist *et al.* 2012) using the following settings: two parallel simultaneous runs over 1 million (nuITS, nuLSU) or 2 million generations (mtSSU), starting with a random tree and employing four simultaneous chains; sampling after 1000 steps. The analyses were run until convergence of the chains was confirmed by the standard deviation of split frequencies reaching 0.01, and the average Potential Scale Reduction Factor (PSRF) value was close to 1. The first 25% of saved data was discarded as 'burn-in'. The consensus tree and posterior probabilities (PP) were calculated from the remainder. The ML analysis was run with RAxML v.8.2.12 (Stamatakis *et al.* 2008) and inferred assuming GTR+G as the nucleotide substitution model. Branch support was calculated by rapid bootstrapping over 1000 pseudoreplicates. All analyses were implemented on the CIPRES Science Gateway v.3.3 (Miller *et al.* 2010). Those clades with posterior probabilities (PP)  $\geq 0.95$  and bootstrap values (BS)  $\geq 0.75$  were regarded as significantly supported. We present only the nuITS tree (Fig. 1) because here the number of taxa was largest (see Table 2). The phylogenetic tree was visualized and edited using FigTree v.1.4.2 (Rambaut 2014). Adobe Illustrator CS3<sup>®</sup> was used for artwork.

## Results

No sequence identical to that of the new species was found from the nucleotide databases, but the closest match was always a member of the *Teloschistaceae*. The percentage identity with the closest taxon ranged from 88% (nuITS) to 95% (mtSSU) and 97% (nuLSU). The phylogenetic analyses did not resolve the position of the new *Caloplaca* species because whereas the nuITS analysis showed a sister relationship with the *Yoshimuria* clade (PP = 1, but BS = 64), the mtSSU analysis suggested a relationship with *Marchantiana occidentalis* (PP = 1, BS = 95). Supported relationships were not found in the nuLSU analysis. The phylogenetic analyses did not reveal any relationship with *Caloplaca* (*Erichansenia*) *epithallina*, a taxon which inhabits *Tephromela*

thalli, nor with any other lichenicolous species of *Caloplaca* for which sequences were available (Fig. 1, Table 1). Although we prefer to retain the generic name *Caloplaca* in the broad sense, we acknowledge that groups of related taxa have been accorded generic rank by various authors. Accordingly, we present these in Fig. 1 as a means of illustrating where the various species groups are positioned in our phylogeny. The results confirm that the lichenicolous habit in *Caloplaca* s. lat. has arisen several times in the phylogeny (indicated with an 'L' after the relevant taxa) and is not confined to any particular group of related taxa. Sequences AF279885, annotated as *Caloplaca cerina*, and KC179123 as *Filsoniana kiamae*, are apparently incorrectly identified (Fig. 1).

## Taxonomy

### *Caloplaca tephromelae* Kantvilas, Suija & Motiej. sp. nov.

Mycobank No.: MB 838715

Species lichenicola, thallus *Tephromelae* incolens, apotheciis lecanorinis vel zeorinis, margo albedo-griseo, pigmentum aurantiacum destituto, hymenio non-insperso, paraphysibus oleovacuolas deficientibus, ascosporis 10–14  $\mu\text{m}$  longis, 5–8  $\mu\text{m}$  latis, septo 5–8  $\mu\text{m}$  crasso recognita.

Typus: Australia, Tasmania, Wind Song Property, northern rim of Callitris Gully, 42°20'55"S, 147°55'03"E, 60 m alt., on thallus of *Tephromela atra*, growing on dolerite outcrops in degraded rough pasture, 22 February 2019, G. Kantvilas 26/19 (HO—holotypus; BILAS, TUF091318—isotypi). GenBank Accession nos: MW483077 (nuLSU), MW483076 (mtSSU). DNA barcode/reference (nuITS) sequence from isotype: GenBank MW485494 / UNITE UDB0778961. UNITE SH3597440.08FU.

