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Neocoleroa metrosideri sp. nov. (Sympoventuriaceae, Venturiales)

PETER R. JOHNSTON & DUCKCHUL PARK
Landcare Research, Private Bag 92170, Auckland 1142, New Zealand
Corresponding author: johnstonp@landcareresearch.co.nz

Neocoleroa metrosideri is described as a new species and phylogenetically it is shown to belong in the Sympoventuriaceae, a recently established family, sister to the Venturiaceae. No other sequences are available for *Neocoleroa* but this new species is morphologically typical of the type species, with distinctive lobed to dichotomously branched, blunt-tipped setae on the superficial pseudothecia. The genus has previously been placed in the Pseudoperisporiaceae. This leaf spotting fungus is known from only a single specimen in an urban park but the same fungus has been detected also from two natural forest sites as an OTU in 454-based high throughput amplicon sequencing from DNA extracted from living leaves of *Metrosideros excelsa*.

Keywords: LSU phylogeny, *Metrosideros excelsa*, New Zealand, *Wentiomyces*, 454 high throughput sequencing

The genera *Neocoleroa* Petrak (1934: 38) and *Wentiomyces* Koorders (1907: 168) have had a tangled taxonomic history. Kirk *et al.* (2008) accepted *Wentiomyces* in the sense of Müller & von Arx (1962) for a group of dimeriaceous fungi with small, globose, dark-walled, setose pseudothecia, developing superficially on a loose basal tomentum of brown-walled hyphae, sometimes with limited invasion of the host leaf tissue, and with hyaline to pale pigmented, 1-septate ascospores. Barr (1997) agreed with Farr (1965) that problems relating to typification of the name *Wentiomyces* mean that it should be considered a *nomen dubium*. Barr (1997) placed the species treated by Müller & von Arx (1962) as *Wentiomyces* in several different genera. Those with lobed to dichotomously branched, blunt-tipped setae and persistent pseudoparaphyses she accepted as *Neocoleroa*, a genus established by Petrak (1934).

Recent authors have used morphology to place *Neocoleroa* and *Wentiomyces* in the Pseudoperisporiaceae (e.g. Barr 1997, Kirk *et al.* 2008), although Barr (1987, as Dimeriaceae) noted that some of these fungi are morphologically close to the Venturiaceae.

This paper describes a new species *Neocoleroa metrosideri*, associated with leaf spots of *Metrosideros excelsa*. Morphologically it fits Barr's concept of *Neocoleroa*. DNA sequences from the type specimen and from cultures grown from single ascospores from the type, place this species in the Sympoventuriaceae, sister to the Venturiaceae in the Venturiales (Zhang *et al*. 2011).

Materials and Methods

The type specimen was examined when fresh, hymenial elements mounted in water; intact pseudothecia and adjacent host leaf tissues were rehydrated in 3% KOH and vertical sections were cut at a thickness of about 10 µm using a freezing microtome and mounted in lactic acid. Mature pseudothecia were crushed gently in 1% streptomycin solution, released ascospores streaked across a water agar plate and after 24 h single germinating ascospores transferred to Difco PDA, 2% Difco MEA, and Difco oatmeal agar plates. Cultures were described after 4 weeks. Specimens have been deposited in the PDD fungarium and ICMP culture collection.

For DNA extraction, three separate extractions were done from single ascomata from three different leaves from PDD 107531 and from a culture derived from germinated ascospores from an ascoma from the collection. DNA was extracted and amplified using a REDExtract-N-Amp Plant PCR Kit (Sigma-Aldrich, USA), following the manufacturer's protocol except that the ascomata were ground in 30 µL extraction solution with a plastic pestle. Amplification primers for ITS were ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993), for LSU were LR0R and LR5 (Vilgalys & Hester 1990, Bunyard *et al*. 1994). The DNA sequences from the all three fruiting bodies and from the culture were identical and have been accessioned in Genbank as KU131677 and KU131678.

Additional LSU sequences were downloaded from Genbank, the taxon selection based on Machouart *et al*. (2014). LSU

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sequences were aligned using MAFFT as implemented in Geneious (Drummond *et al.* 2012), ML analyses were performed with phyML using the GTR model (Guidon *et al*. 2010) as implemented in Geneious, with 1000 bootstrap replications. *Sydowia*, *Dothidea* (Dothideales) and *Myriangium* (Myriangiales) species were used as outgroups.

