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***Megalaria crispisulcans*, a new isidiate species of *Megalaria* (Ramalinaceae) from Aotearoa / New Zealand**

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Abstract

Megalaria crispisulcans A.J. Marshall, Blanchon & de Lange (Ramalinaceae) is described as a new species from populations in Te Ika a Māui / North Island of Aotearoa / New Zealand, and on Rēkohu / Wharekauri / Chatham Island, Aotearoa / New Zealand. The new species is morphologically closely allied to *Megalaria orokonuiana*, from which it is distinguished by its nrDNA ITS sequence and morphologically by the usual presence of a white prothallus, bright green (when fresh) isidiate thallus and dark black apothecia, whose rims are usually crinkled / undulose at maturity. A phylogeny based on nrDNA ITS sequences is presented that recovers *Megalaria crispisulcans* as sister to *M. orokonuiana*. The ecology, habitats and conservation status of *M. crispisulcans* is provided and an update on the conservation status of *M. orokonuiana* provided. A revised key to the Aotearoa / New Zealand species of *Megalaria* is provided.

Keywords

Megalaria; *M. orokonuiana* Fryday & A.Knight; *M. crispisulcans* A.J. Marshall, Blanchon & de Lange; Ramalinaceae; Aotearoa / New Zealand lichenised mycobiota

Introduction

During a survey of 50 lichen plots throughout the Tāmaki Makaurau / Auckland region of Aotearoa / New Zealand (Marshall et al. 2020), a sterile isidiate green crust was collected from several localities. These crusts had no sexual structures or demonstrable thallus chemistry, so we had been unable to put a name to the specimens. In 2020, sometime after initial sampling of the lichen plots and while engaged in a threatened-plant survey on the Maungaroa Track (Figure 1) at Piha in the Tāmaki Makaurau / Auckland region, a fertile specimen was found on nīkau (*Rhopalostylis sapida* H.Wendl. & Drude). Microscopic analysis of that specimen revealed spores similar to those described by Fryday & A.Knight (Fryday & Knight 2012) for *Megalaria orokonuiana* Fryday & A.Knight, a species then known from one location, in the

Orokonui Ecosanctuary, Otago, Te Wai Pounamu / South Island (Figure 1). However, critical examination of the Maungaroa Track specimen and *M. orokonuiana* found that the Maungaroa specimens usually possessed a marginal prothallus, had isidia that were longer and more slender than those of *M. orokonuiana*, and had different apothecial pigment distributions, indicating that it was a different species. Subsequently, more specimens of the new species were discovered at five other locations in the Waitākere Ranges, growing on mahoe (*Melicytus ramiflorus* J.R.Forst. & G.Forst. subsp. *ramiflorus*) and māmāngi (*Coprosma arborea* Kirk) (Figure 2). Further surveys and searches of herbaria have extended the range of the lichen to north Kaipara, Tauranga and south Kawhia in Te Ika a Māui / North Island and the Chatham Islands (Rēkohu / Wharekauri / Chatham Island) (Figure 1).

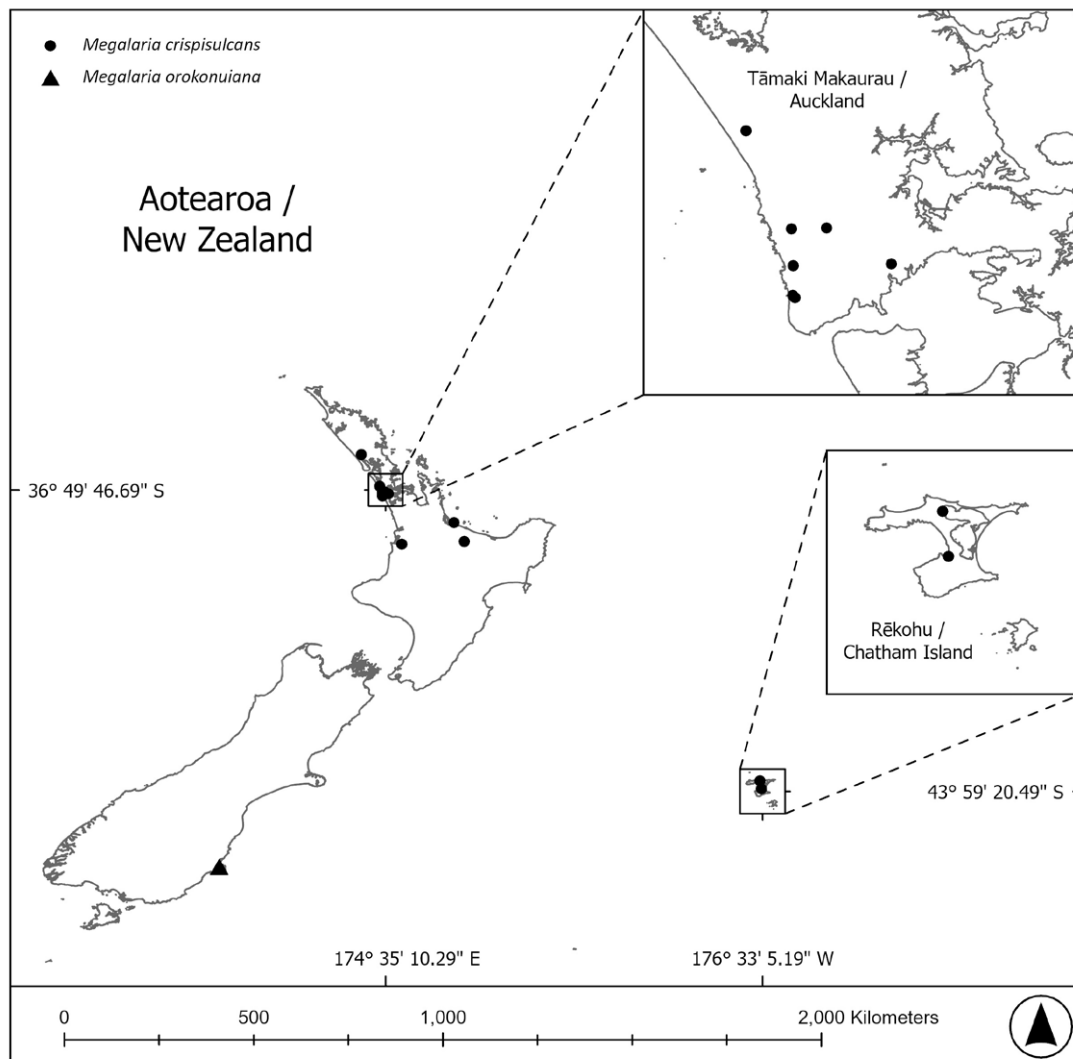


Figure 1. Map showing localities of occurrences of *Megalaria crispisulcans*. Type locality for *Megalaria orokonuiana* shown for reference.