(Figs 2 & 3)

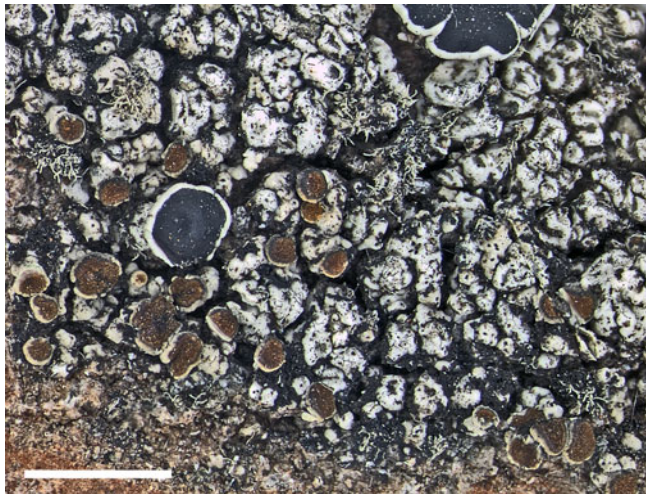
Thallus whitish, areolate, occurring as small islands, bordered by a dark band of prothallus, within the thallus of *Tephromela atra* and *T. granularis*, or  $\pm$ autonomous in close association with these species; medulla patchily I+ blue.

*Apothecia* 0.2–0.7 mm wide, lecanorine to zeorine, scattered over the thallus of the host or crowded together in discrete clusters, roundish or a little distorted due to mutual pressure, sessile, basally constricted; disc orange to dull orange-brown, matt, somewhat coarsely pruinose, persistently plane or a little undulate; thalline margin dull whitish grey, sometimes a little bluish grey to brownish, usually entirely enveloping the apothecia but at times crenulate, incomplete and mainly around the apothecium base, in section 50–80  $\mu\text{m}$  thick, interspersed with crystals that fluoresce white in polarized light and dissolve in K; photobiont trebouxoid, cells  $\pm$ globose, 6–12  $\times$  5–10  $\mu\text{m}$ , extending  $\pm$ continuously beneath the hymenium but absent from the outermost

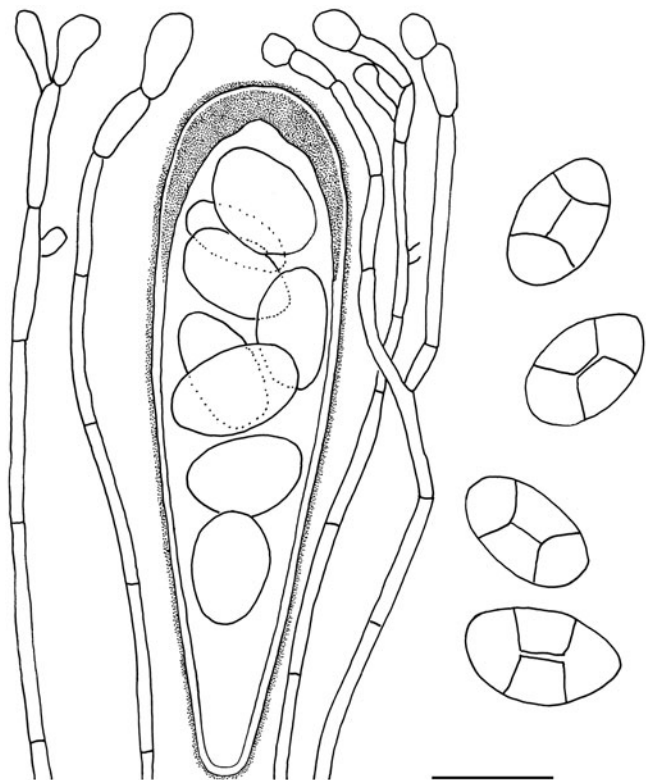


**Fig. 1.** The rDNA ITS-based consensus tree derived by the Bayesian method, showing the position of *Caloplaca tephromelae* within the *Teloschistales*. The branches with Bayesian posterior probabilities (PP)  $\geq 0.95$  and maximum likelihood bootstrap values (BS)  $\geq 75$  indicated above the branches are considered as supported and marked with a thicker line. The supported clades, and clades corresponding to generic rank according to various authors, are collapsed; numbers in brackets after taxon names indicate number of sequences in this clade. Lichenicolous taxa are indicated with 'L' and apparently incorrectly identified sequences with '\*'.  
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**Fig. 2.** *Caloplaca tephromelae* habit, showing the small, lecanorine apothecia with an orange-brown disc, growing on the whitish thallus of *Tephromela atra* (with large lecanorine apothecia with a black disc). Scale = 2 mm.



**Fig. 3.** *Caloplaca tephromelae* anatomy. Paraphyses, *Teloschistes*-type asci with amyloid parts stippled and ascospores (semi-schematic). Scale = 10  $\mu$ m.

