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Host Associations of *Helobdella octatestisaca* (Hirudinida: Glossiphoniidae) and the First Report of this Leech in the United States

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ABSTRACT: *Helobdella octatestisaca* Lai and Chang, 2009, was originally described from Taiwan where it is believed to have been introduced. It has subsequently been reported from throughout much of Mexico. *Helobdella octatestisaca* was collected from a small pond in Walker County, Texas, U.S.A., representing the first report of this species from the United States. *Helobdella octatestisaca* occurred primarily associated with red-eared slider turtles, *Trachemys scripta*, and common mud turtles, *Kinosternon subrubrum*. Individuals of *H. octatestisaca* were frequently found within clusters of juveniles of the common turtle leech, *Placobdella parasitica*, and were often attached to individuals of *P. parasitica*. It was hypothesized that *H. octatestisaca* is utilizing *P. parasitica* as a source of food and that *Helobdella* spp. preferentially associate with turtles, thereby gaining enhanced access to prey items.

KEY WORDS: *Helobdella octatestisaca*, red-eared slider turtle, *Trachemys scriptus*, common mud turtle, *Kinosternon subrubrum*, *Placobdella parasitica*, Hirudinida, Hirudinea, leech, Glossiphoniidae.

Helobdella octatestisaca Lai and Chang, 2009, was originally described from various locations in Taiwan (Lai et al., 2009). Based on the observation that it was common in easily accessible areas but had not been reported in previous surveys, Lai et al. (2009) suggested that *H. octatestisaca* was introduced into Taiwan. This assertion was supported by the molecular data of Oceguera-Figueroa et al. (2010). Salas-Montiel et al. (2014) reported *H. octatestisaca* to be widely distributed throughout central and southern Mexico. All previous reports of *H. octatestisaca* have been of free-living specimens. During July 2015, *H. octatestisaca* was found to be a common symbiotic associate of turtles in a pond in Walker County in East Texas, U.S.A. This represents the first report of this leech from the United States and the first report of this leech from a host.

MATERIALS AND METHODS

Turtles were collected with hoop nets from a small pond in Walker County, Texas (30°45′06.3″N; 95°25′38.9″W), examined for leeches, and released. All leeches were prepared as described by Moser et al. (2006). Representative specimens of each species collected were subjected to molecular analysis according to Richardson et al. (2010) as follows: Purified polymerase chain reaction products were sequenced using HCO2198 and LCO1490 primers (Light and Siddall, 1999) for the cytochrome c oxidase subunit I (CO-I) product by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. Aligned DNA sequences were compared to other leech DNA sequences contained within GenBank to confirm identifications. Representative specimens were deposited in the Division of Invertebrate Zoology, Peabody Museum of Natural History (YPM IZ), Yale University, New Haven, Connecticut, U.S.A.

Leeches were identified by reference to the primary literature (Lai et al., 2009; Moser et al., 2013; Salas-Montiel et al., 2014) and by comparison to DNA sequences contained within GenBank.

RESULTS

Ten of 13 (76.9%) red-eared sliders, *Trachemys scripta,* were infested with 1–114 (mean 45.9) individuals of *Placobdella parasitica* (Say, 1824) (YPM IZ 87915–87926; GenBank KY498612). Seven of 13 (53.8%) red-eared sliders were infested with 1–13 (mean 6.6) individuals of *H. octatestisaca* (YPM IZ 87927–87934; GenBank KY498613). Two of 2 (100%) eastern mud turtles, *Kinosternon subrubrum*, were infested with 6–24 (mean 15.0) individuals of *P. parasitica,* and 1 of 2 eastern mud turtles was infested with 2

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individuals of *H. octatestisaca*. Overall, 13 of 15 (86.7%) turtles examined (13 *T. scripta* and 2 *K. subrubrum*) were infested with leeches. Of these 13 turtles, 7 (53.8%) were coinfested with *P. parasitica* and *H. octatestisaca*, 5 (38.5%) were infested only with *P. parasitica*, and 1 (7.7%) was infested only with *H. octatestisaca*.

