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Pachybrachis holerorum (Coleoptera: Chrysomelidae: Cryptocephalinae), a new species from the Apennines, Italy, identified by integration of morphological and molecular data

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Abstract

Pachybrachis holerorum **n. sp.** is described from the northern Apennines, Italy. The new species is related to *P. karamani* (Weise, 1893), from which it differs in the shape of the median lobe of the aedeagus and in small differences in chromatic pattern. The close relationship with *P. karamani* is confirmed by molecular analyses performed on a fragment of 829 nucleotides of the mitochondrial gene Cytochrome *c* oxidase subunit 1 (*cox1*). The general mixed Yule-coalescent model, developed for species delimitation using single-locus molecular data, was applied to a *cox1* phylogeny in order to test the hypothesis of *P. holerorum* as a separate species. Information on the host plants, acquired during specimen collection, was confirmed from gut content, targeting a fragment of the plastid large subunit of the ribulose-bisphosphate carboxylase gene and the *trnL*(UAA) intron. Besides, the lectotype of *P. karamani* is designated.

Key words: Leaf beetle, internal sac, rectal apparatus, species delimitation, molecular ecology

Introduction

Pachybrachis Chevrolat, 1836, is a widely distributed Holarctic and Neotropical genus of phytophagous insects that reaches maximum species diversity in the Neotropical region. The genus comprises more than 350 described species, with 156 species and subspecies in the Palaearctic (Schöller *et al.* 2010; Sassi 2012). Remarkably, 26 species of *Pachybrachis* have been reported from Italy, 19 in peninsular territories, four in Sardinia and seven (three endemic) in Sicily (Sassi 2012). The Mediterranean area and especially the island territories seem to be particularly rich for *Pachybrachis* (Burlini 1957; Burlini 1959; Daccordi and Ruffo 1971; Daccordi and Ruffo 1975; Sassi 2006), with high endemism in this region (about 20%; Montagna 2011). There are still surprising discoveries to be made, such as the recent discovery of a new taxon in areas thoroughly investigated by entomologists (Montagna 2011). Molecular characters, as well as being useful in the resolution of relationships between taxa, also shed light on their ecology, for example revealing associations between phytophagous insects and their host plants, as well as the role played by symbionts in the host metabolism (e.g. Jurado-Rivera *et al*. 2009; Sabree *et al*. 2012; García-Robledo *et al*. 2013). At present, the knowledge of the host plants of *Pachybrachis* is limited to few species but the genus is regarded as being polyphagous (Jolivet and Hawkeswood 1995; Bienkowski 1999).

Careful study of specimens of *Pachybrachis* from various localities in the North Apennine (Italy), has revealed what appears to be a new species. Subsequent collecting trips were carried out determine the range of this putative new taxon and obtain fresh samples of several *Pachybrachis* species for molecular analyses. The new species is compared with *P. karamani* Weise, 1893, which is the most similar from morphology, and with the less similar *P. salfii* Burlini, 1957, and other species. Molecular comparison of the species is based on the general mixed Yule-coalescent model (Pons *et al*. 2006; Fontaneto *et al*. 2007), developed for species delimitation using single-locus

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