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ROYAL BOTANIC GARDENS VICTORIA

A remarkable species of *Vigneronia* (Roccellaceae *s. lat.*) from the remote southern coast of Tasmania

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Abstract

Vigneronia meridionalis Kantvilas & P.M.McCarthy is described from littoral, siliceous rocks on the South Coast of Tasmania. It is characterised by its robust, solitary or clustered, carbonised lirellae, sparingly branched and anastomosed paraphysoids, *grumulosa*-type asci, 3-septate, non-halonate ascospores, filiform-arcuate conidia, the absence of secondary lichen metabolites, and its saxicolous habit.

Key words: Australia, Lecanographaceae, lichens, lirellae, new species

Introduction

The lichen family Roccellaceae *s.lat.* (including Lecanographaceae as defined by Frisch *et al.* 2014) is well represented in Tasmania, with several genera having been the focus of recent taxonomic study; for example, *Angiactis* (Kantvilas *et al.* 2020a), *Cresponea* (Kantvilas 2020a), *Enterographa* (Kantvilas 2022a), *Lecanactis* (Kantvilas 2022b), *Lecanographa* (Kantvilas 2004), *Mazosia* (Kantvilas 2020b) and *Ocellomma* (Kantvilas *et al.* 2020b). As elsewhere in the world, most of its Tasmanian representatives are comparatively rare taxa or those with distinctive ecologies and distributions. Many species are restricted to climax vegetation types such as rainforest (e.g. *Cresponea subpremnea* (Kantvilas & Vězda) Kantvilas, *Lecanactis abietina* (Ach.) Körb., *L. mollis* (Stirt.) Frisch & Ertz and *Mazosia corticola* Kantvilas) or coastal swampy woodlands (e.g. *Enterographa micrographa* (Nyl.) Redinger), to old individuals of specific tree and shrub species (e.g. *Lecanactis latispora* Egea & Torrente, *Lecanographa nothofagi* Kantvilas, *Ocellomma rediuntum* (Stizenb. ex Hasse) Kantvilas *et al.*), or to highly sheltered niches in vegetation otherwise subject to periodic

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disturbance, especially from fire (e.g. *Chiodecton montanum* Thor, *Cresponea graemeannae* Kantvilas, *Lecanactis scopulicola* Kantvilas).

Many of the traditional genera of the Roccellaceae have been the focus of intensive research, leading to their subdivision into more natural units. Much of the earlier work was based principally on cladistic analyses of morphological and anatomical characters (e.g. Tehler 1990, 1992, 1993). However, the current classification of the Roccellaceae and allied groups rests heavily on molecular analyses, in particular on the work of Ertz & Tehler (2011), Frisch *et al.* (2014) and Ertz *et al.* (2014), with the last in particular describing (or reinstating) as distinct genera several groups from such classical units as *Lecanactis*, *Opegrapha* and *Schismatomma*. Even so, placing individual species into these genera is usually challenging due to the dearth of consistent anatomical, morphological or chemical markers for most of these segregates.

The focus of this paper is a remarkable saxicolous species collected from the remote coast of southern Tasmania. The material is 35 years old and unsuitable for DNA analysis. Consequently, our investigation has relied entirely on a comparative evaluation of morphological, anatomical and chemical data. In order to bring this species to the attention of other lichenologists and local land managers, we tentatively place it in *Vigneronia*, a genus of five species already known from widely scattered locations that include the Galapagos Islands, the Caribbean, Mexico, Chile and Brazil (Ertz *et al.* 2014; Herrera-Campos *et al.* 2019).