Figure 2. Habit of *Megalaria crispisulcans*. (A) Growing on mahoe (*Melicactus ramiflorus* subsp. *ramiflorus*) and adjacent on Waitākere conglomerate, Zion Hill Track, Waitākere Ranges. Photo: A. J. Marshall, July 2021. (B) On māmāngi (*Coprosma arborea*) adjacent to the Houghton Track, Waitākere Ranges. Photo: A. J. Marshall, August 2021. (C) Covering much of the trunk of a large mahoe (*Melicactus ramiflorus* subsp. *ramiflorus*) at the type locality, Hautu Marama / Hodge's Basin, Woodhill. Photo: A. J. Marshall, May 2023.

On the basis of morphological, chemical and molecular differences, we here describe a new species, *Megalaria crispisulcans* A.J. Marshall, Blanchon et de Lange sp. nov. We also explore the phylogenetic relationships of *M. crispisulcans* in relation to other *Megalaria* species, assess the species ecology and propose a threat status using the New Zealand Threat Classification System (Townsend et al. 2008). An updated key to the *Megalaria* species presently recognised in Aotearoa / New Zealand is also provided. For ease of reading, the new species will be referred to as *Megalaria crispisulcans* from here on.

Materials and Methods

Specimens were examined with standard microscopic techniques. The chemical constituents were studied using thin-layer chromatography (Culberson 1972; White & James 1985) using solvent C. Apothecial pigments were studied using the methods of Meyer and Printzen (2000). Voucher specimens were deposited in UNITEC (Herbarium acronyms follow Thiers 2008–onwards), with duplicates of some specimens deposited at AK & MSC. DNA extraction and polymerase chain reaction (PCR) amplification were carried out using a modification of the protocol listed by Hayward et al. (2014). One locus, the nuclear nrDNA ITS, was used for this study utilising the primer pairs ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). Sequencing was performed by the Massey Genome Service (Te Kunenga ki Pūrehuroa / Massey University, Papaioea / Palmerston North, Aotearoa / New Zealand) using an Applied Biosystems model 3730 automated capillary DNA sequencer, using the same primers as for PCR. Forward and reverse reads were automatically aligned, manually curated and then concatenated in Geneious Prime v.2021.0.3.

Phylogenetic analysis

ITS sequences from other *Megalaria* species and outgroups within the *Ramalinaceae* family were downloaded from NCBI GenBank (Table 1). These sequences were aligned with the newly sequenced consensus sequences of the two isidiate Aotearoa / New Zealand *Megalaria* species (Table 1) using the MUSCLE algorithm with default parameters in Geneious Prime v.2021.0.3. On manual inspection of the alignment, there was significant variation between *Megalaria* species and between outgroups. This was due to frequent insertion-deletion (indel) events as well as nucleotide substitutions. Therefore, the alignment was manually edited for minor corrections, and ambiguously aligned regions within the final alignment were excluded using GBLOCKS v. 0.91b (Talavera & Castresana 2007) with the settings of 12 for the minimum number of sequences for a flank position, eight for the maximum number of contiguous non-conserved positions, and five for the minimum length of a block. This resulted in a final alignment consisting of 424 characters.

Pairwise sequence divergences were calculated using uncorrected p distance in MEGA v.11 (Tamura et al. 2021). The most appropriate model of nucleotide evolution was determined in PartitionFinder (Lanfear et al. 2017) using AICc. We consequently applied a general time-reversible model (Rodríguez et al. 1990) with a gamma distribution and a proportion of invariant sites (GTR + G + I) to all phylogenetic analyses. Phylogeny reconstruction was performed using Bayesian inference (BI) and maximum likelihood (ML). Markov Chain Monte Carlo sampling was conducted in MrBayes v.3.2.7 (Ronquist & Huelsenbeck 2003), assuming a GTR + G + I model with six rate categories. Two independent searches were performed using random starting trees and standard Dirichlet priors. Markov chains were run for one million generations, sampling the chains every 1,000 generations. The first 25% of trees ($n = 250$) were discarded as “burn-in”. We assessed convergence and adequate sampling of the posterior distributions by verifying that the standard deviations of split frequencies were below 0.01 and that all posterior distributions resulted in effective sample sizes (ESS) greater than 200. Trees ($n = 1,500$) from the two independent searches were combined and a 50% majority-rule consensus tree was created. The best-scoring ML tree was inferred with RaxML (Stamatakis 2006) implemented via the Geneious Prime plugin, with support determined using 1,000 bootstrap pseudoreplicates. ML and BI trees were visualised in FigTree v.1.4.4.

Results

The final alignment consisted of 424 characters from 22 sequences of 17 taxa. The best-scoring ML tree is shown in Figure 3. A 50% majority-rule consensus tree from a Bayesian analysis resolved a similar topology. However, the Bayesian analysis included a polytomy on the three basal *Megalaria* clades, while ML resolved *Megalaria pachaylenophila* and *Megalaria* sp. (GenBank Accession no. OP698027) as sister to the *M. crispisulcans* and *M. orokonuiana* clade with low support. For both methods, *Megalaria* was reconstructed as a monophyletic genus with significant divergence between species but with low support for some of the more basal clades, reflecting possible saturation or ambiguity within deeper branches of the tree at this gene region. Sequence divergence between the two specimens of *Megalaria crispisulcans* was 0.25%. *M. orokonuiana* and *M. crispisulcans* were resolved as clearly distinct sister taxa with an uncorrected p distance of 12.1% between them. However, support for this sister relationship was low, with bootstrap support and a posterior probability of 37% and 0.54 respectively

