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Leech Parasitism of the Gulf Coast Box Turtle, *Terrapene carolina major* (Testudines: Emydidae) in Mississippi, USA

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Running Title: Leech Parasitism of Box Turtle *Terrapene carolina major*

Abstract

Ten leeches were collected from a Gulf Coast box turtle, *Terrapene carolina major*, found crossing a road in Gulfport, Harrison County, Mississippi, USA. Eight of the leeches were identified as *Placobdella multilineata* and 2 were identified as *Helobdella europaea*. This represents the second vouchered report of leeches from a box turtle. *Helobdella europaea* is reported for the first time associated with a turtle and for the second time from the New World.

Introduction

The first vouchered report of a leech parasitizing a box turtle was that of Richardson *et al.* (2016) who reported 14 individuals of *Placobdella multilineata* feeding on a Gulf Coast box turtle, *Terrapene carolina major*, collected in Gulfport, Harrison County, Mississippi in June 2015. Prior to the report of Richardson *et al.* (2016), the only previous report of leeches from a box turtle was that of Brown (1974), who reported that 7 of 169 (4%) Coahuilan box turtles, *Terrapene coahuila* collected in northern Mexico harbored 1 to 4 small unidentified leeches. *Terrapene coahuila* is the only truly aquatic box turtle (Brown 1974) although substantial aquatic behavior has been documented for *T. carolina* (Belusz and Reed 1969; Summers *et al.* 1998; McDowell *et al.* 2004; Donaldson and Echternacht 2005; Richardson *et al.* 2016). In two instances, Heaton (2017) observed Gulf Coast Box turtles swimming across a 100 m wide seaway in Gulfport, Mississippi. The purpose of this paper is to document the second vouchered report of leeches parasitizing a box turtle from Mississippi.

Materials and Methods

Leeches collected were prepared as described by Moser *et al.* (2006). Specimens were subjected to molecular analysis according to Richardson *et al.* (2010) as follows: Purified PCR products were sequenced using the HCO2198 primer and the LCO1490 primer (Light and Siddall 1999) for the Cytochrome c oxidase subunit I products 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 and in the authors' databases to confirm identifications and deposited in GenBank. Specimens of leeches were deposited in the Peabody Museum of Natural History at Yale University, New Haven, Connecticut (YPM IZ).



Figure 1. Gulf Coast box turtle, *Terrapene carolina major*.

Results

On 29 June 2016, 10 leeches were collected from a Gulf Coast box turtle, *T. carolina major*, found crossing East Taylor Road (30.413235°N, 89.024751°W), Gulfport, Harrison County, Mississippi, USA (Fig. 1). Eight of the leeches were heavily engorged with blood and were identified as *Placobdella multilineata* (YPM IZ 101900-101905) and 2 of the leeches were identified as *Helobdella europaea* (YPM IZ 101859) (Figs. 2,3). This constitutes the second report of *H. europaea* from the New World and the first report of *H. europaea* in association with a turtle.



Figure 2. Individuals of *Placobdella multilineata* and *Helobdella europaea* (arrow) closely associated on the back left inguinal pouch of a Gulf Coast box turtle.



Figure 3. *Helobdella europaea* (YPM IZ 101859). Scale bar = 2 mm

Discussion

Placobdella multilineata is a generalist leech having been reported from 18 species and subspecies of alligators, amphiumas, crocodiles, snakes, and turtles from throughout the southeastern United States and northward through the Mississippi River valley to

Illinois and Iowa (Moser *et al.* 2014; Richardson *et al.* 2016). This constitutes the second report of *P. multilineata* from a box turtle in Mississippi. Richardson *et al.* (2016) reported 14 individuals of *P. multilineata* from a Gulf Coast box turtle collected in Gulfport, Mississippi in June 2015. This second occurrence of these common parasitic leeches on a box turtle supports the assertion of Richardson *et al.* (2016) that *T. carolina* is not merely an incidental host, but rather a competent host for *P. multilineata*. Only 2 of 132 Gulf Coast box turtles examined from 2013 – 2016, including recaptures, were found to harbor leeches. In both the present report and that of Richardson *et al.* (2016) the leeches were collected from a turtle found crossing a road near water. Also, in both instances the turtles were collected during periods of hot weather in the summertime, a time during which these box turtles are occasionally observed inhabiting water (Fig. 4).