15–20  $\mu$ m of the margin; proper excipulum either obscured by the thalline margin or seen as a thin, rather glossy dark brown rim between the thalline margin and the disc, in section 20–50  $\mu$ m thick, cupulate, poorly differentiated from the subhymenium, composed of intertwined, short-celled hyphae with cells 3–6  $\mu$ m wide. *Subhymenium* hyaline, 40–100  $\mu$ m thick in the central part, usually interspersed with minute oil droplets. *Hymenium* 70–90  $\mu$ m thick, hyaline, not interspersed, overlain by a dense band of golden yellow crystals 10–15  $\mu$ m thick that fluoresces

orange-yellow in polarized light; paraphyses 1.5–2  $\mu$ m thick, lacking oil vacuoles, sparsely branched, with apices mostly moniliform and expanding to 2.5–4  $\mu$ m at the apices; asci 8-spored, 45–60  $\times$  13–20  $\mu$ m. *Ascospores* polaribilocular, ellipsoid, 10–14  $\times$  5–8  $\mu$ m; septum 5–8  $\mu$ m.

*Pycnidia* not found.

**Chemistry.** Thallus and apothecial margin K–; apothecial disc K+ crimson (anthraquinone pigments); composition of secondary compounds not analyzed.

**Etymology.** The specific epithet refers to the host of the new lichen.

**Distribution and ecology.** The major host species, *Tephromela atra* is very widespread and common in Tasmania, and ranges from littoral to alpine altitudes. It occurs on a wide range of rock types, usually in exposed situations, in forest, heathland, grassland and highly modified agricultural environments. The sorediate *T. granularis* Kantvilas, on which the new species has also been observed, is more restricted and occurs on dolerite outcrops in low rainfall areas, mostly in open eucalypt forest or in degraded, heavily grazed scrubby pasture. Both host taxa are well represented in herbaria; all available collections (>100) were examined but failed to reveal any further material of the new species. Thus *Caloplaca tephromelae* is still known only from the type locality where it grew on large dolerite outcrops in a highly degraded, roughly-cleared sheep pasture. The first collection made was rather fortuitous, but on revisiting the site the new species was found to be abundant, although extremely localized on just a small number of outcrops. The boulders on which the new species occurs support a diverse suite of foliose and crustose lichens. Major species present include *Caloplaca (Nevilleiella) lateritia* (Taylor) Zahlbr., *Carbonea latypizodes* (Müll. Arg.) Knoph & Rambold, *Flavoparmelia haysomii* (C. W. Dodge) Hale, *Lecanora farinacea* Fée, *Lecidea atromorio* C. Knight, *Monerolechia badia* (Fr.) Kalb, *Paraporpidia leptocarpa* (C. Bab. & Mitt.) Rambold & Hertel, *Punctelia subrudecta* (Nyl.) Krog, *Ramboldia petraeoides* (Nyl. ex C. Bab. & Mitt.) Kantvilas & Elix, *Rhizocarpon geographicum* (L.) DC., *R. reductum* Th. Fr. and numerous species of *Xanthoparmelia*.

**Additional specimen examined.** **Australia: Tasmania:** type locality, 2017, G. Kantvilas 309/17 (HO) [on *T. atra* and *T. granularis*].

### Discussion

Of the approximately 40 lichenicolous *Caloplaca* (in the wide sense) species, sequences are available only for about one third of them (Table 1) and none of these revealed any close relationship with *C. tephromelae* (Fig. 1). Lichenicolous *Caloplaca* species display a wide range of characters with respect to the morphology and coloration of the thallus and apothecia, ascospore form and size, as well as other features. Consequently, we narrowed our detailed comparison of salient morphological and anatomical features to those lichenicolous species with lecanorine apothecia and lacking K+ crimson, anthraquinone pigments in the thallus (Table 3).

Of these morphologically similar, lichenicolous species, *Caloplaca epithallina* Lynge is the only one for which sequences were available, and these indicated that it is not closely related to *C. tephromelae* (Fig. 1). It also differs from *C. tephromelae* in

**Table 3.** Comparison of lichenicolous species of *Caloplaca* with a grey or inapparent thallus and lecanorine apothecia. 'Nd' indicates that no data were given in the reference.

