



Cryptic diversity in Australian stick insects (Insecta; Phasmida) uncovered by the DNA barcoding approach

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Abstract

The barcoding approach was applied to analyze 16 Australian morphospecies of the order Phasmida, with the aim to test if it could be suitable as a tool for phasmid species identification and if its discrimination power would allow uncovering of cryptic diversity. Both goals were reached. Eighty-two specimens representing twelve morphospecies (*Sipyloidea* sp. A, *Candovia annulata*, *Candovia* sp. A, *Candovia* sp. B, *Candovia* sp. C, *Denhama austrocarinata*, *Xeroderus kirbii*, *Parapodacanthus hasenpuschorum*, *Tropidoderus childrenii*, *Cigarrophasma tessellatum*, *Acrophylla wuelfingi*, *Eurycantha calcarata*) were correctly recovered as clades through the molecular approach, their sequences forming monophyletic and well-supported clusters. In four instances, Neighbor-Joining tree and barcoding gap analyses supported either a specific (*Austrocarausius mercurius*, *Anchiale briareus*) or a subspecific (*Anchiale austrotessulata*, *Extatosoma tiaratum*) level of divergence within the analyzed morphospecies. The lack of an appropriate database of homologous *cox1* sequences prevented more detailed identification of undescribed taxa.

Key words: Australian fauna, barcoding gap, cytochrome oxidase I

Introduction

The order Phasmida Leach, 1815 (Hexapoda; Insecta) includes hemimetabolous insects known as stick and leaf insects. Together with Blattodea (cockroaches and termites), Dermaptera (earwigs), Embiodea (web-spinners), Mantodea (praying mantises), Orthoptera (grasshoppers, locusts, bush-crickets and crickets), Plecoptera (stoneflies), Zoraptera (angel insects) and Notoptera [suborders Grylloblattodea (ice crawlers) and Mantophasmatodea (gladiators)], it forms the taxon Polyneoptera, the monophyly of which is quite well recognized on morphological and molecular basis, even if interordinal phylogenetic relationships are still debated (Ishiwata *et al.* 2011, Yoshizawa 2011, Letsch & Simon 2013, Wang *et al.* 2013, Wipfler *et al.* 2014). The order currently includes 13 families, 463 genera and more than 3060 species distributed following a geographical gradient from tropical regions to temperate ones (Brock & Marshall 2011, Kōmoto *et al.* 2011, Brock 2014). The ancestry of the order is controversial; Davis *et al.* (2010), using a super-tree approach, inferred a phylogeny-based origin of Phasmida between the Carboniferous and the Permian, around 290 million years ago (MYA), while Plazzi *et al.* (2011), using complete mitochondrial genome sequence analyses, dated its origin to 150 MYA, in the Upper Jurassic.

Phasmids are known for their cryptic mimicry at all their life stages. Shape, size and colour of the eggs rather resemble plant seeds, while most nymphs and adults mimic leaves or twigs in both morphology and colour. These masters of camouflage represent a clear example of adaptative selection and morphological conservatism, with a wide range of individual variation within some taxa leading inevitably to many problems in species delimitations through the traditional morphological approaches.

Following Brock & Marshall (2011), the order is at present represented by the suborders Agathemerodea (Zompro 2004), Timematodea (Kevan 1982) and Verophasmatodea (Zompro 2004), the last taxon being further