Results

Phylogeny

The relationships within the LSU gene tree (Fig. 1) match those in the multigene phylogeny from Machouart *et al.* (2014), the families Venturiaceae and Sympoventuriaceae being strongly resolved within the Venturiales. *Neocoleroa metrosideri* belongs in the Sympoventuriaceae clade but has no clear relationships within the clade.

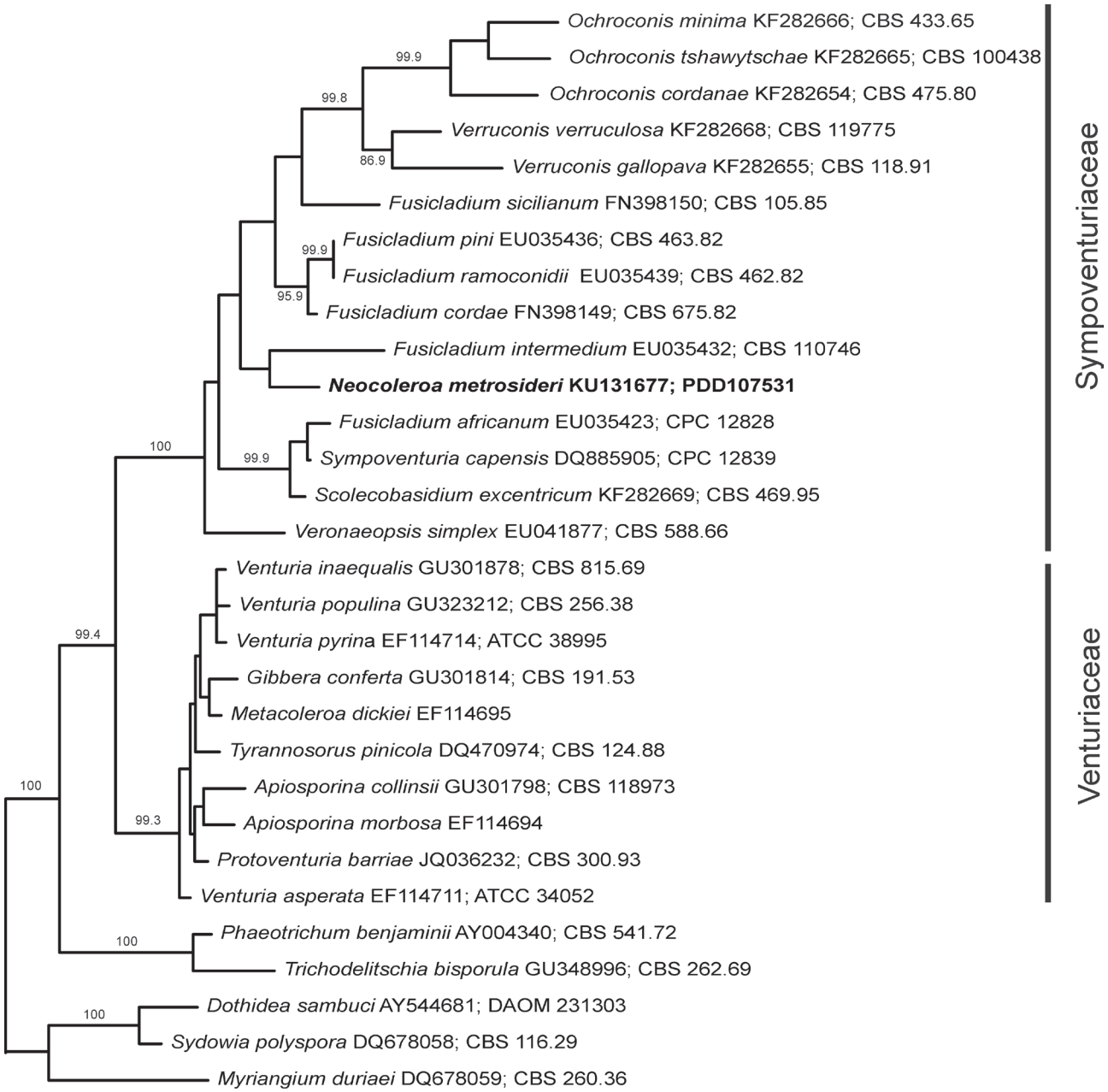

FIGURE 1. ML tree based on LSU sequences, bootstrap values 90% or greater on edges. Taxa selected from Machouart *et al.* (2014) labelled with voucher number Genbank accession numbers for LSU, and voucher numbers where available; *Dothidea sambuci*, *Myriangium duriaei* and *Sydowia polyspora* selected as outgroups. Newly generated LSU sequence from PDD 107531, *Neocoleroa metrosideri*; LSU sequence deposited in Genbank as KU131677.

