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Botryosphaeria ribis-*B. parva* species complex. In: 15th
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BOTRYOSPHAERIA RIBIS-B. PARVA SPECIES COMPLEX

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INTRODUCTION

Botryosphaeria spp. are common endophytes and latent pathogens of woody hosts causing a wide range of symptoms such as shoot blights, cankers and fruit rot. The taxonomy of this genus has been confused due to the similarity of the teleomorph and overlapping characteristics of the anamorphs. (1) Recently DNA sequence data has been used successfully in conjunction with morphological characteristics to resolve taxonomic questions (1, 2). In particular cryptic species such as *B. ribis* and *B. parva*, which have overlapping morphological characteristics, have been clearly separated based upon sequence data (2). However, we now have a number of isolates from eucalypts in Australia that do not fall neatly into either *B. ribis* or *B. parva* suggesting either a species complex or a complex of species. Various scenarios for these isolates were tested the morphological, biological and phylogenetic species concepts.

MATERIALS AND METHODS AND RESULTS

Isolates considered in this study were isolated from eucalypts and other woody plants in Australia. For comparison purposes the type cultures of *B. ribis* (CMW7772 and 7773) and *B. parva* (CMW9080) were also included.

Morphology: Pycnidia of the various isolates were induced on tap water agar overlaid with sterile eucalypt twigs. Both hyaline, fusoid (*Fusicoccum*) and pigmented, irregular (*Dichomera* synanamorph) spore types were found (Fig. 1).

Phylogenetics: Multiple gene genealogies using four gene regions; ITS, EF, CHS, and RPB2, were investigated. The topology of the combined gene tree indicates four distinct well supported clades (Fig. 2).

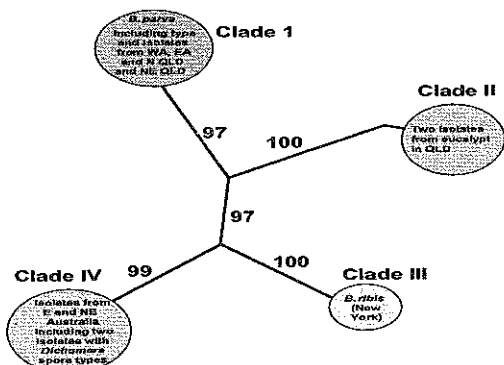


Figure 2. Unrooted phylogram of the most parsimonious tree of 46 steps. Bootstrap values are depicted next to the branch.

Pathogenicity: Pathogenicity tests were conducted on excised stems of *Eucalyptus globulus* (approx 5cm diam.). Stems were underbark inoculated in the center and lesion length determined after 9 days. Pathogenicity varied between isolates but there was no correlation between, phylogenetic grouping, host or geographical region (Fig. 3).

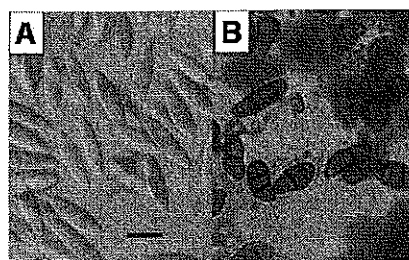


Figure 1 Spore types from the *B. ribis*-*B. parva* complex. (A) Fusiform, hyaline spores. (B) irregular dark spores. Bar = 10 μ m

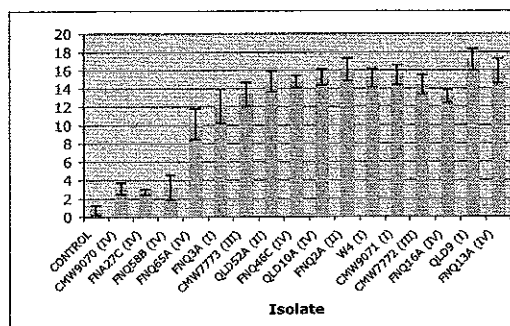


Figure 3 Mean lesion length of excised stems of *E. globulus* stems inoculated with different isolates. Bars represent the standard errors of the mean. Clade number indicated in brackets.

DISCUSSION

In accordance with the Phylogenetic Species Concept, the data based on analysis of 4 gene regions indicates multiple species existing within the *B. ribis*-*B. parva* species complex. However under the more traditional Morphological Species Concept the data indicates the existence of two species (the type specimens of *B. ribis* and *B. parva* are virtually identical based on morphology) (2). As these pathogens cause problems in a wide range of hosts worldwide this becomes a concern as host range may expand and result in "super pathogens". This study highlights problems associated with taxonomy in fungal species-limit of species definition when based on morphology. Further work is currently being done in sequencing the mitochondrial genome to give in-depth population specific information.

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