Zootaxa 2068: 27–46 (2009)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Two new species of *Helobdella* Blanchard 1896 (Hirudinida: Rhynchobdellida: Glossiphoniidae) from Taiwan, with a checklist of hirudinea fauna of the island

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Abstract

In this study, two new species of *Helobdella* leeches, *Helobdella octatestisaca* **n. sp.** and *Helobdella melananus* **n. sp.**, and a newly recorded species, *Helobdella europaea*, are reported. Morphological characters and DNA barcode analysis were used to identify the three new species. The differences between these species and other morphologically congeneric species were also compared. In addition, the potential ecological impact of these species was discussed and a checklist of the hirudinea fauna in Taiwan, including eight species in Family Glossiphoniidae, four in Hirudinidae, two in Haemopidae, two in Haemadipsidae, and two in Salifidae, was provided.

Key words: *Helobdella octatestisaca, Helobdella melananus, Helobdella europaea,* DNA barcode, taxonomy, invasive species, Hirudinea, Taiwan

Introduction

Species of the glossiphoniid genus *Helobdella* Blanchard 1896 are small, dorsoventrally flattened leeches whose ancestors gave up blood-feeding in favour of predation on aquatic invertebrates (Siddall & Borda 2003). They have origins in Central and South America with several species being distributed worldwide by humans. Some species of *Helobdella* have been used as model organisms in annelid developmental biological studies (Weisblat & Huang 2001). The systematics of *Helobdella* was investigated by Siddall & Burreson (1998). The phylogenetic position of the genus was further analyzed, and some species revisions were later confirmed (Light & Siddall 1999; Siddall & Borda 2003; Siddall *et al.* 2005; Siddall & Budinoff 2005). In these studies, both morphological characters including pigmentation patterns and molecular sequences were used to reconstruct the phylogeny of the members of the genus *Helobdella*. However, the uncertainty of pigmentation patterns was raised in these studies and was noted again in later studies. Pigmentation patterns may be reliable identification characters between species in some leech genera; however, they are not stable enough in *Helobdella* (Bely & Weisblat 2006; Siddall & Borda 2003; Mark Siddall, personal communication). In the *Helobdella triserialis* complex, specimens with similar pigmentation patterns and identified as the same species using morphological characters have been demonstrated to be different species by molecular analysis. At the same time, specimens with obviously different colours, pigmentations, and other morphological traits have been shown to be the same species with genetic data (Siddall & Borda 2003; Siddall & Budinoff 2005). Since the morphological characters have been unreliable in the genus *Helobdella*, it is necessary to search for other more reliable characters to avoid the confusion in species identification.

DNA barcoding is the system of species identification using DNA sequences (Hebert *et al.* 2003). In animals, the mitochondrial cytochrome *c* oxidase subunit 1 (COI) sequences have been selected as the DNA barcode for most animal phyla (Hebert *et al.* 2003). Recently, the need for DNA barcoding in the species identification of leeches has been widely discussed (Bely & Weisblat 2006; Siddall *et al.* 2007), including the

Accepted by B. Sket: 4 Mar. 2009; published: 10 Apr. 2009