Taxonomy

Neocoleroa metrosideri P.R. Johnst., *sp. nov.* (Fig. 2)

MycoBank MB 815460

Diagnosis: Differs from *Neocoleroa sibirica* by its host substrate (*Metrosideros* rather than *Vaccinium*) and larger ascospores (18–21 (–24) × (6.5–) 7–8 µm rather than 12 × 2–2.5 µm).

Etymology: refers to host substrate.

Holotype: NEW ZEALAND. Auckland, Glen Innes, Auckland University Tamaki campus (36.883037 S 174.849881 E), on living leaves of *Metrosideros excelsa*, 6 October 2015, P.R. Johnston (PDD 107531, holotype; ex type culture ICMP 21139). GenBank accession numbers KU131678, KU131677.

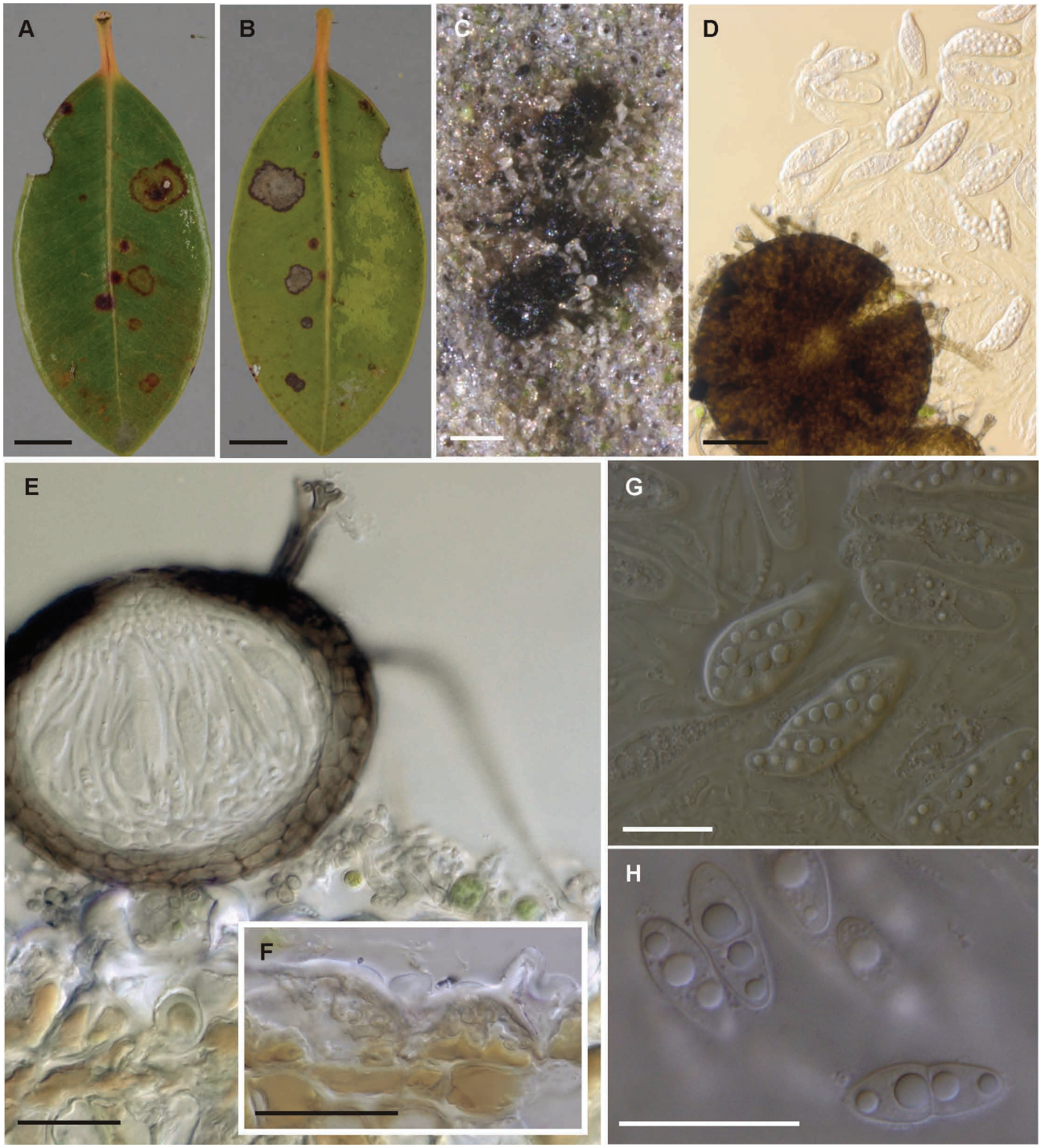

FIGURE 2. *Neocoleroa metrosideri* (PDD 107531). **A**, leaf spots, upper surface of leaf. **B**, leaf spots, lower surface of leaf. **C**, detail of pseudothecia on lower surface of leaf. **D**, crushed pseudothecium showing apically branched setae and released asci. **E**, pseudothecium in vertical section. **F**, detail of fungal tissue within host epidermal cells in vertical section. **G**, asci and pseudoparaphyses. **H**, ascospores. Scale bars, a–b = 5 mm; c = 100 µm; d = 50 µm; e–h = 20 µm.

Leaf spots 3–5 mm diam., round or irregular in shape, barely visible on the upper surface of host leaf, diffuse reddish area with a slightly darker edge; well differentiated on lower surface of host leaf, slightly raised, pale grey with a narrow reddish border. *Pseudothecia* solitary or in small groups on the lower surface. External hyphae sparse, branched, walls darkened, thin, tangled amongst hairs of the leaf tomentum. Fungal hyphae within the epidermal cells of the host, possibly penetrating directly through cuticle. Pseudothecia superficial amongst host leaf tomentum, globose, 0.1–0.15 mm diam., black-walled, sunken and disc-like when mature, small central ostiole; setae 15–25 × 4–5 µm, straight, branched dichotomously several times near the apex, tips of branches rounded, walls pale brown, slightly thickened. Pseudothecial wall 10 µm thick, comprising 2–3 rows of globose to short-cylindric cells with walls barely thickened, darker in outernmost rows of cells. *Hamathecium* with pseudoparaphyses joined both top and bottom, narrow-cylindric, 1.5–2 µm diam., branched and anastomosing, occasionally septate, persistent. *Asci* about 60 × 20 µm, subsaccate to broad-cylindric, foot-like base, broadest in lower half, tapering gradually to rounded apex, bitunicate, fissitunicate, 