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Blastacervulus metrosideri sp. nov. leaf spot on *Metrosideros excelsa* in New Zealand

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Asterinaceae
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pohutukawa

Abstract: A leaf-spotting fungal pathogen common on *Metrosideros excelsa* in New Zealand is described here as *Blastacervulus metrosideri sp. nov*. It has previously been identified in the New Zealand literature as *Leptomelanconium* sp. and as *Staninwardia breviuscula*. The choice of genus for this new species is supported by a phylogeny based on ITS and LSU sequences. It is phylogenetically close to several morphologically similar *Eucalyptus* leaf spotting pathogens.

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INTRODUCTION

A leaf-spotting fungus common on *Metrosideros excelsa* (pohutukawa, *Myrtaceae*) in New Zealand is morphologically similar to a number of leaf-spotting fungi on another myrtaceous host, *Eucalyptus*. These fungi on *Eucalyptus* leaves have been placed in a range of genera, including *Alysidiella*, *Blastacervulus*, and *Staninwardia* (Swart 1988, Sutton 1971, Summerell *et al.* 2006, Cheewangkoon *et al.* 2012), and collectively the diseases they cause on *Eucalyptus* have sometimes been referred to as chocolate spot (e.g. Cheewangkoon *et al.* 2012; Crous *et al.* 2016).

Cheewangkoon *et al.* (2012) presented a phylogeny that resolves a clade of closely-related species of *Eucalyptus* chocolate-spot leaf pathogens that they refer to the genera *Blastacervulus*, *Alysidiella* and *Aulographina*. These taxa form a well-resolved clade, and were placed in the *Asterinaceae* by Giraldo *et al.* (2017). Species in the genera *Alysidiella* and *Blastacervulus* have a similar morphology. They share acervular conidiomata with dry, powdery masses of conidia, the 0–3-septate conidia having thick, verruculate, brown to pale brown walls, base truncate, the conidiogenous cells with a broad conidiogenous locus, sometimes with annellate thickenings. The species accepted by Summerell *et al.* (2006) and Cheewangkoon *et al.* (2012) were distinguished genetically and by differences in conidial size. Crous (2016) later described another genetically similar *Eucalyptus* leaf spotting pathogen as *Blastacervulus eucalyptorum*. *Aulographina* was represented by DNA sequences from the culture CPC 12986 that Cheewangkoon *et al.* (2012) and Giraldo *et al.* (2017) accepted as *A. eucalypti*. *Aulographina eucalypti* differs morphologically from the other chocolate spot pathogens treated by Cheewangkoon *et al.* (2012) in forming a sexual morph and a putatively spermatial asexual morph (Swart 1988, Wall & Keane 1984), and in lacking the acervular conidial morph so distinctive of the other species in this clade. Although the sexual morph of *A. eucalypti* is morphologically typical of *Asterinaceae*, the lack of an acervular asexual morph means that it would be useful to confirm its genetic characterisation with additional specimens.

Staninwardia was first described by Sutton (1971), based on S. *breviuscula*, a *Eucalyptus*-associated fungus from Mauritius that is morphologically similar to the chocolate spot pathogens. Summerell *et al.* (2006) described a second species, *S. suttonii*, on *Eucalyptus* from Australia. Based on DNA sequencing from an ex-type culture of *S. suttonii*, Quaedvlieg *et al.* (2014) place *Staninwardia* in their new family *Extremaceae* (*Capnodiales*), genetically distant from the chocolate spot species of *Alysidiella* and *Blastacervulus* treated by Summerell *et al.* (2006) and Cheewangkoon *et al.* (2012). Another species with similar morphology and associated with similar symptoms on *Eucalyptus* was described by Sutton (1974) as *Leptomelanconium australiense*. Crous *et al.* (2009) recombined it as *Teratosphaeria australiensis*, and Taylor *et al.* (2012) selected an epitype for this species, which again proved to be genetically distant to the chocolate spot species of Cheewangkoon *et al.* (2012).

The New Zealand *Metrosideros* leaf-spotting pathogen was identified tentatively as *Leptomelanconium* sp. by McKenzie *et al.* (1999) and as *Staninwardia breviuscula* by Gadgil & Dick (2006). A record of *S. breviuscula* on *Metrosideros umbellata* may represent the same fungus (Bain 2007), although because of the host difference, this should be confirmed genetically. Based on DNA sequences from a recently obtained culture of the *Metrosideros* pathogen, we describe it here as a new species of *Blastacervulus*.

