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Phylogenetic analysis of *Placobdella* (Hirudinea: Rhynchobdellida: Glossiphoniidae) with consideration of COI variation

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ABSTRACT

Placobdella is a genus of blood-feeding leeches in the family Glossiphoniidae. Historically, species of *Placobdella* have posed difficulty for systematists owing to a lack of informative morphological characters and the preponderance of inadequate or incomplete species descriptions. Here, we conduct a phylogenetic analysis of 55 individuals representing 20 of the 24 currently recognized nominal taxa using COI, ND1, 12S rDNA and ITS sequences under parsimony, maximum likelihood and Bayesian inference. We also examine the isolated COI phylogeny for the genus using an expanded dataset encompassing three additional species not included in the concatenated dataset. Finally, we assess genetic variation at the COI locus to validate initial specimen identifications and estimate how COI variation may reflect species boundaries. We conclude that *Placobdella* is a monophyletic group that places as the sister group to a clade formed by the genera *Haementeria* and *Helobdella*. We discuss the evolutionary implications of several internal relationships that are robustly resolved by all three optimality criteria, paying particular attention to the apparent fluidity of morphological characters exhibited by members of *Placobdella*. We also find preliminary evidence for the presence of cryptic and undescribed diversity within the genus.

1. Introduction

Placobdella Blanchard, 1893 is a genus of sanguivorous, rhynchobdellid (proboscis-bearing) leeches in the family Glossiphoniidae. The genus is almost entirely North American in distribution with one species known from Europe, and one known from Central America (Bielecki et al., 2012; Marrone et al., 2016; Oceguera-Figueroa and Pacheco-Chaves, 2012; Siddall et al., 2005). Members of *Placobdella* are primarily ectoparasitic on turtles, with some specializing on amphibians or aquatic reptiles, though many species will feed opportunistically on other vertebrates including birds, fish and humans (Jones and Woo, 1990; Moser et al., 2010; Siddall and Bowerman, 2006; Siddall and Gaffney, 2004). For some hosts, members of *Placobdella* are vectors of hemogregarine and trypanosome blood parasites (Barta and Desser, 1989; Siddall and Desser, 1990, 1991, 2001).

At present, the genus includes 24 nominal taxa, but this is likely an underestimate of the true diversity (Oceguera-Figueroa and Siddall, 2008; de Carle, unpublished data); see Figs. 1 and 2 for select representative taxa. Many original descriptions of *Placobdella* species were incomplete, lacking, for example, specific type localities (see Moser et al., 2014) or complete morphological descriptions (see Moser et al., 2013b). Species have also been revised without examination of type material, and several taxa have therefore been erroneously synonymized (see Moser et al., 2013c). For several species, type material has been lost, or was never deposited (see Moser et al., 2014, 2013a). Several of these species have been re-described in recent years (Jones and Woo, 1990; Moser et al., 2012a, 2012b, 2013a, 2013b, 2013c, 2014), highlighting the uncertain identities of several members of the genus, and the pervasiveness of inadequate descriptions. In the past decade, six new species of *Placobdella* have been described: *Placobdella ali* Hughes and Siddall, 2007; *Placobdella lamothei* Oceguera-Figueroa and Siddall, 2008; *Placobdella ringueleti* López-Jiménez and Oceguera-Figueroa, 2009; *Placobdella sophieae* Oceguera-Figueroa et al., 2010; *Placobdella kwetlumye* Oceguera-Figueroa et al., 2010; and *Placobdella siddalli* Richardson

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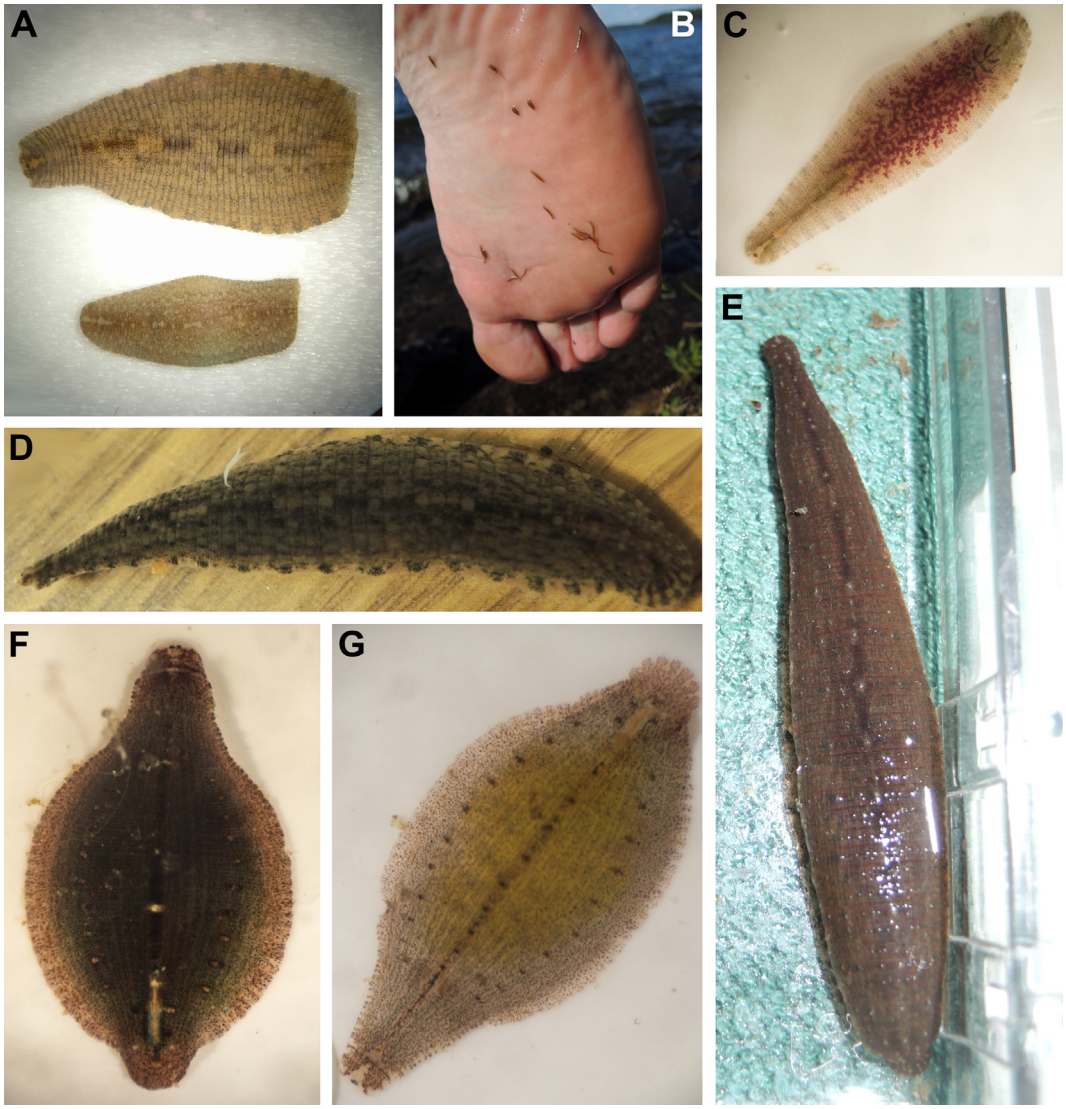