Material and methods

The study is based on a collection by GK that is housed in the Tasmanian Herbarium (HO), and comparisons with published descriptions and reference exsiccata from other regions. Anatomical observations and measurements are based on thin, hand-cut sections of the thallus, apothecia and pycnidia, mounted in water, 10% KOH (K), Lugols Iodine (I), ammoniacal erythrosin and/or Lactophenol Cotton Blue. Application of Lugols Iodine after pretreatment with KOH is abbreviated as KI. The presence of calcium oxalate was established by eluting thin microscope sections with 20% H_2SO_4, which causes a precipitate of needle-like crystals. Ascospore measurements are presented in the format 5th percentile–*average*–95th percentile, with outlying values in brackets and *n* signifying the number of observations. Chemical composition of the new taxon was investigated by thin-layer chromatography using standard methods and solvent A (Orange *et al.* 2010).

For the DNA work, several lirellae were transferred to a Lysing Matrix A tube (MP Biomedicals, Seven Hills, NSW, Australia). The material was ground on a Precellys Evolution (Bertin Instruments, Montigny-le-Bretonneux, France). DNA was then extracted using a phenol-chloroform DNA extraction protocol modified from Zolan & Pukkila (1986), as described in Gueidan *et al.* (2007). Amplifications were attempted on two nuclear ribosomal gene regions: ITS and LSU. For each amplification, raw extract, 1/10 dilution and a 1/100 dilution were used. For ITS, fragments of various sizes were amplified using the primer pairs ITS1F-ITS4, ITS1F-ITS2 and ITS3-ITS4 (Gardes & Bruns 1993; White e*t al.* 1990). For LSU, fragments of various sizes were amplified using the primer pairs LR0R-LR7, LR0R-LR5 and LR0R-LR3 (Vilgalys & Hester 1990). None of the amplifications generated a PCR product visible on gel electrophoresis.

Taxonomy

Vigneronia meridionalis* Kantvilas & P.M.McCarthy, *sp. nov.

MycoBank no. MB860032

Characterised by its saxicolous habit, a thallus lacking secondary lichen metabolites, the carbonised, robust lirellae to 1 mm long and to 0.5 mm wide, occurring singly or in elevated clusters, the sparingly branched and anastomosed paraphysoids, the *grumulosa*-type asci, 3-septate, non-halonate ascospores, 17–28 × 3–5 µm, and the filiform-arcuate conidia, 13–20 × *c.* 0.5 µm.

***Type*: Australia:** Tasmania, South Coast, Prion Beach, 43°32′S, 146°33′E, at sea level, on siliceous rocks, 27 Feb. 1990, *G.Kantvilas 51/90* (holo - HO 00325360).

Thallus epilithic, crustose, effuse, off-white to pale grey or pale grey-brown, patchily pale yellowish brown or rather cream-coloured, dull, continuous to sparingly rimose, smooth to minutely and irregularly uneven, not forming areoles, (0.1–)0.2–0.4(–0.5) mm thick, thickest beneath clusters of ascomata, containing calcium oxalate, ecorticate. *Photobiont Trentepohlia*, dominating a layer *c.* 100 µm thick when adjacent to clusters of ascomata, this subtended by a white, I- medulla 100–300 µm thick; individual cells 10–15 × 8–12 µm, solitary or