Molecular comparison of 607 nucleotides of CO-I revealed an intraspecific difference of 0.0% (0 nucleotides) between one specimen of *H. octatestisaca* collected from a small pond in Walker County, Texas (GenBank XXXXXXXX) and a specimen of *H. octatestisaca* (GenBank HQ179860) collected from South Africa. Sequence data for CO-I of a specimen of *H. octatestisaca* collected from a small pond in Walker County, Texas (GenBank XXXXXXXX) revealed intraspecific differences of 0.2 to 0.3% (1 to 2 nucleotides) when compared to 7 specimens of *H. octatestisaca* collected from Guandu Plain, Taipei, Taiwan (type locality of *H. octatestisaca*) (GenBank FJ000342–FJ000348) and intraspecific differences of 1.7% to 2.0% (10–12 nucleoides) when compared to 5 specimens of *H. octatestisaca* collected from Mexico (GenBank HQ179855–HQ179859). In contrast, CO-I sequence data of a specimen of *H. octatestisaca* collected from a small pond in Walker County, Texas (GenBank XXXXXXXX) revealed interspecific differences of 16.4 to 16.8% (99–102 nucleotides) when compared to 6 specimens collected from the type locality of *Helobdella modesta* (New Haven, Connecticut) (GenBank JF319988–JF319993) and an interspecific difference of 15.9% (96 nucleotides) when compared to a specimen of *Helobdella stagnalis* (GenBank AF329041) collected from the United Kingdom.

Individuals of *H. octatestisaca* were frequently associated with clusters of juvenile *P. parasitica* and were often observed to be attached to individuals of *P. parasitica* although it is not clear if they were actively feeding.

DISCUSSION

The occurrence of *H. octatestisaca* in Texas constitutes a considerable extension of the known range of this species and the first report of this species from the United States. Previous North American reports of *H. octatestisaca* have been restricted to central and southern Mexico (Salas-Montiel et al., 2014). Molecular comparison of the specimens of *H. octatestisaca* collected in this study to sequences from GenBank confirm the assertions of Lai et al. (2009), Oceguera-Figueroa et al. (2010), and Salas-Montiel et al. (2014) that this leech is widely distributed and appears to have been broadly introduced.

This represents the first instance of *H. octatestisaca* being reported from turtles, although representatives of *Helobdella* spp. are frequently found on turtles, usually in low numbers (Readel et al., 2008; Davy et al., 2009) and it is generally accepted that these associations do not represent parasitism (Sawyer, 1986; Siddall and Borda, 2003; Richardson et al. 2010, 2015). The paradigmatic view is that *Helobdella* spp. were derived from blood-feeding ancestors that switched to predation on aquatic invertebrates (Siddall and Borda, 2003). Richardson et al. (2015) suggested that the association of *Helobdella* spp. with turtles may be a manifestation of some vestige of an ancestral physical association that may have been retained, especially if selective advantages are conferred by the association (Davy et al., 2009). Such advantages may include provision of an efficient dispersal mechanism, protection from predators, and/or enhanced access to prey, including other leeches (Sawyer, 1972; Davey et al., 2009).

The high prevalence and intensity of *H. octatestisaca* on turtles in the present study suggests that the association is more than coincidental, especially given the fact that relatively few free-living specimens were collected from the site. It appears that *H. octatestisaca* was preferentially associating with the turtles. Given the high degree of co-occurrence of *H. octatestisaca* with *P. parasitica,* the frequent occurrence of individuals of *H. octatestisaca* within clusters of young juvenile *P. parasitica,* and the fact that they are often attached to individuals of *P. parasitica*, we hypothesize that *H. octatestisaca* is utilizing *P. parasitica* as a source of food and that *Helobdella* spp. preferentially associate with turtles, thus providing enhanced access to their prey.

Leeches commonly feed on other leeches, although it is not clear whether they are feeding on the body fluids of the leeches, crop contents, or both. Lai et al. (2009) observed that individuals of *H. octatestisaca* were occasionally found in groups attached to the body surface of the predatory leech *Whitmania laevis* (Baird, 1869). Mann (1961) indicated that "*Helobdella*,. . ..sucks the body fluids of invertebrates such as snails and insect larvae. It is really a specialized predator rather than a parasite, as it normally kills the host." Nevertheless, it is not clear if the relationship between *H. octatestisaca* and *P. parasitica* is one of parasitism or predation. Sawyer (1972) indicated that *Helobdella* spp. feed on snails, small oligochaetes,

aquatic insects, and possibly other leeches, rather than snails. Sawyer (1972) was unable to get individuals of *H. modesta* to feed on snails and commonly found individuals of *H. modesta* attached to other leeches (e.g., *Placobdella picta*, *Haemopis grandis*, *Haemopis marmorata*, and *Macrobdella decora*) but never observed *H. modesta* feeding on leeches to which they were attached. Laboratory studies are warranted to further investigate the relationship between *H. octatestisaca* and other leeches and to gain greater understanding concerning the feeding preferences of *Helobdella* spp.

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