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**Abstract:** A new lichen species, *Tephromela baudiniana* Kantvilas & Elix, is described from coastal rocks on Kangaroo Island, South Australia. It is characterised chiefly by its unique chemistry, comprising the rare metabolites, 9-O-methylalternariol and alternariol. The species is compared with other saxicolous species of the genus occurring in Australia.

Keywords: alternariol, biodiversity, lichens, new species, South Australia, Tephromelataceae

## Introduction

This paper is a further contribution to the documentation of the lichens of Kangaroo Island, South Australia (Elix & Kantvilas 2013a, b; Kantvilas 2016; Kantvilas & Elix 2014; Kantvilas & Kondratyuk 2013; Kantvilas & van den Boom 2013, 2015; Kantvilas & Wedin 2015; McCarthy & Kantvilas 2013a, b, 2016 a, b), and describes a new species of the cosmopolitan genus Tephromela M.Choisy (Tephromelataceae). Tephromela is characterised by a crustose thallus with a unicellular, trebouxioid photobiont, apothecial ascomata, eight-spored, Biatora- to Lecidella-type asci, and simple, hyaline, non-halonate ascospores; the hymenium is invariably pigmented a diagnostic crimson, referred to as atra-red by Meyer & Printzen (2000). Detailed descriptions and illustrations of the genus and a discussion of its affinities are provided by Fryday (2011), Spribille et al. (2011), Kantvilas (2015) and many others, and are not repeated here. Species of Tephromela may be lichenicolous or occur freeliving on a wide range of substrata, including rocks, bark and wood. They tend to be defined by thalline characters (e.g. presence or absence of soredia, etc.), apothecial anatomy and morphology, ascospore size and thallus secondary chemistry. The apothecia can be highly variable, ranging from immarginate to biatorine to "lecanorine". The seemingly lecanorine apothecial margin, a feature of the many species ascribed to the T. atra group, has been interpreted by Spribille et al. (2011) and Kantvilas (2015) as a thalline cushion that subtends the apothecium and may envelop it at the margins.

Variation in the widespread *T. atra* group was investigated at a global scale by Muggia *et al.* (2013), who found that DNA sequence data do not support

some of the phenotypically distinct entities that have been described as species. They considered that some of the variability with respect to apothecial and ascospore size, substratum preference and thallus chemistry may be better interpreted as locally developed features that are part of the infra-specific diversity of one highly variable taxon. Subsequently, however, Cestaro et al. (2016) demonstrated that at least in one segregate of T. atra, the North American species T. pacifica Björk & Muggia, differences in chemical composition were underpinned by phylogenetic data. In Australia (and elsewhere), variation, particularly chemical variation, continues to be treated at species rank (see Kalb 2004, 2008; Elix 2012, 2013a, b; Elix & Kalb 2006, 2008) and its taxonomic importance requires further detailed investigation. The new species described below represents a further example of this variation.

# Material and methods

The study is based on collections by the first author housed in the Tasmanian Herbarium (HO). Anatomical and morphological observations were undertaken using light microscopy, with thin hand-cut sections mounted in water, 10% KOH, 50% HNO3, lactophenol cotton blue, ammoniacal erythrosin and Lugol's iodine, with and without pretreatment with KOH. Ascospore measurements are based on 30 observations and are presented in the format: least value–*average*–highest value. Chemical composition was investigated by thinlayer chromatography using standard methods (Orange *et al.* 2001; Elix 2014). Nomenclature of ascus types essentially follows Hafellner (1984) and Kantvilas (2009); that of pigments follows Meyer & Printzen (2000).

## Tephromela baudiniana Kantvilas & Elix, sp. nov.

*Tephromelae atrae* (Hudson) Hafellner similis sed 9-O-methylalternariolum et alternariolum, substantias chemicalias adhuc in *Tephromela* ignotas, continenti differt.

**Typus:** South Australia, Kangaroo Island: Rocky River Track, c. 250 m from mouth of river, 35°58'S 136°39'E, 10 m alt., on coastal rocks on the bank of a fast-flowing fresh-water stream, 25 Sep. 2015, *G. Kantvilas* 511/15 & B. de Villiers (holo: HO 581706; iso: AD).