MATERIALS AND METHODS

Conidia from the fresh collection subsequently dried and stored as fungarium specimen PDD 108694 were suspended in streptomycin solution and streaked across a water agar plate. After 24 h germinating conidia were removed and transferred to 2 % Difco potato dextrose agar (PDA) plates. The cultures had a consistent macromorphological appearance and one was

later selected and stored as ICMP 21883. DNA was extracted from mycelium from this culture, and ITS and LSU sequences generated following the methods of Johnston & Park (2013). The sequences were aligned with *Alysidiella*, *Blastacervulus* and *Aulographina* ITS and LSU sequences from Cheewangkoon *et al.* (2012), *Blastacervulus eucalyptorum* from Crous *et al.* (2016), *B. eucalypti* from Cheewangkoon *et al.* (2009) and additional *Asterinaceae* LSU sequences selected from sister clades in the Giraldo *et al.* (2017) phylogeny, with *Venturia populina* as the outgroup (Table 1). For taxa with both ITS and LSU sequences available, the sequences were concatenated, an alignment carried out using MAFFT v. 1.3.7 as implemented in Geneious R10 (Kearse *et al.* 2012), and a ML phylogenetic tree generated using PhyML v. 3.2.2 (Guindon *et al.* 2010) with the GTR model as implemented in Geneious R10, with support values estimated using 1 000 bootstrap replicates.

Dried specimens were rehydrated using 3 % KOH, conidia and conidiogenous cells examined in 3 % KOH in squash mounts, and the excised acervuli sectioned at about 10 µm thickness using a freezing microtome and the sections mounted in lactic acid.

RESULTS

Based on the taxa and genes sampled, there is no clear genetic difference that can be used to distinguish *Alysidiella* from *Blastacervulus* (Fig. 1). Based on published descriptions, species in the two genera are also very similar morphologically (Swart 1988, Summerell *et al.* 2006, Crous *et al.* 2016). We have chosen to refer our new species to the older genus *Blastacervulus*.

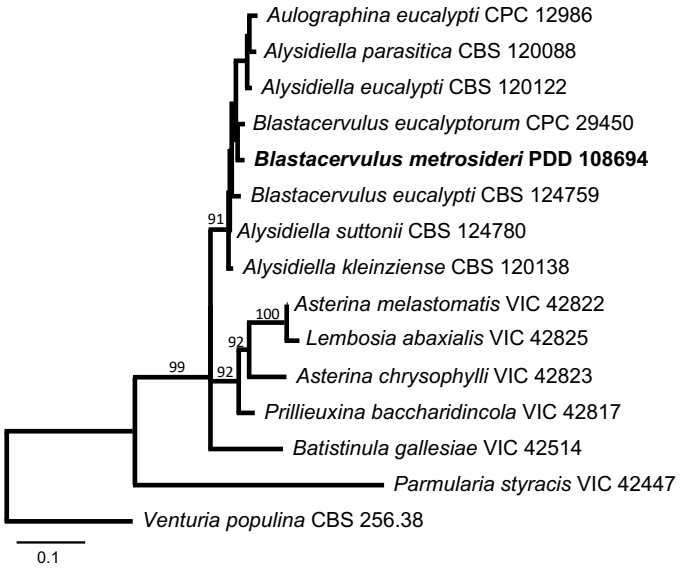

Fig. 1. PhyML maximum likelihood tree based on concatenated ITS and LSU sequences. Bootstrap support values are provided where greater than 90 %. The novel species described here is indicated in **bold** text.

Blastacervulus metrosideri P.R. Johnst. ***sp. nov.***
MycoBank MB829588. Fig. 2.

Etymology: Refers to the host plant.

Diagnosis: Differs from the type species *Blastacervulus eucalypti* in host preference and conidial size and shape.

Typus: **New Zealand**, Auckland, Glen Innes, Auckland University Tamaki Campus, on living leaves of *Metrosideros excelsa* (*Myrtaceae*), 5 Oct. 2017, *P.R. Johnston* (**holotype** PDD 108694; ex-type culture ICMP 21883).

