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Elucidating the *Ramularia eucalypti* species complex

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Abstract



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The genus *Ramularia* includes numerous phytopathogenic species, several of which are economically important. *Ramularia eucalypti* is currently the only species of this genus known to infect *Eucalyptus* by causing severe leaf-spotting symptoms on this host. However, several isolates identified as *R. eucalypti* based on morphology and on nrDNA sequence data of the ITS region have recently been isolated from other plant hosts, from environmental samples and also from human clinical specimens. Identification of closely related species based on morphology is often difficult and the ITS region has previously been shown to be unreliable for species level identification in several genera. In this study we aimed to resolve this species-complex by applying a polyphasic approach involving morphology, multi-gene phylogeny and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). Six partial genes (ITS, ACT, TEF1- α , HIS3, GAPDH and RPB2) were amplified and sequenced for a total of 44 isolates representing *R. eucalypti* s.lat. and closely related species. A multi-gene Bayesian phylogenetic analysis and parsimony analysis were performed, and both the resulting trees showed significant support for separation of seven species in *R. eucalypti* s.lat., including two previously described (*R. eucalypti* and *R. miae*), four novel species here described (*R. haroldporteri*, *R. glennii*, *R. mali* and *R. plurivora*) and one undescribed *Ramularia* species (sterile). Additionally, *Mycosphaerella nyssicola* is newly combined in *Ramularia* as *R. nyssicola*. Main mass spectra (MSPs) of several *R. eucalypti* s.lat. strains were generated using MALDI-TOF MS and were compared through a Principal Component Analysis (PCA) dendrogram. The PCA dendrogram supported three clades containing *R. plurivora*, *R. glennii*/*R. mali* and *R. eucalypti*/*R. miae*. Although the dendrogram separation of species differed from the phylogenetic analysis, the clinically relevant strains were successfully identified by MALDI-TOF MS.

Keywords: MYCOSPHAERELLACEAE; PLANT PATHOGEN; SPECIES COMPLEX; SYSTEMATICS

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