Character	<i>C. tephromelae</i>	<sup>1,3,6</sup> <i>C. epithallina</i>	<sup>2</sup> <i>C. interna</i>	<sup>5</sup> <i>C. lecanorae</i>	<sup>1,4</sup> <i>C. magni-filii</i>
Host	<i>Tephromela</i>	various saxicolous lichens on non-calcareous substrata	<i>Circinaria</i>	<i>Lecanora leprosa</i>	<i>Miriquidica nigroleprosa</i>
Apothecium diameter (mm)	0.2–0.7	0.3–1	to 0.5	0.25–0.45	0.2–0.4
Disc colour	orange to dull orange-brown	dark orange to ferruginous red, often blackening	orange	orange-brown to red-brown	brick red to rust red
Thalline margin	dull whitish grey, to bluish grey, to brownish, sometimes crenulate	thick, persistent or excluded, greyish to blackish red or ferruginous brown to ferruginous red	orange to reddish orange	white, soon excluded	concolorous with the disc
Proper margin	obscured or thin, glossy dark brown	inapparent	inapparent	inapparent	inapparent
Hymenium thickness (µm) / inspersion	70–90 / not inspersion	50–60 / nd	50–70 / nd	80–95 / nd	to 70 / nd
Amyloid reaction of apothecial medulla	yes	no	nd	no	yes
Ascospore size (µm) / septum width (µm)	10–14 × 5–8 / 5–8	11–12.5 × 7–8 / 2–3.5	12–15 × 3–4.5 / 3–4.5	11.5–14.5 × 6.5–7.5 / 5–6	9.5–12 × 7–12 / 2.5–3.5
Width of apices of paraphyses (µm)	2.5–4	3–4	to 5	to 2	to 4

<sup>1</sup>Hansen *et al.* (1987); <sup>2</sup>Nimis & Poelt (1987); <sup>3</sup>Øvstedal *et al.* (2009); <sup>4</sup>Poelt (1958); <sup>5</sup>Seavey & Seavey (2012); <sup>6</sup>Søchting *et al.* (2008)


that its apothecial margin is concolorous with the disc, the hymenium is thinner (to 60 µm thick) and the ascospores, although of a similar size to those of *C. tephromelae*, have a septum only 2–3.5 µm thick (Hansen *et al.* 1987; Øvstedal *et al.* 2009). This Northern Hemisphere species is known to occur on *Tephromela*, as well as on some other saxicolous crustose and foliose lichens.

The most similar species to *C. tephromelae* morphologically is *C. lecanorae* F. Seavey & J. Seavey, described from *Lecanora leprosa* Fée in Florida. This species differs in having smaller apothecia (0.25–0.45 mm wide), with a thalline margin that is soon excluded and an inapparent proper margin (Seavey & Seavey 2012), as well as in the lack of a medullary iodine reaction (F. Seavey, personal communication; Table 3). Despite our efforts, material of this species for molecular analysis and comparison could not be obtained. Two further lichenicolous *Caloplaca* species with lecanorine apothecia lack anthraquinone pigments in the thallus: *Caloplaca interna* Poelt & Nimis differs in having a narrower ascospore septum (2–3.5 µm), wider paraphyses tips (to 5 µm) and a thalline apothecial margin containing anthraquinone pigments, and occurs on *Circinaria* species on calcareous substrata (Nimis & Poelt 1987); *Caloplaca magni-filii* differs by its strongly convex apothecia with darker apothecial discs, the thalline margin containing anthraquinone pigments, and the broadly ellipsoid to rounded ascospores with a narrow septum (2.5–3.5 µm), and occurs on *Miriquidica nigroleprosa* (Poelt 1958; Hansen *et al.* 1987). Both species occur only in the Northern Hemisphere.

Although the genus *Caloplaca* is very species-rich in Australia and Tasmania, no lichenicolous species have been reported so far from this region. Amongst the autonomous taxa, the critical characters of the new taxon, notably the whitish thallus lacking

anthraquinones and the lecanorine to zeorine apothecia, are also uncommon and seen only in the corticolous *C. bastowii* S. Y. Kondr. & Kärnefelt and the saxicolous *C. kilcundaensis* S. Y. Kondr. & Kärnefelt. Both of these taxa differ from *C. tephromelae* by having significantly larger apothecia (to 1.2 mm wide), a hymenium and subhymenium densely inspersioned with oil droplets, and ascospores with a narrower septum (at most to 4 µm wide) (Kantvilas 2016). The latter differs further in having a thallus with a distinct brownish tinge and apothecia that are mostly biatorine and only secondarily develop a thalline margin.

**Acknowledgements.** We thank Jean Jarman for the photograph and preparing the figures for publication. Frederick Seavey is thanked for supplying additional details for *Caloplaca lecanorae*. Financial support for AS was provided by the European Regional Development Fund (Centre of Excellence EcolChange) and an Estonian Research Council grant (PRG1170). Rasmus Puusepp (Tartu) is thanked for laboratory work. Fieldwork in Tasmania where the new species was discovered was supported by Jane and Tom Teniswood (Wind Song, Tasmania) and the Friends of the Tasmanian Museum and Art Gallery.

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