



Multivariate ratio analysis and DNA markers reveal a new Australian species and three synonymies in eucalypt-gall-associated *Megastigmus* (Hymenoptera: Megastigmidae)

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Abstract

The genus *Megastigmus* Dalman, 1820 (Hymenoptera: Megastigmidae) contains potential biocontrol agents of the invasive eucalypt galling chalcid *Leptocybe* spp. (Hymenoptera: Eulophidae), with several species reported in various parts of the world. Species discrimination is challenging due to intraspecific morphological variation, difficulty in measuring sizes of body parts, and the lack of information regarding the global distribution of parasitic *Megastigmus*. We used two species commonly associated with *Leptocybe* in its native range to review taxonomic methods and determine the most reliable morphological characters in species delimitation. We examined size variation of body characters, and conducted species discrimination using multivariate ratio analysis, mitochondrial Cytochrome c oxidase subunit 1 (COI) and nuclear 28S rDNA (28S) sequences. Morphological traits were effective in species delimitation yet revealed high variation in several characters employed in current keys. Knowledge generated on morphology and DNA justified the description of a new species, *M. manonae*, sp. n., the first record of *M. pretorianensis* in Australia, and revised diagnostic characters for *M. zvimendeli*. Based on these diagnostic characters and molecular data, we synonymize three species (*M. judikingae*, syn. n., from Australia, *M. sichuanensis*, syn. n., from China and *M. icipeensis*, syn. n., from Kenya) with *M. zvimendeli*. Our findings highlight the importance of molecular markers in assisting taxonomic decision-making and the need for coordinated work in identifying *Megastigmus* associated with *Leptocybe* spp.

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