(Figure 3). There is a high degree of divergence within *Megalaria*, with sequence divergences ranging between 12% and 25%, while divergences between *Megalaria* and outgroup taxa were similar (range 11% to 28%). This significant divergence within *Megalaria* is consistent with findings from other phylogenetic studies (Ekman 2001, Kistenich et al. 2018, Wang et al. 2019, van den Boom & Magain 2020). The low support for clades within *Megalaria* and the substantial divergence between *Megalaria* species suggests that ITS by itself is insufficient to resolve relationships of taxa within *Megalaria* and its relatives. Nevertheless, our phylogeny demonstrates similar groupings of *Megalaria* taxa to those found previously with clades consisting of *M. laureri* and *M. grossa*, and another consisting of *M. alleniae*, *M. pulvereae*, *M. yunnanensis* and *M. phayapipakiana* recovered with high support. While our findings are sufficient to resolve *M. crispisulcans* as a new species, our analysis suggests that further investigation of the relationships within *Megalaria* at additional gene regions that are less prone to indel events, and with further taxa, would be beneficial.

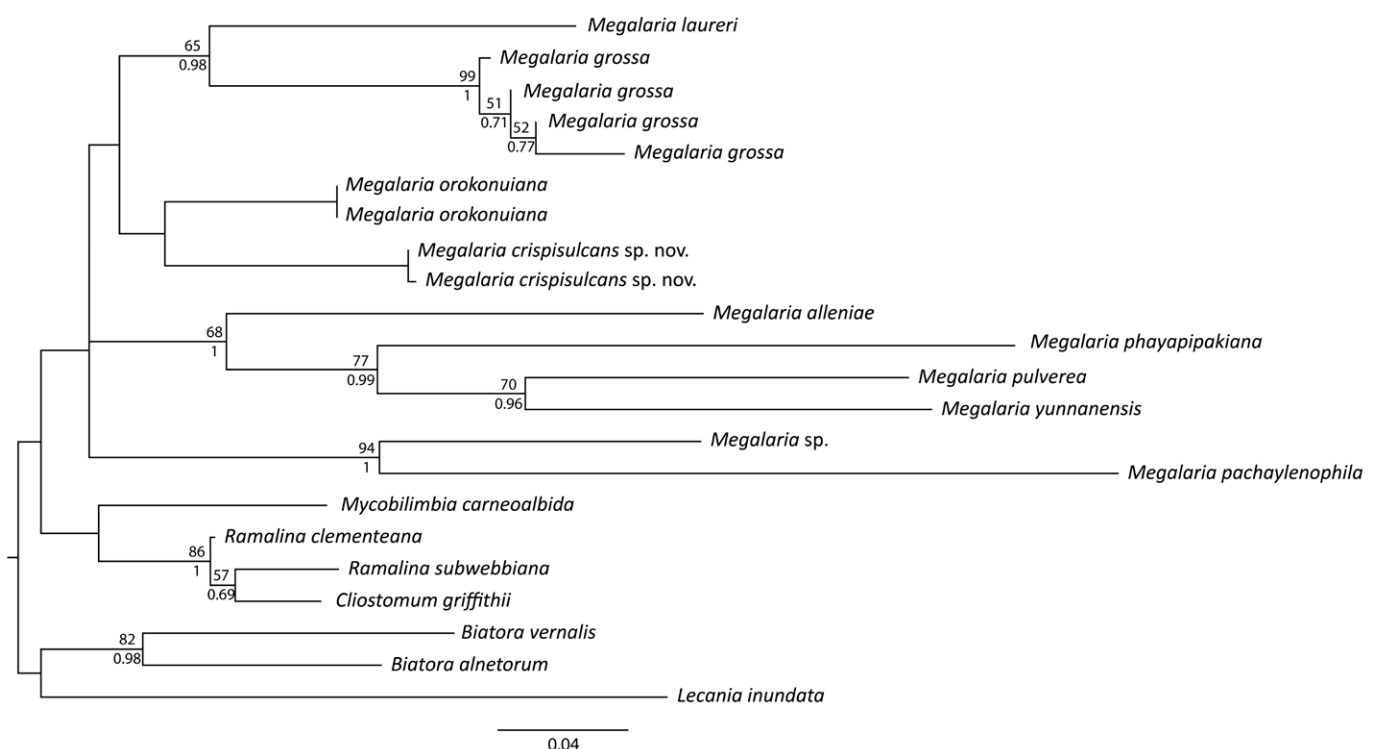


Figure 3. Maximum likelihood best-scoring phylogram of the *Megalaria* genus based on 424 characters of the ITS1, 5.8S, and ITS2 rRNA gene region and produced using RaxML. Support values are given in the order of maximum likelihood bootstrap support $\geq 50\%$ above the branches and Bayesian posterior probabilities ≥ 0.6 below the branches. Bootstrap and posterior probability values for the *M. crispisulcans* / *M. orokonuiana* clade were 37% and 0.54 respectively.