in filaments of up to six cells; interstitial hyphae 2–3 µm thick. *Prothallus* not apparent. *Ascomata* moderately numerous, lirelliform, solitary or clustered; solitary lirellae brown-black to dull black, semi-immersed in the thallus, occasionally more prominent, to 1 mm long and 0.5 mm wide, simple or sparingly branched, epruinose, with blunt ends and an inconspicuous disc that is usually slit-like or occasionally slightly open; ascomatal clusters prominent on small cushions of thallus tissue, rounded to somewhat irregular, 1–1.5 (–2.5) mm wide, each with up to 3–5(–7) contiguous or overlapping lirellae. *Thalline exciple* absent. *Proper exciple* thick, black, closed below the hypothecium; in solitary ascomata 80–150 µm thick laterally, 140–250 µm thick at the base, K+ greenish when very thinly sectioned. *Epihymenium* hyaline to pale brown, 10–20 µm thick. *Hypothecium* hyaline to pale brown, 25–50 µm thick, inspersed with oil droplets. *Hymenium* 80–120 µm thick, not inspersed with granules or oil droplets, I+ soon becoming red-brown, KI+ medium blue. *Paraphysoids* sparingly branched and anastomosed (especially distally), long-celled, (1–)1.5–2(–2.5) µm thick; apices neither swollen nor pigmented. *Asci* 52–70 × 13–16 µm [*n* = 20], approximating the *grumulosa*-type (after Egea & Torrente 1994): cylindrical, 8-spored, with walls and tholus essentially non-amyloid except for a highly reduced amyloid ring, visible when young surrounding a minute ocular chamber; stalk gradually tapering. *Ascospores* hyaline, 3-septate, biseriate or irregularly massed in the ascus, narrowly oblong to fusiform, slightly to strongly curved (most submature and some mature ascospores straight), not constricted at the septa, (17–)19.5–*23.3*–27(–28) × (3–)3.5–*3.9*-4.5(–5) µm [*n* = 68]; cells of ± equal size throughout spore ontogeny (i.e. microcephalic); perispore lacking; apices ± rounded, occasionally subacute; contents clear or minutely granular, occasionally guttulate. *Pycnidia* moderately numerous, black, semi-immersed, solitary, convex, conical or hemispherical, *c.* 0.1 mm wide. *Conidia* hyaline, simple, filiform, usually arcuate, 13–20 × *c.* 0.5 µm, with blunt apices. *Chemical composition*: no substances detected by TLC. Figs 1 & 2.

Etymology

The specific epithet, meaning 'southern', alludes to the geographic distribution of the species.

Taxonomic position

Freshly collected specimens (up to 12 months old) appear to be mandatory for obtaining usable DNA extracts from Arthoniales taxa (e.g. see Ertz & Tehler 2010; Frisch *et al.* 2014), and so, not surprisingly, our efforts to obtain molecular data from the single, 35 year-old specimen were unsuccessful. Thus, the taxonomic classification of the new species relies solely on an evaluation of morphological and anatomical characters.

Several genera in the Roccellaceae *s. lat.* develop lirellate ascomata but, in most, including *Enterographa* and some species of *Lecanographa*, *Schismatomma* and *Paralecanographa*, the exciple is not, or only partly or weakly carbonised. However, in both *Gyrographa* and *Vigneronia*, exciple carbonisation is extreme and complete to the extent that several species currently placed in these genera were originally included in *Opegrapha* and removed principally on the basis of molecular data (Ertz *et al.* 2014). These authors referred to them as "roccelloid *Opegrapha* species", differing anatomically from *Opegrapha* by their asci and non-halonate ascospores, and it was on these genera that we concentrated our attention. Both have a carbonised, K± olive exciple and 3-septate, non-halonate ascospores. *Gyrographa* is further characterised by having clustered ascomata with ± open lirellae and a gyrose disc, as well as shortly bacilliform conidia, 4–7 x 0.3–0.8 µm, with gyrophoric acid present in some species (Ertz *et al.* 2014; McCarthy 2022). *Vigneronia* differs by having closed lirellae, a non-gyrose disc and filiform conidia, and its chemistry can include gyrophoric, lecanoric and/or roccellic acids and erythrin; pruinose ascomata are common (Ertz *et al.* 2014, Herrera-Campos *et al.* 2019). In our view, the closed lirellae and filiform conidia of our species suggest its affinities are closest to *Vigneronia*.

