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Identification of *Thielaviopsis paradoxa*

Final Report

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By P. R. Johnston and D. Park, Landcare Research

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Reviewed by:	Approved for release by:
Bevan Weir Scientist Landcare Research	Ilse Breitwieser Portfolio Leader – Characterising Land Biota Landcare Research

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Executive Summary

This technical report was carried out to identify if samples of fungus from New Zealand, which were held under the name *Thielaviopsis paradoxa,* were still regarded as *T. paradoxa* using current taxonomy. Findings were:

- *Thielaviopsis paradoxa* (=*Ceratocystis paradoxa*) does not occur in New Zealand; none of the International Collection of Microorganisms from Plants (ICMP) isolates originally identified as *T. paradoxa* represent that species in its current sense.
- One of the cultures originating from New Zealand represents *Thielaviopsis musarum*, the other represents *T. ethacetica*.
- *Thielaviopsis ethacetica* has often been confused with *T. paradoxa*, and probably represents many of the historical *T. paradoxa* reports from a range of hosts in other regions.
- *Chalaropsis thielavioides* (=*Thielaviopsis thielavioides*) does not occur in New Zealand.
- The NZFungi database biostatus statements have been updated to reflect these results.

The outcomes of this work will be used to inform risk assessment work within MPI by providing scientific evidence currently lacking on the presence of this fungus in New Zealand.

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1 Introduction

The fungal pathogen *Thielaviopsis paradoxa* (= *Ceratocystis paradoxa*) is a potential hazard on the salacca (snakefruit) from Indonesia pathway. It also appears relatively frequently as a potential hazard in other pathways. To assess its potential risk, it is necessary to determine whether it is already present in New Zealand. There are records of this species from New Zealand, but the taxonomy has changed and it is not clear whether the samples that have been recorded here in the past are still regarded as *T. paradoxa*. This project checks the identification of specimens identified as *T. paradoxa* (and its synonyms) in the ICMP culture collection, using DNA sequences.

2 Background

Thielaviopsis paradoxa has been reported as present in New Zealand (Pennycook & Galloway 2004, as *Ceratocystis paradoxa*), but recent molecular studies clarifying relationships within the *T. paradoxa* complex (Mbenoun *et al.* 2014, as *C. paradoxa*) mean that the historical, morphologically-based New Zealand records need to be reassessed using DNA sequence data. Mbenoun *et al.* (2014) stated "Few morphological differences could be used as a diagnostic tool to distinguish species within the *C. paradoxa* complex. Sexual structures are not known for all species and are not commonly observed in nature.... the shape and size of asexual structures mostly overlap. DNA sequences are therefore critically important for accurate identification of species in this complex."

Following de Beer *et al.* (2014), the genus *Ceratocystis* is now treated in a more limited sense than has been used in the recent past, restricted to *C. fimbriata* and its relatives. *Ceratocystis paradoxa* and the complex of similar species are accepted in the genus *Thielaviopsis*, with the type species *T. ethcetica*. Another *Thielaviopsis* species recorded as present in New Zealand, *T. thielavioides* (as *Chalara theilavioides* in Pennycook & Galloway 2004), is now accepted as the type species of the genus *Chalaropsis*.

To clarify the identity of the *C. paradoxa*-like isolates in ICMP, DNA sequences were generated for ITS, β-tubulin and TEF, and incorporated the ICMP isolates into the phylogeny of Mbenoun et al. (2014). The origin of the isolates in ICMP were also clarified, some of which had been derived from imported fruit at the border.

3 Methods

Five ICMP cultures identified as either *Ceratocystis paradoxa* or *Chalara thielavioides* were grown on 2% Difco malt extract agar (MEA) and in liquid malt extract broth. After 7 days the morphology of the cultures on agar was examined and photographs prepared of the cultures and the asexual spores. No sexual fruiting structures were observed.

Because of potential confusion about the source of some cultures, whether from PEQ or from the field in New Zealand, the origin of the ICMP isolates was confirmed by examining original documentation provided at the time of deposition in ICMP.

For DNA sequencing, mycelium from the liquid cultures was harvested, dried, then ground in 400µL lysis buffer (Qiagen, USA) with a plastic pestle followed by incubation for 2h at 55°C. Then 220µL lysed solution was loaded into the QIAxtractor Robot (Qiagen, USA) and DNA extraction performed with a Qiagen DX reagent pack and tissue extraction protocol. DNA

sequences for ITS, β-tubulin and TEF-1α were generated using the amplification primers from Mbenoun *et al.* (2014).

The sequences were concatenated and a phylogenetic analysis performed using our newly generated sequences together with selected taxa and isolates from Mbenoun *et al.* (2014). The 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 100 bootstrap replications.

4 Results and Discussion

Molecular identification and origin of the ICMP cultures sequenced are summarised in Table 1. Figure 1 provides a phylogeny of the ICMP cultures plus selected isolates of related species. The NZFungi biostatus statements have been updated to reflect these results, and the DNA sequences will be deposited in Genbank (sequences are provided in Appendix 1). Figure 2 illustrates the morphology of the isolates sequenced; these match the descriptions of these taxa from Mbenoun *et al.* (2014), supporting the DNA sequencing.