8-spored, forming near base of pseudothecia. *Ascospores* 18–21 (–24) × (6.5–) 7–8 µm (average 20.2 × 7.3 µm), 1–septate, constricted at septum, upper cell wider than the lower cell, tapering to rounded ends, hyaline. Growth in culture very slow, colonies 1.5–3 mm diam. after 4 weeks on standard agar such as PDA, MEA, and Oatmeal agar. Cultures dark grey-brown, mostly immersed in agar with cottony dark aerial mycelium. Mycelium dark walled, some cells slightly swollen. No conidia observed.

Discussion

DNA sequences place *Neocoleroa metrosideri* in the Sympoventuriaceae (Venturiales). Although DNA sequences are not available for the type species of *Neocoleroa*, *N. sibirica* Petrak (1934: 38), the highly distinctive setae, as well as other features of the sexual morph morphology are consistent with this genus in the sense it is accepted by Barr (1997), based on the description of Petrak (1934). *Neocoleroa* had previously been placed in the Pseudoperisporiaceae (Dothideomycetes incertae sedis), many members of which are morphologically similar to Venturiales. Barr (1987) noted that where known, asexual morphs of the Pseudoperisporiaceae are coelomycetous and Venturiaceae are hyphomycetous, otherwise the descriptions provided by Barr for the two families (Pseudoperisporiaceae as Dimeriaceae) are extremely similar.

The only species placed previously in the Sympoventuriaceae known to form a sexual morph is *Sympoventuria capensis* Crous & Seifert (Crous *et al.* 2007: 32) (Machouart *et al.* 2014). Although the form of the fruiting body, the position it develops in relation to the host issue, and reported ecology differs between *Neocoleroa* and *Sympoventuria*, based on the description of Crous *et al.* (2007) they share similar asci, hyaline ascospores, and persistent pseudoparaphyses.

A specimen of this fungus has been found at only one site on trees in an urban setting in Auckland City, however the same fungus has been detected as an OTU from a 454-based high throughput amplicon sequencing project using DNA extracted from living *Metrosideros excelsa* leaves from natural *M. excelsa* forests at two sites (unpubl. data). The two sites sampled by amplicon sequencing (Rangitoto Island and Waihi Beach) are about 120 km apart, suggesting that this fungus is widespread in *M. excelsa* forests in the north of New Zealand.

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