Asci have proved to be a very useful diagnostic character in the Roccellaceae since the work of Egea & Torrente (1994), and these have been applied with success in studies of Tasmanian taxa (e.g. Kantvilas 2004). The *grumulosa*-type ascus was first described by Egea & Torrente (1994) and is based on the widespread European/Macaronesian taxon *Paralecanographa grumulosa* (Dufour) Ertz & Tehler (≡ *Lecanographa grumulosa* (Dufour) Egea & Torrente) (see Egea *et al.* 1993; Egea & Torrente 1994). It is cylindrical to narrowly clavate, with a thin, faintly KI+ blue or KI- wall, a slightly

thickened tholus and, when young, a small ocular chamber. In young asci, there is a small, faintly amyloid ring enveloping the ocular chamber but, as the ascus develops, this becomes compressed into an amyloid band adjacent to the ascoplasm. This ascus type is illustrated by Egea & Torrente (1994) for *P. grumulosa* and by Kantvilas *et al.* (2020a) for *Angiactis banksiae* (Müll.Arg.) Kantvilas & Stajsic, with the latter source showing the development of the ascus as the amyloid ring becomes compressed.

In the Australian lichen biota, the *grumulosa*-type ascus is also seen in the recently described *Gyrographa fecunda* P.M.McCarthy from eastern New South Wales (McCarthy 2022). Unfortunately, in describing *Vigneronia*, Ertz *et al.* (2014) did not describe the asci in detail, whereas Herrera-Campos *et al.* (2019) provided only the most cursory and to some extent ambiguous description. Having discounted *Gyrographa* on anatomical/morphological grounds, we also briefly considered *Lecanographa* as a placement for the new