Leaf spots on upper surface of living leaves, initially red-brown with narrow yellow margin, becoming darker, almost black with age, round, up to 4 mm diam. Discolouration of the spots sometimes extending through to the lower leaf surface. Apart from the cells associated with the acervuli, fungal tissue is sparse within the leaf, forming a narrow plate of hyaline, thin-walled hyphae about 3–5 µm diam between the cuticle and epidermis. Little or no fungal hyphae is present more deeply in the leaf. The colour of the spots is associated with deposition of tannins or other compounds within the intact epidermal and palisade cells of the host. *Acervuli* develop within the spots on the upper leaf surface, 0.1–0.3 mm diam, upper wall black, breaking open irregularly to expose the black, powdery conidial mass. In vertical section acervuli develop between the cuticle and the epidermal cells, with the upper and lower walls comprising 2–3 rows of angular cells 4–7(–10) µm diam. *Conidiogenous cells* line the inside of both the upper and lower walls, solitary, 6–8 × 4–5 µm, cylindrical, conidiogenous locus broad, apical, often with several thickened and slightly flaring annellations. *Conidia* cylindrical, base truncate, apex broadly rounded, 1–3(–9)-septate, 1-septate 8–10 µm long, 2-septate 10–14 (–18) µm long, 3-septate 13–16 (–19) µm long, 4-septate 16–19 µm long, 9-septate up to 33 µm long × 3.5–6 µm wide, walls thickened, dark brown, finely verruculate.

Culture characteristics: Cultures on PDA about 15–25 mm diam after 20 wk. Margin of colony uneven, surface black, convoluted, lumpy and cracked, finely felted, brown pigment diffusing into agar. Cells in the mycelium near the edge of the colony starting to become swollen and to develop thick and dark walls, cells in the older parts of the colony almost all short, broad-cylindric, with walls thick, dark, smooth, hyphae partly disarticulating.

Additional materials examined: **New Zealand**, Auckland, Leigh, on *Metrosideros excelsa*, 30 Mar. 1924, *E.G. Bollard*, (PDD 43314); Te Henga, on *M. excelsa*, 25 Mar. 1949, *J.M. Dingley* (PDD 15909); Titirangi Beach, on *M. excelsa*, 3 Dec. 1963, *F.J. Morton & J.D. Read* (PDD 30158); Langholm, on *M. excelsa*, 3 Dec. 1963, *F.J. Morton & J.D. Read* (PDD 30159); Auckland City, Mt Albert Rd, on *M. excelsa*, Feb. 1994, *P.R. Johnston* (PDD 64252); Glen Innes, Auckland University Tamaki Campus, on *M. excelsa*, 5 Oct. 2016, *P.R. Johnston* (PDD 108727); Glen Innes, Colin Maiden Park, on *M. excelsa*, 23 Jan. 2019, *P.R. Johnston* (PDD 116628); Coromandel: Port Charles, between wharf and Big Sandy Bay, on *M. excelsa*, 26 Mar. 1989, *P.R. Johnston* (PDD 55197); Port Charles, on *M. excelsa*, 28 Mar. 1993, *P.R. Johnston* (PDD 62168); Port Charles, Little Sandy Bay, on *M. excelsa*, Nov. 1993, *P.R. Johnston & E.M. Gibellini* (PDD 64249); Port Charles, Big Sandy Bay, on *M. excelsa*, 29 Dec. 1993, *P.R. Johnston & E.M. Gibellini* (PDD 64236); Port Charles, Big Sandy Bay, on *M. excelsa*, 24 Oct. 1994, *P.R. Johnston & E.M. Gibellini* (PDD 64251); Northland: Bay of Islands, Black Rocks off Moturoa, north west islet of Crater Rim group, on *M. excelsa*, 23 Jan. 1990, *R.E. Beever* (PDD 56841); Westland: Hokitika, Hokitika Hospital, on *M. umbellata* cultivated plant, 22 May 2007, *B.H. Doherty* (NZFS 5422).