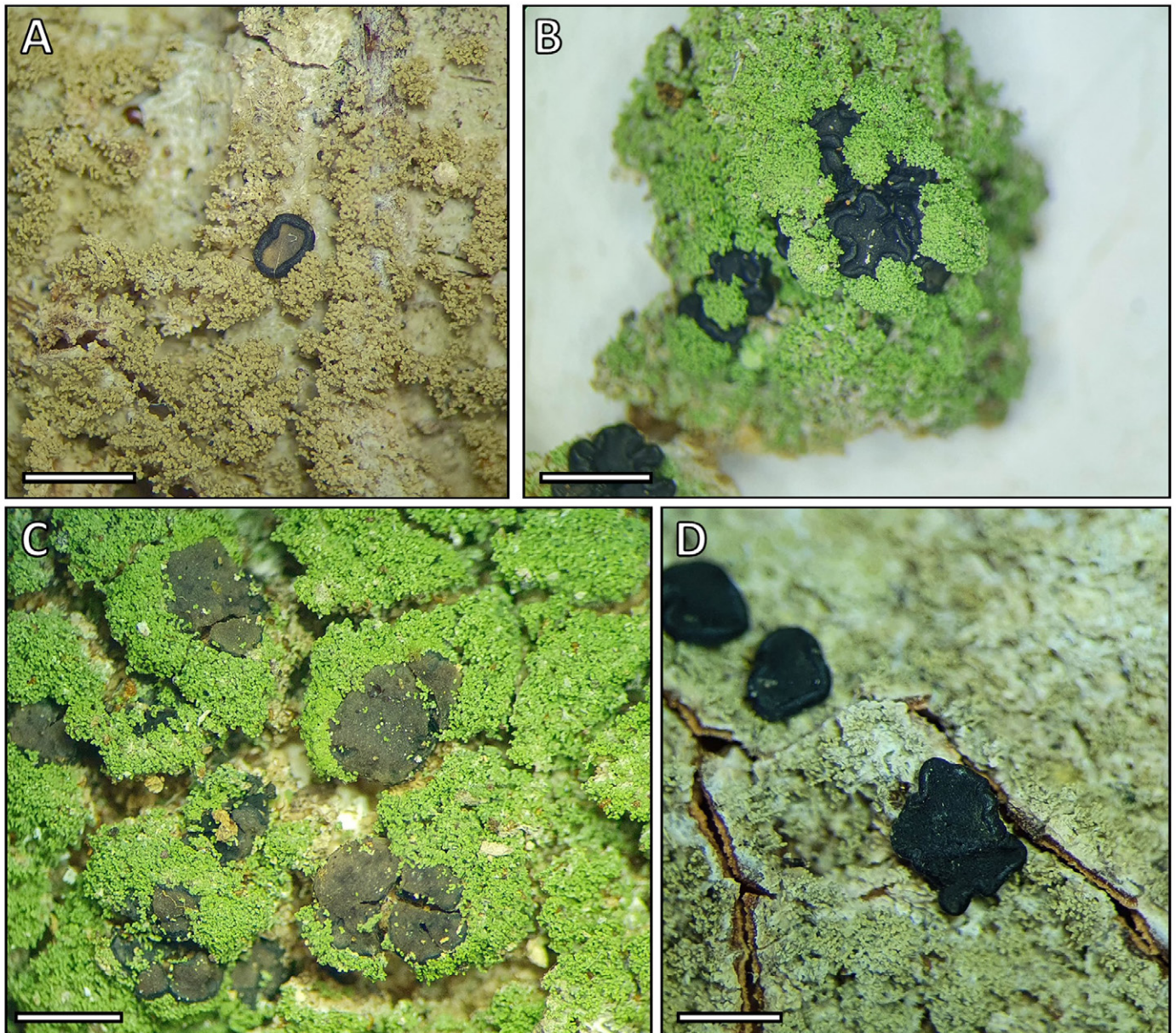


Figure 4. Variation in the thallus and apothecia of *Megalaria crispisulcans*. (A) A packeted specimen that had lost much of the vibrance in colour, becoming more muted over time. The apothecium is strongly cupular with a pronounced proper margin, scale = 1 mm. Photo: A. J. Marshall, August 2021. (B) A specimen with a highly contorted apothecial rim. The apothecia are often hidden beneath crowded isidia in the species, scale = 1 mm. Photo: A. J. Marshall, July 2023. (C) Crowded apothecia with pale epithecium, lacking a pronounced proper margin, scale = 1 mm. Photo: A. J. Marshall, August 2021. (D) Margins of apothecia are often irregular-crenulate and here the epithecium is black, concolourous with the proper margin, scale = 1 mm. Photo: A. J. Marshall, August 2021.

Taxonomy

Megalaria Hafellner, *Beihefte zur Nova Hedwigia* 79: 302 (1984)

Mycobank No. MB 25649

Megalaria crispisulcans A.J.Marshall, Blanchon et de Lange sp. nov.

Mycobank No. 849858

Holotype: New Zealand, North Island, Auckland, Hautu Marama / Hodge's Basin, 36°45'38.94 S 174°24'46.09 E, 58 m, bark, on mahoe, 12.v.2023, A.J. Marshall (AJM80) & C.D. Kilgour (UNITEC 14109. Isotype: AK).

Diagnosis: *Megalaria crispisulcans* is distinguished from other New Zealand *Megalaria* by its nrDNA ITS sequence (OR575319, OR57320), and morphologically by the normally present white prothallus, which extends up to

5 mm from the thallus; by the bright green (when fresh) isidiate thallus and dark black apothecia, whose rims are usually crinkled / undulose at maturity. It is most similar to *Megalaria orokonuiana*, from which it differs by the more prominent, coralloid rather than granular, isidia; these 0.05–0.07 mm diameter in *M. crispisulcans* and 0.1–0.15 mm diameter in *M. orokonuiana*, and 0.1–0.5 mm long in *M. crispisulcans* and 0.1–0.2 mm long in *M. orokonuiana*. The proper margins of the apothecia of *M. orokonuiana* when mature are usually immarginate, whereas those of *M. crispisulcans* are distinctly undulose contorted. The ascospores of *Megalaria crispisulcans* are 25–30(35) × (8–)12.5–15 µm, whereas those of *M. orokonuiana* are (20–)25–30 × 12–14 µm.

Etymology: The epithet “*crispisulcans*” refers to the often (but not always) undulating, crinkled apothecial rim of the species (Stearn 2004). Formed from ‘*crispus*’ meaning undulating, kinky or wavy and ‘*sulcatus*’ meaning furrowed or grooved (Figure 4).

Description: Corticolous, sometimes saxicolous lichen. Marginal prothallus usually present, this white, up to 5 mm wide. Thallus effuse, 1.0–1.5 mm thick, composed of aggregated green to olivaceous (grey-brown on storage) coralloid isidia, 0.05–0.07 mm diameter, 0.1–0.5 mm long. Photobiont green, cells irregular, 4–8 µm across. Apothecia solitary to crowded, sessile, sometimes partially hidden within isidia, black, 1.0–2.6 mm diameter, flat to slightly concave with a smooth, thin (0.1–0.2 mm) proper margin, becoming slightly convex and contorted when mature. Hymenium 125–160 µm tall, composed of thin (1.0–1.5 µm wide), simple paraphyses, not or slightly swollen at the apex; separating in K but ±conglutinate at the epihymenium; epihymenium thin, olivaceous grey (no discernible reaction with K, H, or N). Hypothecium dark brown or blue-black in places, heavily pigmented and indistinct from exciple (N+ red bleeding into hymenium, other tests negative). Ascus *Biatora*-type, cylindrical, 90–120 × 30–35 µm; ascospores 1-septate, broadly ellipsoid, often slightly constricted at septum, 25–30(35) × (8)12.5–15 µm (n = 38). Exciple cupular, composed of radiating hyphae ca. 1–2 µm wide, 75–150 µm wide laterally, blue-black (K+ green, H+ blue/green, N+ magenta, cinereorufa-green), outer 5–50 µm hyaline with blue-black pigmentation often bleeding in at base. Conidiomata not observed. Chemistry: K–, C–, Pd–. TLC: negative.

