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Revision of the Immaculatus Group of *Culicoides* Latreille (Diptera: Ceratopogonidae) from the Australasian Region with description of two new species

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Abstract

The Immaculatus Group of *Culicoides* encompassing four species from Australia, New Caledonia, Fiji, Solomon Islands, New Guinea and the Malay archipelago is revised. A diagnosis for the group, descriptions of males and females of *C. shivasi* sp. n. and *C. collessi* sp. n., a description of the male of *C. immaculatus* Lee & Reye, a redescription of the female of *C. immaculatus* and a diagnosis of *C. agas* Wirth & Hubert together with keys for their specific determination are presented. Specific separation of the morphologically similar *C. shivasi* and *C. immaculatus* is supported by DNA barcodes (mitochondrial cytochrome oxidase I or COI) and nuclear carbomoylphosphate synthetase (CAD) sequence data.

Key words: Biting midges, Australasia, *Culicoides agas*, *Culicoides immaculatus*, *Culicoides shivasi*, *Culicoides collessi*

Introduction

Dyce (1996) first proposed a grouping of species related to *C. immaculatus* Lee and Reye containing *C. agas* Wirth & Hubert and two undescribed species. He subsequently considered the group endemic to the Australasian region (Dyce 2001) and provided illustrations of the wings of five taxa belonging to the group (one remains undescribed) (Dyce *et al.* 2007).

Recent work has shown that DNA barcoding and genetic analysis of the mitochondrial cytochrome oxidase I (COI) gene can be useful in distinguishing *Culicoides* species that are difficult to separate morphologically (Linton *et al.* 2002; Dallas *et al.* 2003; Pagès & Sarto i Monteys 2005; Nolan *et al.* 2007; Pagès *et al.* 2009). The majority of these studies have concentrated on the two subgenera of greatest veterinary importance, *C.* subg. *Avaritia* Fox and *C.* subg. *Culicoides* Latreille, although data for species from a wider range of subgeneric groupings has been generated (Matsumoto *et al.* 2009) indicating that mitochondrial DNA is likely to be widely useful for DNA barcoding and identifying species throughout the genus. Nuclear genetic markers can be used to provide independent tests of species hypotheses generated by mitochondrial COI barcoding (Dayrat 2005). This approach to species diagnostics can also provide novel insights into the evolutionary history of focal taxa, particularly when patterns of incongruence are detected between independent genomic markers (Dayrat 2005; Roe & Sperling 2007).

In this paper a diagnosis is proposed for the Immaculatus Group *sensu* Dyce *et al.* (2007). A first description of the male and a redescription of the female of *C. immaculatus*, diagnoses for *C. agas*, descriptions of two new species and keys for the specific determination of species in this group are provided. DNA barcodes (partial mitochondrial COI gene sequences) and nuclear DNA sequence data are presented in support of species status and to serve as molecular diagnostics.