Figure 4. Gulf Coast box turtle, *Terrapene carolina major*, soaking in water.

Helobdella europaea was originally described by Kutschera (1985) as *Helobdella striata* from a fast-running stream in southern Germany. Upon discovery that the South American leech *Helobdella triserialis* var. *striata* preoccupied the name *striata*, Kutschera (1987) renamed the German species as *H. europaea*. Since its original description from Germany, *H. europaea* has been reported from Australia (under the junior synonym *Helobdella papillornata*), New Zealand, South Africa, Hawaii, Taiwan, Germany, the Netherlands, Spain, Hungary and Alameda and Sacramento Counties in California, USA (Govedich and Davies 1998; Kutschera 2004; Siddall and Budinoff 2005; Bely and Weisblat 2006; Lai *et al.* 2009; Reyes-Prieto *et al.* 2013; Pfeiffer *et al.* 2014; Málnás *et al.* 2016).

Robust morphological and molecular analysis of the genus *Helobdella* led Siddall and Budinoff (2005) to conclude that species of the genus *Helobdella* originated

Leech Parasitism of Box Turtle *Terrapene carolina major*

in South America and that reports of *H. europaea* from around the globe may have been a result of accidental introductions with common aquatic invasive plant species. This view has been widely accepted (Bely and Weisblat 2006; Lai *et al.* 2009; Reyes-Prieto *et al.* 2013). Furthermore, Siddall and Budinoff (2005) found that *H. europaea* is a sister species to *Helobdella triserialis sensu stricto* from Bolivia and that the *europaea/triserialis* cluster is sister to *Helobdella cordobensis* from Chile.

As pointed out by Siddall and Borda (2003), *Helobdella* spp. descended from ancestors that appear to have switched from being sanguivores to being predators of aquatic invertebrates with aquatic mollusks and oligochaetes being popular prey items (Siddall and Budinoff 2005). Although virtually identical molecularly, based on the COI analysis conducted by Siddall and Budinoff (2005), there is some discrepancy in the literature concerning feeding habits of *H. europaea*. Govedich and Davies (1998) reported that specimens of *H. europaea* from Australia feed exclusively on gastropod snails whereas specimens of *H. europaea* from Germany demonstrated to be more catholic in feeding habits, rapidly capturing prey items such as oligochaetes and sucking their body fluids with the aid of their proboscides (Kutschera and Wirtz 2001; Pfeiffer *et al.* 2004). In addition to aquatic snails, German *H. europaea* were reported to readily feed on oligochaetes (*Tubifex* sp.), insect larvae (*Chironomus* sp.), and isopods (*Asellus aquaticus*) (Kutschera 2004).

The current report represents the first report of *H. europaea* associating with a turtle. As pointed out by Richardson *et al.* (2017), leeches of the genus *Helobdella* are often encountered in low numbers on turtles (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, 2017). It has been pointed out that the association of *Helobdella* spp. with turtles may be a manifestation of an ancestral physical association that may have been retained, especially if selective advantages are conferred by the association (Davey *et al.* 2009; Richardson *et al.* 2015). Access to prey, including other leeches (Sawyer 1972; Davey *et al.* 2009) is one such possible advantage. Richardson *et al.* (2017) commonly found individuals of *Helobdella octatestisaca* within clusters of young *Placobdella parasitica*, often attached to *P. parasitica*, on turtles in a Texas pond. This led Richardson *et al.* (2017) to the hypothesis that *H. octatestisaca* was utilizing *P. parasitica* as a source of food and that *Helobdella* spp. may preferentially associate with turtles, thus providing

enhanced access to their prey. Richardson *et al.* (2015) reported individuals of *Helobdella modesta*, *Helobdella papillata*, and *Helobdella lineata* from snapping turtles, stinkpot turtles, and painted turtles from Massachusetts and Connecticut, all of which are common hosts of *Placobdella* spp. The present finding of *H. europaea* along with *P. multilineata* on a box turtle is consistent with the hypothesis that *Helobdella* spp. may associate with turtles as a mechanism to provide enhanced access to prey.

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