Representative Specimens (out of 14 seen):

Aotearoa / New Zealand, Te Ika a Māui / North Island: Northland, Maungaraho Rock, P.J. de Lange 15596, A.J. Marshall & D.J. Blanchon, 15 Jul 2017, UNITEC 14143; Waitākere Ranges, Maungaroa Ridge Track, Piha, A.J. Marshall s.n., P.J. de Lange & D.J. Blanchon, 12 Jun 2020, UNITEC 12902; Waitākere Ranges, Maungaroa Ridge Track, Piha, A.J. Marshall s.n., P.J. de Lange & D.J. Blanchon, 12 Jun 2020, UNITEC 12918 (MSC); Waitākere Ranges, Fenceline Track, A.J. Marshall s.n. & C.D. Kilgour, 20 May 2021, UNITEC 12901; Waitākere Ranges, Zion Ridge Track, A.J. Marshall s.n. & C.D. Kilgour, 1 Jul 2021, UNITEC 12904; Waitākere Ranges, Zion Hill Track, A.J. Marshall s.n. & C.D. Kilgour, 2 Jul 2021, UNITEC 12903 (MSC); Chatham Islands: Rēkohu / Wharekauri / Chatham Island, Te Whanga, Blind Jim’s, P.J. de Lange CH4141, 27 Jun 2021, UNITEC 12908; Mangāpe Creek Bush, P.J. de Lange CH4417, 31 May 2008, UNITEC 14144.

Distribution

Megalaria crispisulcans is thus far known only from Te Ika a Māui / North Island and from Rēkohu / Wharekauri / Chatham Island. At the time of writing (August 2023) it is known best from the Waitākere Ranges, where it was first recognised, and also from where it has been most widely collected, Woodhill Forest. Outside the Waitākere Ranges and Woodhill Forest, the species is known from Northland (Maungaraho Rock), Bay of Plenty (Mount Maunganui and Lake Rotongata), and South Kawhia (Awaroa Scenic Reserve, Prasad et al. 2022). On Rēkohu / Wharekauri / Chatham Island the species has been collected twice: in 2008 as part of a mixed collection of lichens sampled from inihina (*Melicytus chathamicus* (F.Muell.) Garn.-Jones) from Mangāpe Creek, and, in 2021, also from inihina growing on the shore of Te Whanga. The disjunct nature of these occurrences is unlikely to be natural. It is likely that *M. crispisulcans* occurs elsewhere in Aotearoa / New Zealand. As was frequently noted by Galloway (2007) for the majority of lichens he recognised for Aotearoa / New Zealand, more regional collecting of *Megalaria* is needed.

Recognition

Megalaria crispisulcans is distinguished from other Aotearoa / New Zealand *Megalaria* by its bright green (when fresh) isidiate thallus, which is usually surrounded by a white prothallus of up to 5 mm wide (Figures 2,