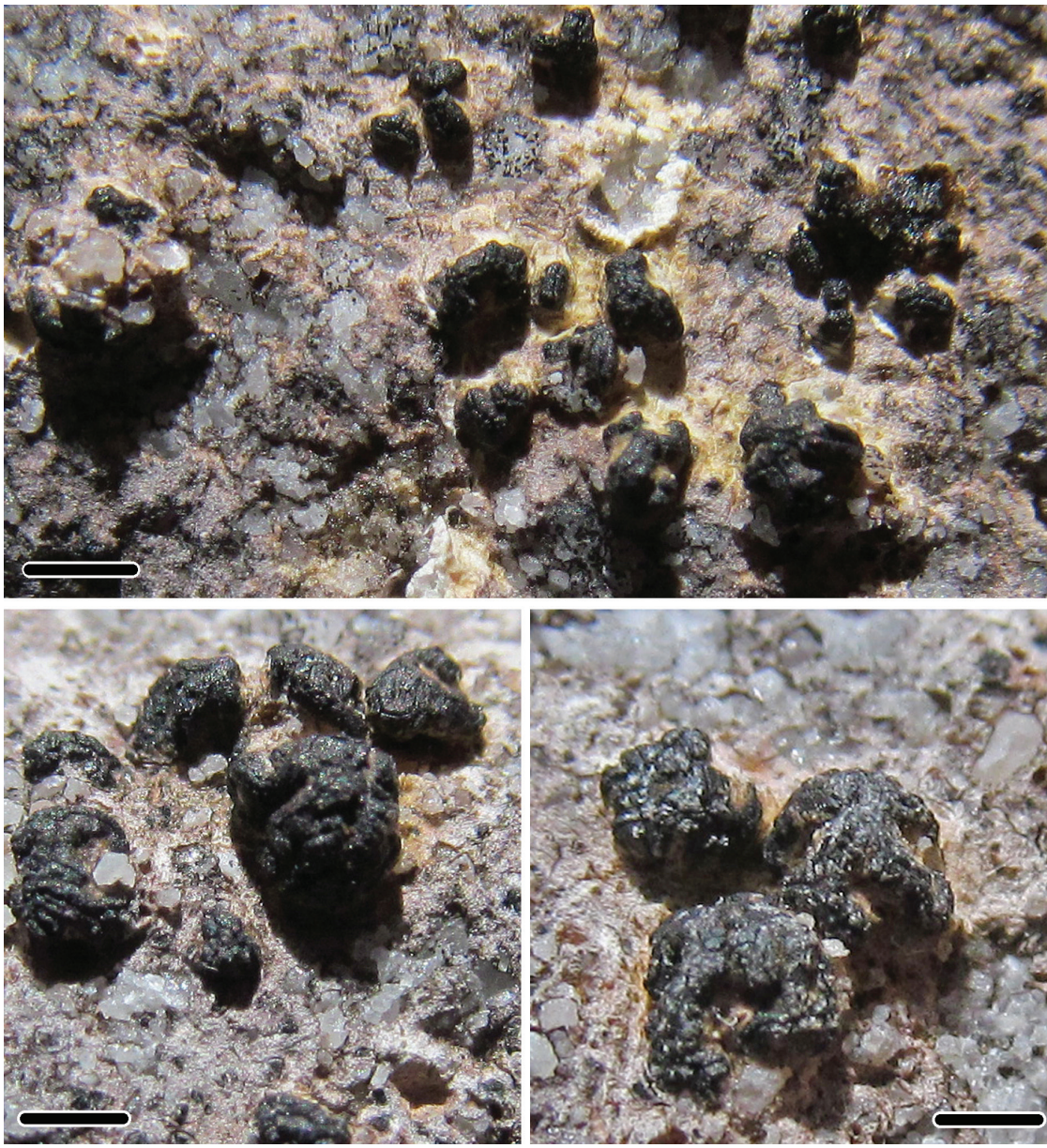

Figure 1. *Vigneronia meridionalis* (holotype). Thalli and mainly clustered ascomata. Scales: 1 mm.

species, not least because it is already represented in Australia by two taxa (McCarthy 2023). However, in this genus, as represented by the *L. lyncea* (Sm.) Egea & Torrente (the type species) as well as by the Tasmanian endemic *L. nothofagi* Kantvilas, the asci differ by lacking an amyloid ring throughout all stages of development. Thus, in the absence of any contradictory information, we elect to describe the new species in *Vigneronia* as a genus of best fit.

In contrast with the Tasmanian lichen, the other five known species of *Vigneronia* are corticolous in tropical and subtropical latitudes, and all display distinctive and quite different combinations of thallus chemical composition, ascomatal morphology and anatomy, as well as excipular, hymenial and ascospore dimensions. Thus, *V. spieri* (Aptroot & Sparrius) Ertz & Bungartz, *V. cypressi* (R.C.Harris) Ertz & Tehler and *V. mexicana* Herrera-Campos *et al*. all have thickly pruinose ascomata

