



First report of *Thyronectria pinicola* causing twig death of radiata pine in Chile

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Radiata pine (*Pinus radiata*) is native to central and southern coastal California, and widely planted throughout the southern hemisphere, most notably in Australia, Chile and New Zealand. This species, grown on more than 1.4 million hectares in Chile, represents the basis of the national forestry industry, and is therefore, a valuable resource that is subjected to ongoing surveillance activities by the Servicio Agrícola y Ganadero (SAG). During the course of forest health surveys completed in August 2016, the pycnidial stage of a Nectriaceae species was detected on dead twigs of radiata pine (Fig. 1), in a four year old plantation located in the county of Coihueco, region of Bío Bío (37°25'00" S, 72°15'21" W). Conidia extruded out of the pycnidia in a yellow coloured cirrus were hyaline, ellipsoidal to oblong, nonseptate, measuring 3.1 × 1 µm on average (Fig. 2). The fungus was cultured on potato dextrose agar (PDA) and synthetic nutrient agar (SNA) for morphologic and molecular analysis (Fig. 2).

To identify accurately the specimen of Nectriaceae found, the internal transcribed spacer (ITS), part of the translation elongation factor 1-alpha (EF1α) and β-tubulin 2 gene (TUB2) of one isolate (SAG-66098) were amplified and sequenced using the universal primer pairs ITS1/ITS4, EF1-728/EF2 (O'Donnell *et al.*, 1998; Carbone & Kohn, 1999) and TUB2Fd/TUB4Rd (Aveskamp *et al.*, 2009) respectively. The sequences obtained were deposited in the NCBI database under GenBank Accession Nos. KY514388 (ITS), KY514389 (EF1α) and KY514390 (TUB2). BLAST analysis showed 99-100% identity with several isolates of *Thyronectria pinicola* (KP751375, JF832573 and JF832863). A multi-locus phylogenetic analysis using criteria of maximum parsimony with the software PAUP v4.0b10 confirmed that the SAG-66098 isolate clustered with the *T. pinicola* clade (bootstrap = 100%) (Fig. 4). Morphological observations carried out on SNA were concordant with the anamorphic state for the species in culture (Hirooka *et al.*, 2012).

The pathogenicity of the isolate was tested by inoculation of 10 (one-year-old) healthy radiata pine seedlings in April 2017. The seedlings stems were wounded using a sterile scalpel 3 cm above soil level and inoculated with a mycelial plug (5 mm diameter) from a seven-day-old culture grown on PDA. Another 10 seedlings used as control were inoculated with sterile agar plugs. The plugs were sealed with paraffin film. Seedlings were kept outdoors with temperatures ranging from 6.2 to 32.2°C, and watered as needed. After three weeks, necrotic lesions were observed on the inoculated seedlings and the fungus was consistently re-isolated from all

lesions. Control seedlings remained healthy (Fig. 5).

According to the SMML Fungus-Host Distribution Database, *Thyronectria pinicola* is common throughout the northern hemisphere (Farr & Rossman, 2017). On lacebark pine this pathogen causes canker disease in China (He *et al.*, 2016). In South America, there are no published records of the presence of *T. pinicola* in any country. Therefore, the diagnosis of this fungus represents the first report for Chile and South America, with a clearly pathogenic role on radiata pine.

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Figure 1

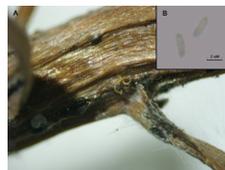


Figure 2



Figure 3

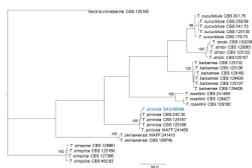


Figure 4



Figure 5

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