### Mycobank number: MB820508.

Thallus free-living, markedly rimose-areolate, whitish grey, esorediate, forming extensive, irregular, rather discontinuous patches to 10 cm or more in extent, essentially comprised of numerous irregularly circular thalli c. 10 mm wide that coalesce; individual areoles irregularly rhomboid, 0.4-1.5 mm wide, 0.7-1 mm thick, plane, verruculose or bullate; prothallus not developed, but actively expanding thallus margins often discoloured dark bluish grey at the very edge. Apothecia scattered, roundish, basally constricted, "lecanorine", 0.5-1 mm wide; disc black, glossy, plane to undulate to convex, epruinose; thalline "margin" typically prominent but occasionally not developed, entire, often slightly inrolled, persistent, concolorous with the thallus, in section to 100-200 µm thick. Proper excipulum to 20-60 (-100) µm thick, thickest at the base, becoming excluded at the edges, pale to intense yellow-brown, K± intensifying yellow, N+ intense brownish orange. Hypothecium to 20-45 µm thick, hyaline to pale yellowish, thickest in the centre of the apothecium, tapering towards the margins and sometimes ± excluded. Hymenium 90-130 (-170) µm thick, not inspersed or with occasional oil droplets, crimson red, K+ intensifying red, N+ orange (atrared), persistently coherent in water, K and N, with

an intensely *atra*-red pigmented epihymenial layer. *Paraphyses* 2–3 µm thick, stout, simple; apices not or gradually expanded to 6 µm wide, with a thick coat of gel and heavily caked and conglutinated with pigment. *Asci* clavate, 43–60 × 12–18 µm, of the *Biatora*-type: having a well-developed amyloid tholus, a masse axiale with ± parallel flanks and a rounded apex, and a darker, more intensely amyloid edge adjacent to the masse axiale. *Ascospores* hyaline, ellipsoid, 8–10.7–13 × 4.5– 6.4–9 µm. *Pycnidia* not found. **Fig. 1.** 

*Chemistry.* Thallus K+ yellow, C–, PD–, UV+ blue; containing atranorin (minor), 9-*O*-methylalternariol (major) and alternariol (minor).

*Etymology.* The specific epithet commemorates the French navigator Nicolas Baudin who made the second European landing on Kangaroo Island in 1802 (including just to the north of type locality) and who charted and named many of the island's features.

**Remarks.** Although Muggia *et al.* (2013) questioned the rigorous application of chemical characters in the delimiation of *Tephromela* species, this criterion underpins the taxonomy of the genus in Australia where 27 species are recorded (McCarthy 2016); see Elix (2013a) for an identification key. It is the unusual secondary chemistry of the new species that distinguishes it unequivocally from other, morphologically highly similar, saxicolous species (Table 1). *Tephromela baudiniana* is chemically unique within the genus. Alternariol and 9-O-methylalternariol occur very rarely as major metabolites in lichens and are known as such only in *Pertusaria praecipua* A.W.Archer & Elix from Papua New Guinea (Archer & Elix 1998). This chemical character is highly significant. Alternariol is derived biosynthetically from a single, non-reduced



Fig. 1. Tephromela baudiniana (holotype). A Habit, scale = 2 mm; B Detail, scale = 1 mm.

	secondary chemistry (key compounds)	asexual propagules	apothecial morphology	ascospore size	distribution
<b>T. arafurensis</b> Rambold	atranorin, perlatolic and glomelliferic acids	esorediate	± immarginate to "lecanorine"	$9-14 \times 5-7 \ \mu m^1$	northern Australia (NT, WA)
<b>T. atra</b> (Huds.) Hafellner	atranorin, $\alpha$ -collatolic, alectoronic and bourgeanic (±) acids	esorediate	"lecanorine"	$11-17 \times 5.5-9 \ \mu m^2$	widespread, cosmopolitan
<b>T. baudiniana</b> Kantvilas & Elix	atranorin, alternariol, 9-O-methylalternariol	esorediate	"lecanorine"	8–13 × 4.5–9 μm	Kangaroo Is (SA)
<b>T. buelliana</b> (Müll.Arg.) Kalb	atranorin, α-collatolic acid		± immarginate to "lecanorine"	$10-14 \times 5.5-9  \mu m^3$	SA, Vic, Brazil
<b>T. granularis</b> Kantvilas	atranorin, $\alpha$ -collatolic, alectoronic and bourgeanic (±) acids	sorediate	"lecanorine"	$1015\times510\mu\text{m}^2$	Tas
<b>T. korundensis</b> (Räsänen) Kalb	atranorin	esorediate	$\pm$ immarginate	$6.512\times69\mu\text{m}^{1}$	NE QId
<b>T. lillipillensis</b> Elix	atranorin, alectoronic acid	esorediate	"lecanorine"	$7.5-14  imes 6-7.5 \ \mu m^{1}$	NSW, Qld
<b>T. promontorii</b> (Zahlbr.) Kalb	atranorin, α-collatolic and alectoronic acids	esorediate	± immarginate	$713  imes 5.57  \mu m^4$	SA, NT, Vic, South Africa
* <b>T. skottsbergii</b> (Darb.) Fryday	atranorin, α-collatolic and alectoronic acids	esorediate	"lecanorine"	$10-17 \times 5-8 \ \mu m^1$	Qld, NSW, Chile
T. stenosporonica Elix & Kalb	atranorin, stenosporonic acid	esorediate	"lecanorine"	$11-13 \times 7-9 \mu\text{m}^1$	northern Australia (NT, WA)
T. territoriensis Elix & Kalb	atranorin, physodic acid	esorediate	"lecanorine"	$7.58\times56.5~\mu\text{m}^1$	NT

Table 1. Salient features of saxicolous, free-living species of Tephromela in Australia.