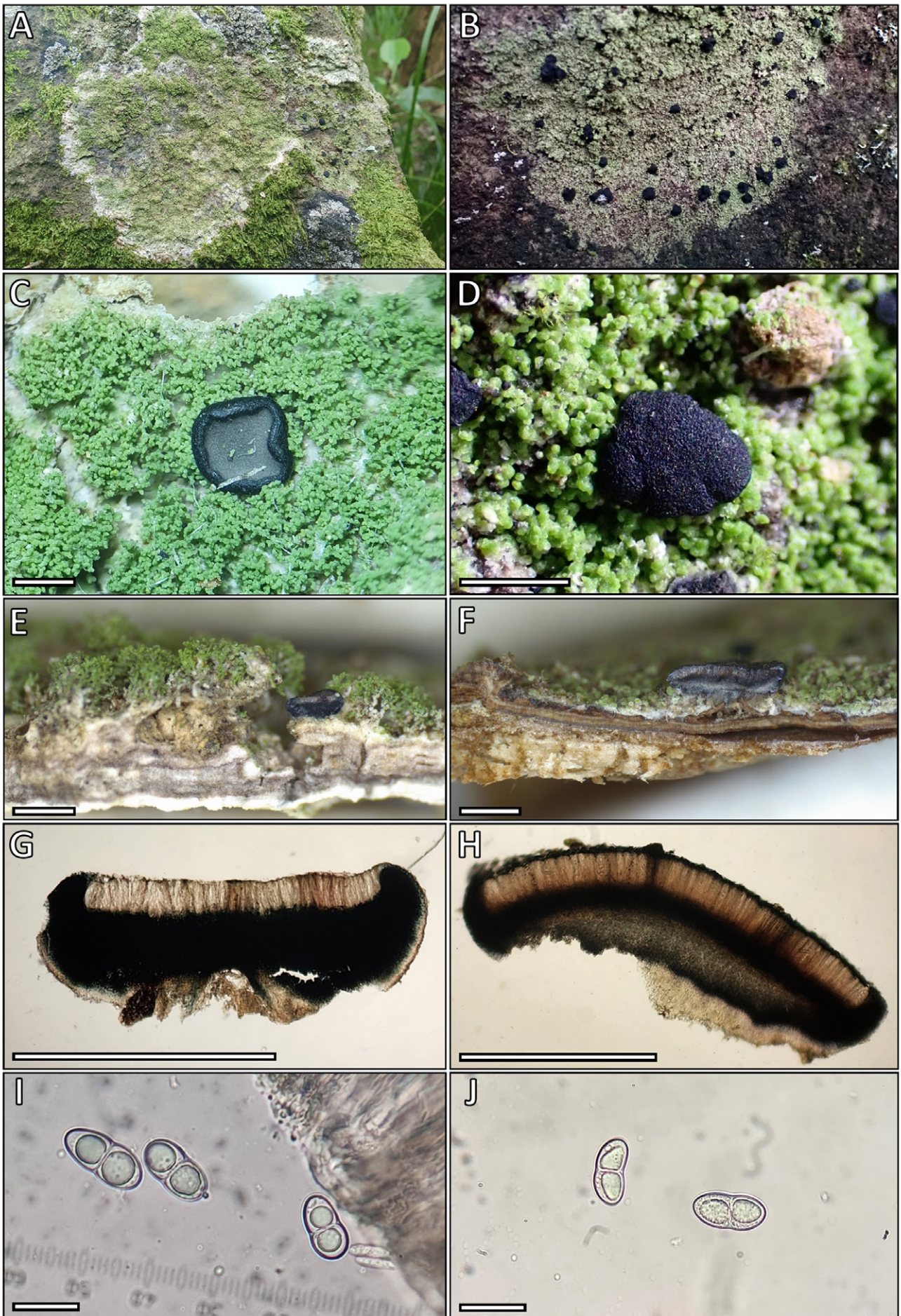


Figure 5. Comparison showing differences and similarities between *Megalaria crispisulcans* and *M. orokonuiana*. (A) *M. crispisulcans* growing on andesite on the Zion Hill Track, Waitākere Ranges, the white prothallus of the species is distinct. Photo: A. J. Marshall, June 2021. (B) *M. orokonuiana* on basalt, lacking the obvious prothallus of *M. crispisulcans*. Photo: P. J. de Lange, May 2023. (C) Close-up of thallus and typical apothecium of *M. crispisulcans*, with distinct cupular exciple and coralloid isidia that often surround or even bury the apothecium. Photo: A. J. Marshall, June 2021. (D) A mature apothecium of *M. orokonuiana*, tending towards being convex with the exciple indistinct, isidia are granular and both shorter and wider than those observed in *M. crispisulcans*. Photo: P. J. de Lange, May 2023. (E) Cross-section through substrate, thallus and apothecium of *M. crispisulcans*, the branched coralloid nature of the isidia can be clearly seen, the apothecium attached to the substrate with overarching isidia, scale = 1 mm. Photo: A. J. Marshall, August 2023. (F) Cross-section through substrate, thallus and apothecium of *M. orokonuiana*, here the isidia are short and granular, the apothecium on top, scale = 1 mm. Photo: A. J. Marshall, August 2023. (G) Cross-section through the apothecium of *M. crispisulcans*, the hypothecium a single pigmented layer and epithecium pigmentation at the tips of the paraphyses diffuse, scale = 1mm. Photo: A. J. Marshall, August 2023. (H) Cross-section through the apothecium of *M. orokonuiana*, the hypothecium bi-layered and epithecium pigmentation concentrated around swollen tips of the paraphyses, scale = 1 mm. Photo: A. J. Marshall, August 2023. (I) Spores typical of *M. crispisulcans*, scale = 25 µm. Photo: A. J. Marshall, August 2021. (J) Spores typical of *M. orokonuiana* are very similar to those of *M. crispisulcans*, scale = 25 µm. Photo: A. J. Marshall, August 2023.

5). Infertile material is more often observed and has a superficial resemblance to a species of *Phyllospora* Müll. Arg., sterile material of which is also green, often isidiate and has a distinctive white prothallus. The similarity is so striking that we have found sterile specimens of *Megalaria crispisulcans* filed in herbaria as *Phyllospora* spp. (see below). The apothecia of *Megalaria crispisulcans* vary significantly in morphology and density (Figure 4). The only *Megalaria* species that could be considered similar are *M. spodophana* (Nyl.) D.J.Galloway, which is described by Galloway (1985) as having a minutely isidiate-furfuraceous thallus, but this species has much smaller ascospores ((13.5–)15–17(–19) × (5–)6–8.5 µm) and lacks a prothallus, and *M. orokonuiana*, which tends to lack an obvious prothallus, has more granular and shorter isidia, and different apothecial structure and pigmentation (Figure 5). The results of our phylogenetic analysis, albeit limited to a single nucleotide marker (nrDNA ITS), places *M. crispisulcans* and *M. orokonuiana* as sister taxa, and indeed our initial Waitākere Ranges discoveries were placed within *M. orokonuiana*. The species have a superficial similarity (Figure 5). However, morphologically *M. crispisulcans* differs from *M. orokonuiana* by the isidia that are prominent and distinctly coralloid (0.05–0.07 mm diameter, 0.1–0.5 mm long) rather than granular (0.1–0.15 mm diameter, 0.1–0.2 mm long) in *M. orokonuiana*. Further, the apothecia of *M. orokonuiana* when mature are usually immarginate, whereas those of *M. crispisulcans* are distinctly undulose contorted. The ascospores of *Megalaria crispisulcans* are 25–30(35) × (8–)12.5–15 µm, whereas those of *M. orokonuiana* are (20)25–30 × 12–14 µm, and the hypothecium a single pigmented layer in *M. crispisulcans*, but distinctly bi-layered in *M.*

orokonuiana (Figure 5).

Sterile specimens of *Megalaria crispisulcans* have been confused with sterile *Phyllospora*, presumably due to the usual presence of a prothallus. There is a striking similarity, such that we found additional specimens of *Megalaria* residing as undetermined *Phyllospora* in herbarium collections. Clear distinction in sterile specimens is not always possible, though *Phyllospora* have squamulose rather than crustose thalli (Galloway 2007).