Table 1: ICMP cultures sequenced with name as deposited, accepted name following DNA sequencing, and notes on origin

ICMP	Name as deposited	Accepted name	Notes on origin
2116	*Chalara thielavioides*	*Chalaropsis thielavioides*	PEQ specimen from rose from Australia, isolated 1969
2036	*Ceratocystis paradoxa*	*Thielaviopsis musarum*	pure culture from banana imported from Ecuador, isolated 1966
5789	*Ceratocystis paradoxa*	*Thielaviopsis musarum*	PEQ specimen from imported banana fruit, isolated 1977
15221	*Ceratocystis paradoxa*	*Thielaviopsis musarum*	isolated from roots and crown of banana from Great Barrier Island, NZ, 2003
13062	*Ceratocystis paradoxa*	*Thielaviopsis ethacetica*	isolated from wheat leaf spots, from Oamaru, NZ, 1996

4.1 Notes on species

4.1.1 Thielaviopsis paradoxa

Not known to occur in New Zealand, see notes under *T. ethacetica*.

4.1.2 Thielaviopsis ethacetica

This is the first report of *T. ethacetica* for New Zealand. Mbenoun *et al.* (2014) noted that *T. paradoxa* (as *Ceratocytis paradoxa*) has in the past often been confused with *T. ethacetica* (as *C. ethacetica*). They considered *T. paradoxa* to have a more restricted distribution than previously accepted, and that the commonly reported, cosmopolitan *Thielaviopsis* species with a broad host range is probably *T. ethacetica*.

4.1.3 Chalaropsis thielavioides

Not known to occur in New Zealand. Reported from New Zealand by Pennycook & Galloway (2004) but the basis for that report is not known. No New Zealand voucher material is available in PDD or ICMP and we could find no earlier literature reports under *C. thielavioides* or any of its synonyms.

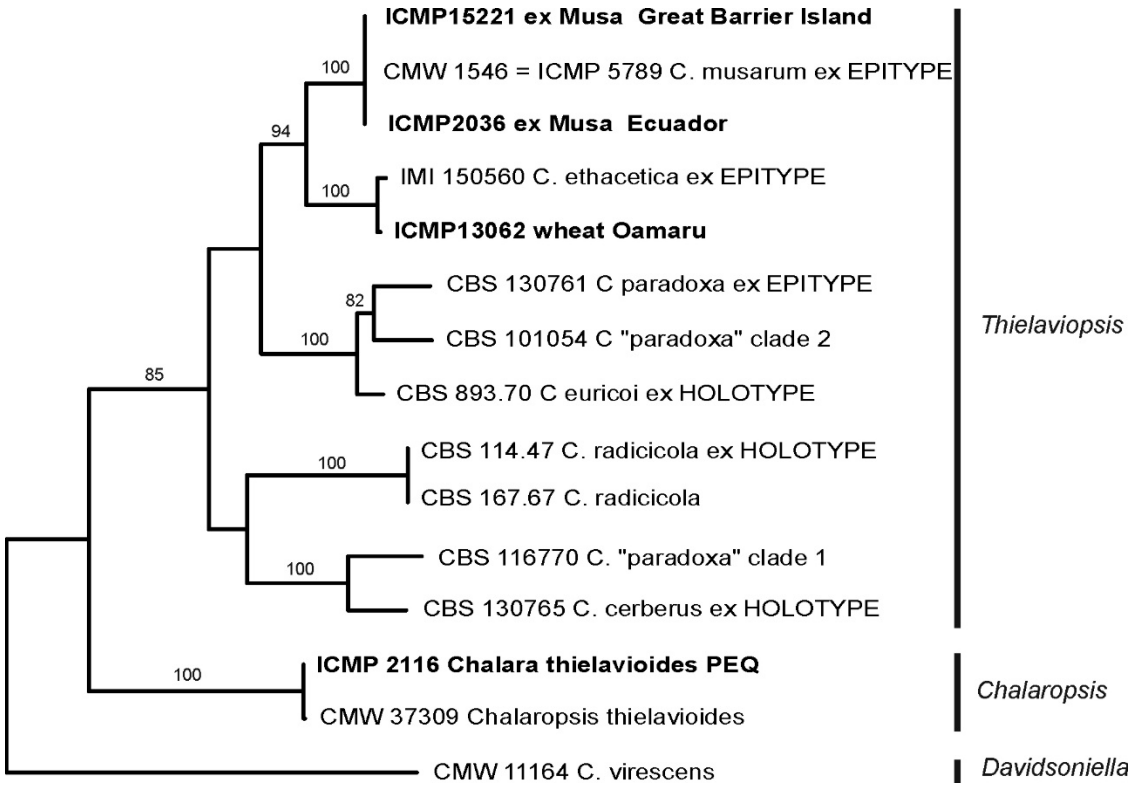

Figure 1: Phylogeny **based on concatenated ITS, β**-tubulin and TEF sequences, including ICMP isolates originally identified as *Certaocystis paradoxa* and *Chalara thielavioides* (= *Thielaviopsis thielavioides*) and representative isolates from the *C. paradoxa* complex cited in Mbenoun et al. (2014). ICMP isolates sequenced as part of this project in bold. Bootstrap values >80 shown. The generic names on the right are those accepted by de Beer et al. (2014). *Davidsoniella virescens* is selected as the outgroup based on the Mbenoun et al. (2014) phylogeny.