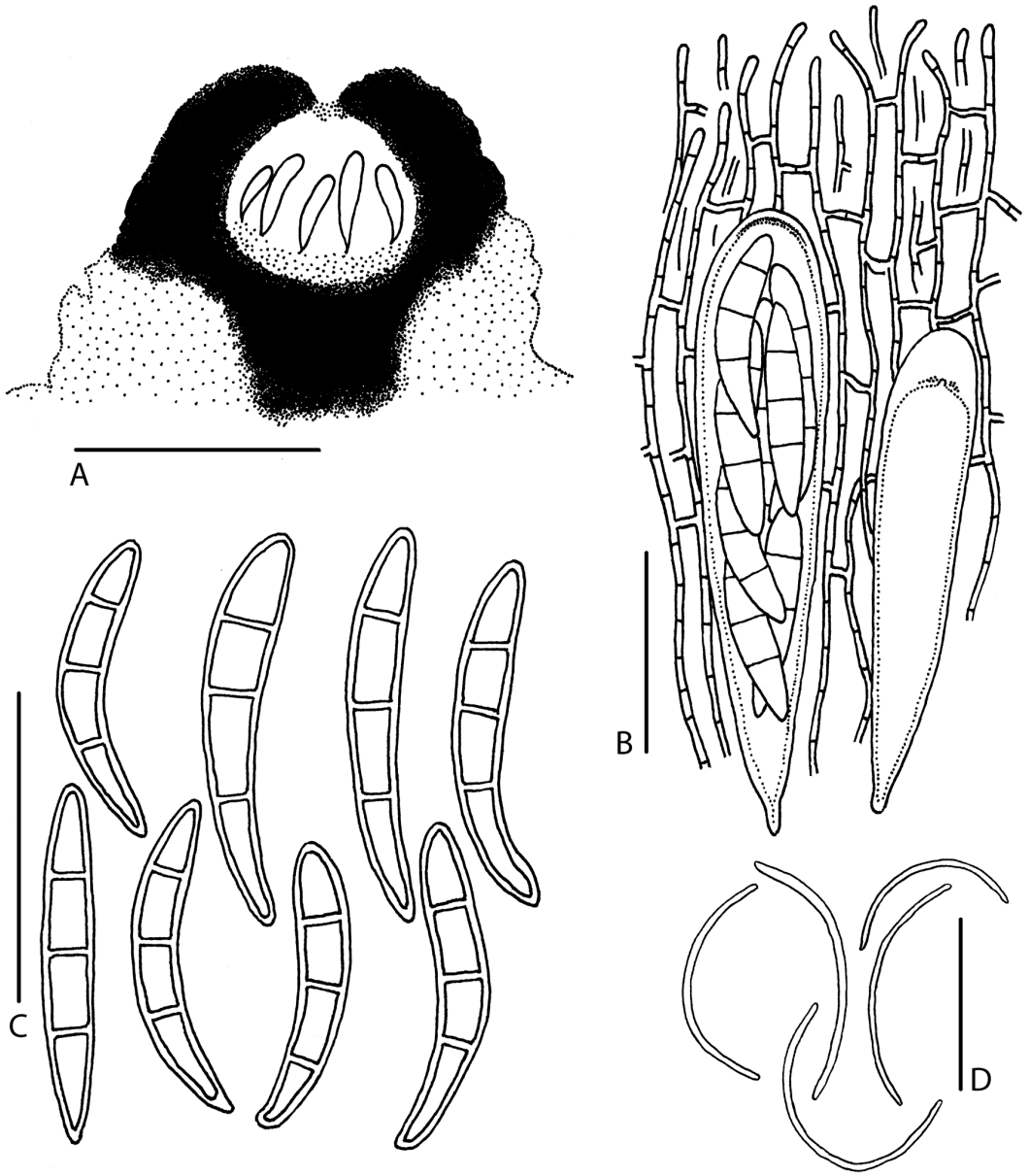

Figure 2. *Vigneronia meridionalis* (holotype). A: Sectioned ascoma (semi-schematic); B: Mature and immature asci and paraphysoids, with amyloid parts stippled; C: Ascospores; D: Conidia. Scales: A = 0.2 mm; B, C = 20 µm; D = 10 µm.

and contain erythrin with roccellic (±) and gyrophoric acids, roccellic acid (±) or lecanoric acid respectively (Ertz *et al.* 2015; Herrera-Campos *et al.* 2019). *Vigneronia robustula* (Nyl.) Ertz & Tehler and *V. caceresiana* (Kalb & Aptroot) Lücking & Herrera-Campos also have pruinose ascomata, but their chemical composition has not been determined (Ertz *et al.* 2015; Herrera-Campos *et al.* 2019). In this context, the new Tasmanian species is characterised by a unique suite of characters.

Distribution and ecology

The single specimen of *Vigneronia meridionalis* was collected fortuitously on siliceous, seashore rocks on Tasmania's remote South Coast and, despite extensive exploration of Tasmania's lichen flora, it has not been seen subsequently. The collection is from coarse, metamorphosed sedimentary rock, the type that characterises much of Tasmania's South-West. As no further ecological information can be gleaned from the specimen, this species remains one of several remarkable, rare or overlooked taxa that is known only from the type specimen. Although several other lirellate, saxicolous lichens (e.g. *Enterographa ophiolithica* Kantvilas, *Opegrapha spodopolia* Nyl.) can occur in littoral habitats in Tasmania, especially in sheltered overhangs, none are likely to be confused with the new species due to their ascospores having more than three septa.

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