Ecology

Megalaria crispisulcans is known from a range of habitats and vegetation associations (Figure 2). At the type locality, Hautu Marama / Hodge's Basin, Woodhill Forest, *M. crispisulcans* is common on large mahoe that grow within a 48 ha dune forest remnant surrounded by plantation forestry. Here the species is common in partially shaded sites (though characteristically found infertile). In the Waitākere Ranges, on the Fenceline Track the species was also noted on mahoe and māmāngi in association with *Porina exocha* (Nyl.) P.M.McCarthy, *Pseudocyphellaria rufovirescens* (C.Bab.) D.J.Galloway, *Sticta lacera* (Hook.f. & Taylor) Müll.Arg., *Pyrenula microcarpa* Müll.Arg, *Leptogium aucklandicum* Zahlbr. and *Thelotrema lepadinum* (Ach.) Ach.. At Piha it has also been observed growing on nīkau (*Rhopalostylis sapida*) on the Kitekite Track among a similar vegetation association, and interestingly at two locations south of Karekare on kōwhai (*Sophora fulvida* (Allan) Heenan & de Lange) and saxicolous on Waitākere conglomerate adjacent to a mahoe, on which it was also growing

(Figure 2). The largest population seen in the Waitākere Ranges was on the Houghton Track, where *Megalaria crispisulcans* was commonly observed in a stand of māmāngi stretching over an area of several hundred metres. *Megalaria crispisulcans* has been observed growing on andesite at Maungaraho Rock, Northland, and also on rock near the summit of Mauao / Mount Maunganui, Bay of Plenty, although we were unable to collect a specimen at the second location. On Rēkohu, *M. crispisulcans* was also discovered as a fragment within a larger collection of crustose and foliose lichens sampled from an inihina tree growing at Pana / Blind Jim's. This collection lacked apothecia, but a further investigation of lichen collections made from Rēkohu in 2008 by one of the authors found another specimen of *M. crispisulcans* also collected from inihina. The vegetation at Pana / Blind Jim's is a treeland left by past livestock grazing, which is now fenced to exclude stock and is dominated by hikoā karamu (*Coprosma chathamica* Cockayne), kopi (*Corynocarpus laevigatus* J.R.Forst. & G.Forst.) and mataira (*Myrsine chathamica* F.Muell.) that grow between a series of exposed limestone outcrops and Te Whanga lagoon. Associated lichens included *Arthonia* Ach., *Hyperphyscia adglutinata* (Flörke) Hafellner, H.Mayrhofer & Poelt, *Lecanora kohu* Printzen, Blanchon, Fryday & de Lange, *Micarea* Fr., *Physcia adscendens* H.Olivier, *Pyrenula* Ach., and *Ramalina peruviana* Ach.. The fertile Rēkohu specimen, also from inihina, was part of a mixed collection containing *Megalaria grossa* (Pers. ex Nyl.) Hafellner and *Megalospora gompholoma* (Müll.Arg.) C.W.Dodge subsp. *gompholoma*, and was collected at Mangāpe Creek Bush. Both specimens, being accidental incorporations, provide little indication of the species abundance; clearly further surveys for it there could be illuminating.

Conservation Status

Megalaria crispisulcans is thus far known from 13 locations – 11 from Te Ika a Māui / North Island (Northland, Auckland, Bay Of Plenty) and two from Rēkohu / Wharekauri / Chatham Island – and there is now sufficient data on the species' ecology to propose a conservation status. As with other lichen assessments, though, the critical issues remain: defining what is an individual, what is a population and what the trends of those populations are (de Lange et al. 2012; de Lange et al. 2018; de Lange 2021). In practice, obtaining such data is problematic, and Aotearoa / New Zealand

assessments of cryptogamic plants and animals, algae and fungi have therefore resorted to using approximations (in most cases) of area of occupancy and population trend data (see de Lange et al. 2018; de Lange et al. 2020; Nelson et al. 2019; Rolfe et al. 2019). Accepting these constraints, we have estimated the total area of occupancy of *M. crispisulcans* at 15 ha, spread over 13 locations. Trend data for this species is not available but, as employed by de Lange (2021) for *Lecanora kohu*, an approximation of trend can be obtained from examining the ecology of the phorophytes utilised by the species.

From our observations, aside from the occasional saxicolous occurrences, *Megalaria crispisulcans* is primarily a corticolous species, of moderately well-lighted places, colonising the bark of *Sophora fulvida*, inihina, mahoe, māmāngi, nīkau and rawirinui in mostly regenerating forest. Although we have no information on abundance on Rēkohu / Wharekauri / Chatham Island, within the Waitākere Ranges and at Maungaraho Rock *M. crispisulcans* is locally common. In all of the Waitākere locations, the lichen has been found on phorophytes growing in forest that has regenerated from open ground left following the logging of kauri (*Agathis australis*) forest (Esler & Astridge 1974). While the average longevity of the range of phorophytes is by and large unknown, with the possible exceptions of māmāngi and *Sophora fulvida*, the other species, notably inihina, mahoe, nīkau and rawirinui are unlikely to live for much longer than 300 years (de Lange 2007; de Lange 2014; Enright & Watson 1992; Wardle 1991).

From this, there is sufficient information to propose a conservation status for *Megalaria crispisulcans*. Thirteen populations are now known, collectively occupying an area of 15 ha, in well-lighted places within regenerating vegetation, where the lichen mostly colonises the bark of hosts that can live for up to 300 years. Using the New Zealand Threat Classification System, there are two possible classifications for *M. crispisulcans*. If one accepts that the current distribution of the species is “unnatural resulting from land clearance” then *M. crispisulcans* qualifies as “Threatened / Nationally Vulnerable” Pathway B (Townsend et al. 2008, p. 21). Under this scenario *M. crispisulcans* qualifies, as the total area of occupancy (c.15 ha) is <100 ha, and the population is unlikely to decline over the next ten years and so is judged ‘stable’ (Criteria S3, T1). Alternatively, the species qualifies for listing as “At Risk / Naturally Uncommon” (Townsend et al. 2008, p. 24) if one accepts that the current distribution has not been caused by recent or past human disturbance.

It is our view, though, that the highly disjunct nature of *Megalaria crispisulcans* occurrences is not natural, but accidental, reflecting that the species has not been critically surveyed for. We hypothesise that formal publication of the species *M. crispisulcans* will stimulate interest within the Aotearoa / New Zealand lichenologist community, resulting in further discoveries elsewhere in the country, and we see nothing in its ecological preferences (i.e., seemingly well-lighted places in regenerating forest, where it colonises a range of phorophytes and rocks) that would seriously threaten the species. In that context, assessing the species as 'Threatened / Nationally Vulnerable' seems both alarmist and unnecessary. While it is true that much of the habitat occupied by the species has been eliminated by past, and in some cases ongoing, land clearance, there are also numerous secure areas of that vegetation still present throughout the country. Therefore, we propose that *M. crispisulcans* be assessed as 'At Risk / Naturally Uncommon'. To that proposed assessment we suggest the qualifiers 'DPS' [Data Poor: Size] and 'DPT' [Data Poor: Trend] are appended, as we still lack accurate information on the population size or trend. While this proposed assessment will need to be ratified at the next New Zealand Lichen Threat Assessment meeting, we hope that the information provided in this paper will stimulate further survey and collection of the species outside the areas in which we report it here.