Data sources. <sup>1</sup> Elix (2009); <sup>2</sup> Kantvilas (2015); <sup>3</sup> Vainio (1890); <sup>4</sup> Elix (2013a).

\*T. skottsbergii is characaterised further by having an hymenium inspersed with oil droplets.

polyketide chain, whereas  $\alpha$ -collatolic and alectoronic acids, found in the widespread, morphologically similar *T. atra*, are derived from two partially reduced polyketide chains (Armaleo *et al.* 2011; Saha *et al.* 2012).

The new species has proved to be technically challenging to study. Although seemingly abundantly fertile, most apothecia sectioned proved to be devoid of mature asci or almost so, and literally dozens of sections were required to compile the observations necessary for a comprehensive description. Kantvilas (2015) noted that the ascospores of *Tephromela* may vary significantly in size, depending on their stage of development. In the present work, most measurements were of relatively young spores still within the ascus, and so their dimensions may have been underestimated. The thallus of the new species included numerous crystals detached from the rock substrate, which protrude through the upper surface or are enclosed within the medulla. We have not observed this in previous studies of the genus, even in collections from highly crystalline rocks such as granite, and it is not clear whether this is a product of the species or the substrate. No pycnidia were located, and the abundant speck-like features on the surface of the thallus proved to be mainly apothecial initials or crystalline inclusions.

Morphologically, *T. baudiniana* falls within the very broad range of variation exhibited by *Tephromela atra*, which is characterised by containing atranorin plus  $\alpha$ -collatolic and alectoronic acids, usually together with bourgeanic acid. However, the very "chinky" rimose thallus, the absence of a prothallus and the relatively

small apothecia place it at the extreme of variation for that species.

**Distribution and ecology.** The new species is known only from the type locality where it was locally very abundant. It grew on dry, exposed siliceous rocks lining the course of a fast-flowing fresh water stream, but close to its mouth and therefore also subject to marine influences. Thus associated lichen species included truly littoral species such as *Caloplaca gallowayi* S.Y.Kondr., Kärnefelt & Filson, *Catillaria austrolittoralis* Kantvilas & van den Boom and *Opegrapha spodopolia* Nyl., as well as species more typical of inland, non marine habitats, for example, *Xanthoparmelia australasica* D.J.Galloway and *Amandinea* spp.

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#### References

- Archer, A.W. & Elix, J.A. (1998). The lichen genus *Pertusaria* (lichenised Ascomycotina) in Papua New Guinea: three new species and two new reports. *Mycotaxon* 69: 311–318.
- Armaleo, D., Sun, X. & Culberson, C.F. (2011). Insights from the first putative biosynthetic gene cluster for a lichen depside and depsidone. *Mycologia* 103: 741–754.

- Cestaro, L., Tønsberg, T. & Muggia, L. (2016). Phylogenetic data and chemical traits characterize a new species in the lichen genus Tephromela. Herzogia 29: 383-402.
- Elix, J.A. (2009). Tephromela. Flora of Australia 57: 74-83.
- Elix, J.A. (2012). New species and a new record of Tephromela (lichenized Ascomycota) from Australia. Australasian Lichenology 71: 3–11.
- Elix, J.A. (2013a). Further new species and new records of Tephromela (lichenized Ascomycota) from Australia. Australasian Lichenology 72: 22–31.
- Elix, J.A. (2013b). New crustose lichen taxa (lichenized Ascomycota) from Australia. Australasian Lichenology 73: 45-54.
- Elix, J.A. (2014). A catalogue of standardized thin-layer chromatographic data and biosynthetic relationships for lichen substances, 3rd edn. (Published by the author: Canberra).
- Elix, J.A. & Kalb, K. (2006). Two new species of Tephromela (Lecanoraceae, lichenized Ascomycota) from Australia. Australasian Lichenology 58: 27-31.
- Elix, J.A. & Kalb, K. (2008). Additional new lichen taxa (lichenized Ascomycota) from Australia. Australasian Lichenology 63: 30-36.
- Elix, J.A. & Kantvilas, G. (2013a). New taxa and new records of Amandinea (Physciaceae, Ascomycota) in Australia. Australasian Lichenology 72: 3–19.
- Elix, J.A. & Kantvilas, G. (2013b). New taxa and new records of Buellia sensu lato (Physciaceae, Ascomycota) in Australia. Australasian Lichenology 73: 24–44.
- Fryday, A.M. (2011). New species and combinations in Calvitimela and Tephromela from the southern subpolar region. Lichenologist 43: 225-239.
- Hafellner, J. (1984). Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien Lecanoraceae und Lecideaceae. Beihefte zur Nova Hedwigia 79: 241-371.
- Kalb, K. (2004). New or otherwise interesting lichens. II. Bibliotheca Lichenologica 88: 310-329.
- Kalb, K. (2008). New or otherwise interesting lichens. IV. Sauteria 15: 239-248.
- Kantvilas, G. (2009). The genus Mycoblastus in the cool temperate Southern Hemisphere, with special reference to Tasmania. Lichenologist 41: 151–178.
- Kantvilas, G. (2015). Observations on the genus Tephromela (lichenised Ascomycetes) in Tasmania, with the description of a new species. Herzogia 28: 430-444.
- Kantvilas, G. (2016). A synopsis and key for the lichen genus Caloplaca on Kangaroo Island, with the description of two new species. Journal of the Adelaide Botanic Gardens 29: 53-69.
- Kantvilas, G. & Elix, J.A. (2014). Additions to the genus Lecidella (lichenised Ascomycetes: Lecanoraceae). Journal of the Adelaide Botanic Gardens 27: 41-45.
- Kantvilas, G. & Kondratyuk, S.Y. (2013). New species of Caloplaca (lichenized Ascomycetes: Teloschistaceae) from Kangaroo Island. Journal of the Adelaide Botanic Gardens 26: 9–14.

