

Abstract

Botryosphaeria spp. have been found around the world on numerous hosts. Recent work has indicated that *Botryosphaeria ribis* and *B. parva* may not be distinct species but rather represent the extremes in a complex. It has been difficult to separate these species based on phenotypic, molecular or niche associations. In this thesis, a selection of isolates, representing the diversity in the whole group, were chosen from a larger subset. This thesis sought to uncover the true status of *B. ribis*-*B. parva*. Four possible scenarios were hypothesized:

- a) they were never two distinct species and are instead one species
- b) they are a species complex in the process of speciation
- c) they were two distinct species that have never developed reproductive isolation mechanisms and when brought back into contact with each other hybridized and formed a species complex.
- d) There are many species

Multiple gene genealogies using five gene regions, ITS, EF, CHS, RPB2 and ACT, were investigated. The topology of each generated from each individual gene region reflected a different phylogenetic relationship between isolates in the *B. ribis*-*B. parva* complex. The trees were incongruent and could not be combined. This inability to combine the data sets indicated recombination amongst isolates from these two “species”. Isolates were classified as either *B. parva*, *B. ribis* or “other”, depending on whether they grouped with the type specimens of each “species” or not. Based on this there were isolates that consistently grouped with types of *B. parva* and *B. ribis* and there were isolates contained a combination of characters from both *B. parva sensu stricto* and *B. ribis sensu stricto*. The reticulate nature of each tree topology indicated recombination amongst isolates, lending weight to the scenario hypothesising one species.

SSR data was used to help support the conclusions drawn from the multiple gene genealogies and to try and identify possible gene flow and assess level of genetic diversity amongst isolates. Eight SSR markers that had previously been developed for *B. parva* were used. Distance analysis indicated no association with host or geographic location. The data indicated limited gene differentiation amongst isolates from the Australasian region suggesting the movement throughout the region on eucalypts. Whilst there was evidence of gene flow between South Africa and Australasia, the highest diversity and most unique alleles were found in South Africa from a group of isolates collected from a native *Syzygium* species. Perhaps this could be the origin of *B. parva*. The I_A values indicated clonality despite high genotypic diversity (73

different genotypes out of 100 isolates). The SSR data did not support the maintenance of two distinct species of *B. ribis* and *B. parva*.

Pathogenicity tests showed no significant differences between isolates previously identified as *B. parva sensu stricto* and *B. ribis sensu stricto*, one group of isolates did show a significant reduction in pathogenicity but no correlation between host or geographical region could be made to support this. Both hyaline, fusoid (*Fusicoccum*) and pigmented, irregular (formerly *Dichomera*) spore types were found amongst isolates in this group however, there were no differences between isolates of the types of *B. parva* and *B. ribis*. The appearance of *Dichomera* spore type is very interesting and further investigations of *Dichomera*-like spores in the genus *Botryosphaeria* should be undertaken. Phenotypic evidence does not support the maintenance of two distinct species, nor does it provide any evidence of hybridisation.

There are no significant, consistent morphological differences, multiple gene genealogies generate reticulate incongruent phylogenies (indicating recombination) and SSR data indicates substantial gene flow and shared alleles. Under the Morphological Species Concept, the Biological Species Concept and the Phylogenetic Species Concepts the separation of two distinct species is not supported. **Therefore the results of this thesis indicates that *B. parva* should be synonymized into *B. ribis* (*B. ribis* was described before *B. parva*).**