The situation on Rēkohu / Wharekauri / Chatham Island, in isolation from the rest of Aotearoa / New Zealand, is less positive. There the species is known from fragmented, accidental collections made while sampling pyrenocarpous lichens. At one site, Pana / Blind Jim's, where *Megalaria crispisulcans* was found in 2021, a second search in July 2023 found that the phorophyte had fallen in Te Whanga and died, along with the associated lichen mycobiota. No further *Megalaria crispisulcans* occurrences were noted there. The other collection, made in 2008, came from an area that is more secure from vegetation loss than Pana / Blind Jim's, Mangāpe Creek, and that location should be a priority for survey. It should be noted, though, that there are now numerous lichen collections from Rēkohu / Wharekauri / Chatham Island, and our analysis of these has not found further *Megalaria crispisulcans* specimens. It may well be that this species is genuinely scarce there.

While we have reasonable information for *Megalaria crispisulcans*, the situation for *M. orokonuiana*, assessed as 'Data Deficient' by de Lange et al. (2018), remains unchanged beyond that we have an additional location

at Mopanui, just outside the Type Locality (Orokonui), near Ōtepoti / Dunedin, Te Wai Pounamu / South Island. Further, the species has been reported from Australia (McCarthy & Elix 2016), and though its abundance there has not been ascertained it seems to be widespread across parts of New South Wales and Victoria, so the qualifier 'SO' [Secure Overseas] may now apply. During our May 2023 visit to Orokonui and Mopanui we noted that *Megalaria orokonuiana*, where seen, was locally common, though no comprehensive survey for it was undertaken throughout either location. As with *M. crispisulcans*, it is likely, now that both species are more clearly delimited and illustrated, that further occurrences of *M. orokonuiana* will be discovered by the Aotearoa / New Zealand lichen community.

Key to Species of *Megalaria* presently recognised in Aotearoa / New Zealand

Fryday & Lendemer (2010) showed that three of the species (*M. semipallida*, *M. subcarnea* and *M. variegata*) transferred to *Megalaria* by Galloway (2004) and included in Galloway (2007) were referable to other genera, and also showed that *M. obludens* was the correct name for *M. imshaugii*. Here we provide an updated key to reflect those changes and to also include *M. orokonuiana* and *M. crispisulcans*.

1. Saxicolous 2.
Corticolous/lignicolous 5.
2. Ascospores small, 12–16(–18) × 6–8 µm *Megalaria obludens*
Ascospores large, 20–36 × 10–17 3.
3. Thallus isidiate 4.
Thallus ± endolithic, lacking isidia *Megalaria macrospora*
4. White prothallus usually present, isidia coralloid, hypothecium indistinct *M. crispisulcans*
Prothallus usually absent, isidia granular, hypothecium distinct *M. orokonuiana*
5. Thallus isidiate or sorediate 6.
Thallus lacking isidia or soredia 9.
6. Isidiate/isidiate-furfuraceous 7.
Sorediate *Megalaria pulverea*
7. Isidiate, spores (20)25–35 µm 8.
Isidiate-furfuraceous, spores <20 µm *Megalaria spodophana*
8. White prothallus usually present, isidia coralloid, hypothecium indistinct *M. crispisulcans*
Prothallus usually absent, isidia granular, hypothecium distinct *M. orokonuiana*
9. Apothecia immarginate *Megalaria sublivens*
Apothecia marginate 10.
10. Apothecial discs ± pruinose, at least when young *Megalaria melanotropa*
Apothecial discs epruinose 11.
11. Thallus grey-white; marginal prothallus black; hypothecium colourless, 55 µm thick *Megalaria maculosa*
Thallus olive-green; marginal prothallus usually absent; hypothecium brown-black, 200–250 µm thick *Megalaria grossa*

Data accessibility statement

No additional database

Alan M. Fryday: Writing – review and editing (equal)

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Tables

Table 1. List of species included in the ITS phylogenetic analyses conducted in this study.

Species	GenBank accession no.
<i>Mycobilimbia carnealbida</i> (Müll. Arg.) S.Ekman & Printzen	MK812691
<i>Lecania inundata</i> (Hepp ex Körb.) M.Mayrhofer	AM292690
<i>Biatora alnetorum</i> S.Ekman & Tønsberg	MH818375
<i>Biatora vernalis</i> (L.) Fr.	OQ717335
<i>Cliostomum griffithii</i> (Sm.) Coppins	AF282076
<i>Megalaria pachaylenophila</i> Phraphuchamngong, Buaruang & Lumbsch	OP698020
<i>Megalaria</i> Hafellner sp.	OP698027
<i>Megalaria laureri</i> (Hepp ex Th.Fr.) Hafellner	AF282075
<i>Megalaria orokonuiana</i> Fryday & A.Knight†	OR575317
<i>Megalaria orokonuiana</i> Fryday & A.Knight†	OR575318
<i>Megalaria crispisulcans</i> sp. nov. †	OR575319
<i>Megalaria crispisulcans</i> sp. nov. †	OR575320
<i>Megalaria grossa</i> (Pers. ex Nyl.) Hafellner	MK811839
<i>Megalaria grossa</i> (Pers. ex Nyl.) Hafellner	MZ159555
<i>Megalaria grossa</i> (Pers. ex Nyl.) Hafellner	MK812151
<i>Megalaria grossa</i> (Pers. ex Nyl.) Hafellner†	OR575321
<i>Megalaria alleniae</i> Lendemmer & McMullin	NR153467
<i>Megalaria pulvereae</i> (Borrer) Hafellner & E.Schreiner	KX660735
<i>Megalaria yunnanensis</i> C.X.Wang & L.Hu	MK348528
<i>Megalaria phayapipakiana</i> C.X.Wang & L.Hu	NR182746
<i>Ramalina subwebbiana</i> (Nyl.) Hue	GU827292
<i>Ramalina clementeana</i> Llimona & Werner	MN811263

† New samples sequenced for this study.

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