- Kantvilas, G. & van den Boom, P.P.G. (2013). A new saxicolous species of Catillaria (lichenized Ascomycetes: Catillariaceae) from Southern Australia. Journal of the Adelaide Botanic *Gardens* 26: 5–8.
- Kantvilas, G. & van den Boom, P.P.G. (2015). Observations on some calcicolous species of Lecania A.Massal. (lichenised Ascomycetes: Ramalinaceae) in southern Australia. Journal of the Adelaide Botanic Gardens 29: 15–21.
- Kantvilas, G. & Wedin, M. (2015). Lichenicolous species of the Ascomycete genus Arthonia Ach. from Kangaroo Island. Journal of the Adelaide Botanic Gardens 29: 1-6.
- McCarthy, P.M. (2016). Checklist of the lichens of Australia and its island territories. (Australian Biological Resources Study: Canberra). Version 22 January 2016. http://www.anbg.gov. au/abrs/lichenlist/introduction.html. [accessed: 20 March 2017].
- McCarthy, P.M. & Kantvilas, G. (2013a). Psoroglaena halmaturina sp. nov. (lichenized Ascomycota: Verrucariaceae) from Kangaroo Island, South Australia. Journal of the Adelaide Botanic Gardens 26: 1-4.
- McCarthy, P.M. & Kantvilas, G. (2013b). Two new species of Sarcogyne (lichenized Ascomycota: Acarosporaceae) from central and southern Australia. Journal of the Adelaide Botanic Gardens 26: 15-21.
- McCarthy, P.M. & Kantvilas, G. (2016a). Thelidium robustum sp. nov. (lichenized Ascomycota: Verrucariaceae) from Kangaroo Island, South Australia. Journal of the Adelaide Botanic Gardens 29: 37-40.
- McCarthy, P.M. & Kantvilas, G. (2016b). A new species of Anisomeridum (Monoblastiaceae) from Kangaroo Island, South Australia. Australasian Lichenology 79: 16-19.
- Meyer, B. & Printzen, C. (2000). Proposal for a standardized nomenclature and characterization of insoluble lichen pigments. Lichenologist 32: 571-583.
- Muggia, L., Pérez-Ortega, S., Fryday, A., Spribille, T. & Grube, M. (2013). Global assessment of genetic variation and phenotypic plasticity in the lichen-forming species Tephromela atra. Fungal Diversity 64: 233–251.
- Orange, A., James, P.W. & White, F.J. (2001). Microchemical methods for the identification of lichens. (British Lichen Society: London).
- Saha, D., Fetzner, B., Burkhardt, B., Podlech, J., Metzler, M., Dang, H., Lawrence, C. & Fischer, R. (2012). Identification of a polyketide synthase required for alternariol (AOH) and alternariol-9-methyl ether (AME). Formation in Alternaria alternata. PLoS ONE 7(7): e40564. doi:10.1371/journal. pone.0040564
- Spribille, T., Goffinet, B., Klug, B., Muggia, L., Obermayer, W. & Mayrhofer, H. (2011). Molecular support for the recognition of the Mycoblastus fucatus group as the new genus Violella (Tephromelataceae, Lecanorales). Lichenologist 43: 445-466.
- Vainio, E.A. (1890). Étude sur la classification naturelle et la morphologie des lichens du Brésil, II. Acta Societatis pro Fauna et Flora Fennica 7(2): 1–256.



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