Fig. 1. Selected specimens of *Placobdella.* Photos by D. de Carle or S. Kvist except where specified. (A) Two preserved specimens of *Placobdella rugosa* showing disparate patterns of dorsal pigmentation (Manitoba, Canada; ROMIZ I11553 [top] and ROMIZ I11587 [bottom]); (B) Live juvenile specimens of *Placobdella rugosa* during collecting (Minnesota, USA; photo by A. Oceguera-Figueroa); (C) Live, recently fed juvenile *Placobdella rugosa* with crop caecae visualized by the bloodmeal (Ontario, Canada; ROMIZ I10254); (D) Live adult *Placobdella rugosa* with spermatophore attached to caudal surface (Ontario, Canada; ROMIZ I10476); (E) Live adult *Placobdella* sp. 1 (Saskatchewan, Canada; ROMIZ I11464), this specimen represents the same species as specimens ROMIZ I11277 and ROMIZ I11494 (see Discussion); (F) Live *Placobdella phalera* (Ontario, Canada; ROMIZ I10190); (G) Live *Placobdella picta* with eggs on ventral surface giving the specimen a yellow tinge (Ontario, Canada; ROMIZ I10257).

and Moser, 2017 (Hughes and Siddall, 2007; López-Jiménez and Oceguera-Figueroa, 2009; Oceguera-Figueroa et al., 2010; Richardson et al., 2017).

There is a great deal of uncertainty surrounding the taxonomy of *Placobdella*, stemming largely from a lack of phylogenetically informative morphological characters. Sawyer (1986) described *Placobdella* as including species that possess triannulate mid-body somites, two pairs of coalesced eyespots, two pairs of compact salivary glands, and one pair of bacteriomes – structures that house bacterial endosymbionts – inserting into the oesophagus. Despite these seemingly clear synapomorphies, phylogenetic evaluation of Glossiphoniidae by Light and Siddall (1999) recovered *Placobdella* as paraphyletic due to the inclusion of the genera *Oligobdella* Moore, 1918 and *Desserobdella* Barta and Sawyer, 1990 which exhibit biannulate mid-body somites and diffuse salivary glands, respectively. In a subsequent study, these genera were designated junior synonyms and subsumed within *Placobdella* (Siddall et al., 2005). In addition, constituent species have been variously assigned to the genera *Haementeria* de Filippi, 1849, *Actinobdella* Moore, 1901; *Clepsine* Savigny, 1822 and *Batracobdella* Viguier, 1879 (Barta and Sawyer, 1990; Moser et al., 2012b; Oceguera-Figueroa, 2012). Furthermore, Autumn (1936) considered *Placobdella* a junior synonym of *Haementeria,* resulting in some nomenclatural complications. As currently defined, the genus *Placobdella* encompasses all glossiphoniid species bearing one pair of cecate bacteriomes, bilobate ovaries, and two coalesced pairs of eyespots (Siddall et al., 2005).

Recently, the unique microbiomes and secretions of *Placobdella* have been investigated. The endosymbiotic bacteria housed in bacteriomes of *Placobdella* species were shown to represent a lineage of alphaproteobacteria (*Reichenowia* Siddall, Perkins and Desser, 2004) related to Rhizobiaceae (Siddall et al., 2004). Although many other glossiphoniids house bacterial endosymbionts in similar structures, *Reichenowia* species are known only from *Placobdella*, and the two taxa likely share close co-evolutionary histories (Perkins et al., 2005; Kvist et al., 2011). Additionally, the peptides