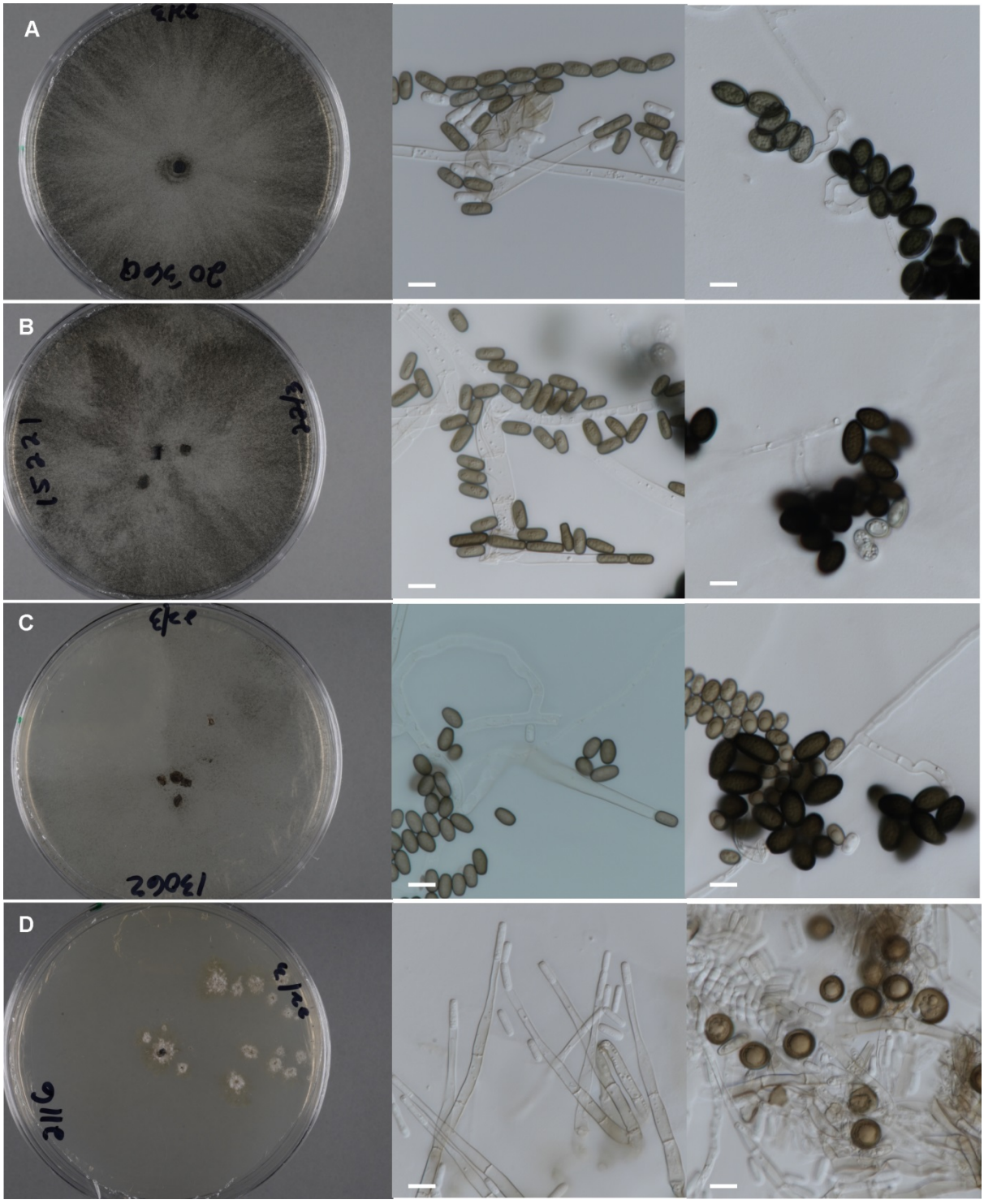

Figure 2: Cultures (MEA, 7 days, 90 mm petri dish), conidia and conidiophores, and aleurioconidia. A, ICMP 2036, *Thielaviopsis musarum*; B, ICMP 15221, *T. musarum*; C, ICMP 13062, *T. ethacetica*; D, ICMP 2116, *Chalaropsis theilavioides*. **Scale bars = 10 µm.**

5 References

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Appendix 1 – DNA sequences

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