Notes: Symptoms that match those associated with *Blastacervulus metrosideri* are very common on *Metrosideros excelsa* wherever it grows in New Zealand. The literature cited in

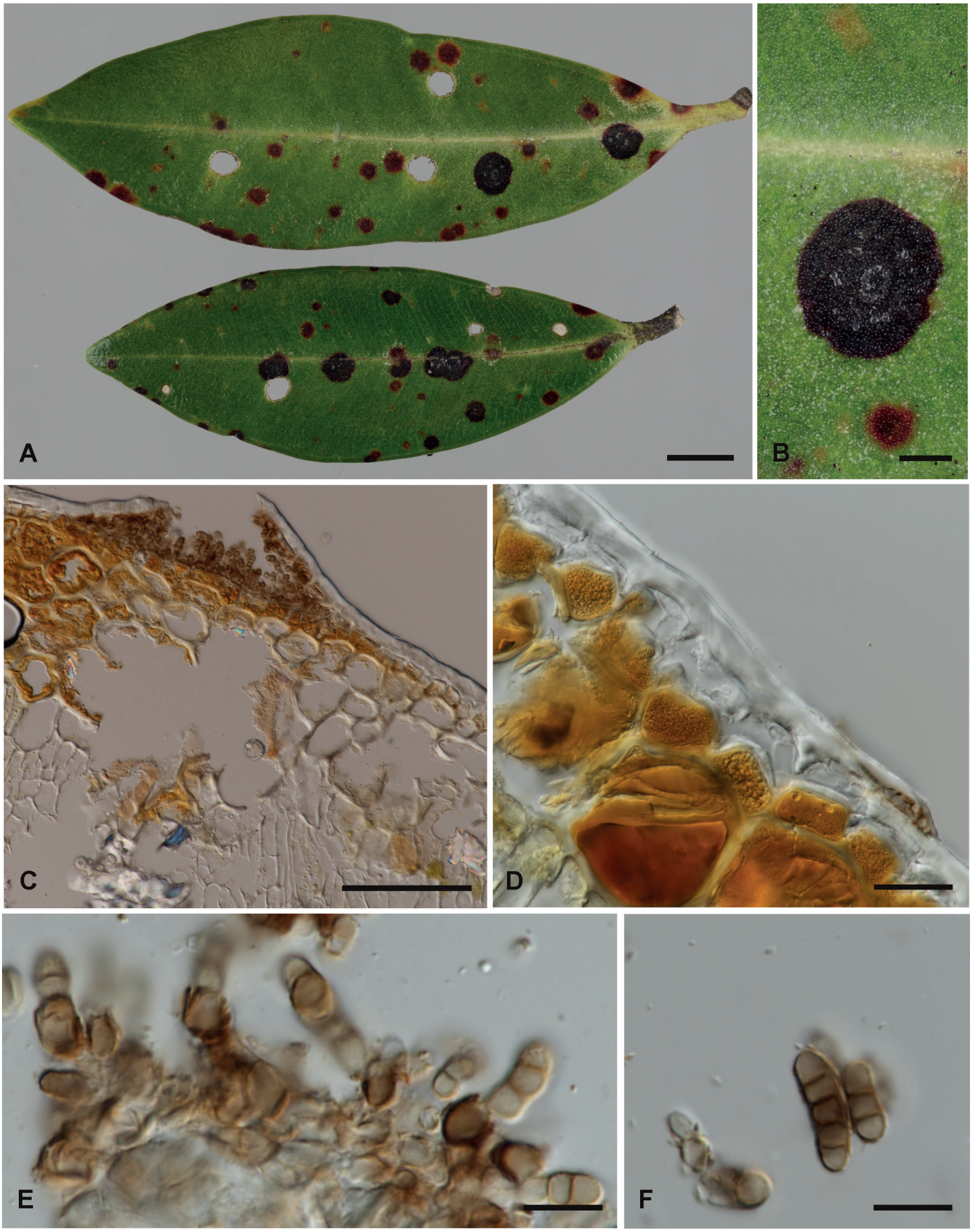

Fig. 2. *Blastacervulus metrosideri*. **A.** Immature and mature spots on *Metrosideros excelsa* leaf. **B.** Detail of one of the mature spots, with several individual erumpent acervuli. **C.** Acervulus in vertical section. **D.** Leaf in vertical section with incipient acervulus, apart from the dark-walled cells of the acervulus, fungal tissue restricted to a single layer of hyaline cells beneath the cuticle. **E.** Conidiogenous cells. **F.** Conidiogenous cell and released conidia. A, B, E, F — PDD 116628; C, D — PDD 108694. Scale bars: A = 5 mm; B = 1 mm; C = 100 µm; D = 20 µm; E, F = 10 µm.

Table 1. Specimens and GenBank accession numbers of sequences used for the phylogeny in Fig. 1.

Species	Voucher	Country, Collector	Host	Reference	ITS	LSU
Alysidiella eucalypti	CBS 120122	Uruguay, M.J. Wingfield	*Eucalyptus dunnii*	Crous *et al.* (2006)	DQ885893	DQ885893
Alysidiella kleinziense	CBS 120138	South Africa, Z.A. Pretorius	*Eucalyptus* sp.	Crous *et al.* (2007a)	EF110616	EF110616
Alysidiella parasitica	CBS 120088	South Africa, P.W. Crous	*Eucalyptus* sp.	Crous *et al.* (2007a)	DQ923525	DQ923525
Alysidiella suttonii	CBS 124780	Cyprus, A. van Iperen	*Eucalyptus* sp.	Cheewangkoon *et al.* (2012)	HM628774	HM628777
Asterina chrysophylli	VIC 42823	Brazil, A.L. Firmino	*Henriettea succosa*	Guatimosim *et al.* (2015)	—	KP143738
Asterina melastomatis	VIC 42822	Brazil, A.L. Firmino	*Miconia* sp.	Guatimosim *et al.* (2015)	—	KP143739
Aulographina eucalypti	CPC 12986	Australia, A. Carnegie	*Eucalyptus cloeziana*	Cheewangkoon *et al.* (2012)	HM535599	HM535600
Batistinula gallesiae	VIC 42514	Brazil, A.L. Firmino *et al.*	*Caesalpinia echinata*	Guatimosim *et al.* (2015)	—	KP143736
Blastacervulus eucalypti	CBS 124759	Australia, B.A. Summerell	*Eucalyptus robertsonii*	Cheewangkoon *et al.* (2009)	GQ303271	GQ303302
Blastacervulus eucalyptorum	CPC 29450	Australia, P.W. Crous	*Eucalyptus decipiens*	Crous *et al.* (2013)	KY173390	KY173484
Blastacervulus metrosideri	ICMP 21883	New Zealand, P.R. Johnston	*Metrosideros excelsa*	This paper	MK547091	MK547100
Lembosia abaxialis	VIC 42825	Brazil, A.L. Firmino	*Miconia jucunda*	Guatimosim *et al.* (2015)	—	KP143737
Parmularia styracis	VIC 42447	Brazil, R.W. Barreto	*Styrax ferrugineus*	Guatimosim *et al.* (2015)	—	KP143728
Prillieuxina baccharidinicola	VIC 42817	Brazil, O.L. Pereira	*Baccharis* sp.	Guatimosim *et al.* (2015)	—	KP143735
Venturia populina	CBS 256.38, IMI 163996	Italy, E.J.H. Nijhaf	*Populus* x *canadensis*	Crous *et al.* (2007b), Schoch *et al.* (2009)	EU035467	GU323212

the Introduction shows that *Eucalyptus* has several superficially similar leaf-spotting fungi, and more intensive study of the *M. excelsa* associated fungi may reveal a greater diversity of species than currently recognised. For example, *Teratosphaeria* spp. were commonly detected from environmental DNA sequences from *M. excelsa* leaves (unpubl. data), and the symptoms caused by *Teratosphaeria australiensis* are similar to those associated with *B. metrosideri* (Sutton 1974, as *Leptomelanconium australiense*; Taylor *et al*. 2012). Commonly, the blastacervulus-like spots seen in the field are sterile, making a definitive identification based on morphology impossible.

The single, small specimen on *Metrosideros umbellata* (NZFS 5422) has markedly paler spots than those on *M. excelsa* and its acercvuli are smaller. Microscopically, this specimen appears to match those from *M. excelsa*. Additional specimens are needed to determine whether the macroscopic differences are consistent, and DNA sequences from a specimen on *M. umbellata* would confirm whether *M. metrosideri* occurs on more than one species of *Metrosideros*.

The holotype specimen was selected because a culture and DNA sequences were derived from it, but this specimen is not large. To examine the morphology, particularly nice specimens include PDD 30158 and PDD 116628.

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