

Rapid Communication

The alien Asian leech *Barbronia weberi* (Blanchard, 1897) (Hirudinea: Salifidae) reported from two disjunct localities in North Carolina, United States, with observations on its biology and potential for laboratory research

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Received: 16 November 2017 / Accepted: 5 February 2018 / Published online: 18 February 2018

Handling editor: Vadim E. Panov

Abstract

The alien Asian leech *Barbronia weberi* (Blanchard, 1897) was discovered in two widely separate localities in coastal North Carolina, USA, during an eco-systematic study of the Erpobdellidae of this region. Both populations display key external characteristics of this easily recognizable species, most significantly two accessory pores on the venter and gonopores separated by 4 ½ annuli. Of taxonomic significance, the accessory pores are not always detectable, but arise during maturation, with the posterior pore developing first. The question arises whether these populations are truly disjunct (i.e., two introductory events) or have a continuous distribution (i.e., one introductory event). Toward an answer, we propose exploiting differences in the respective cocoons between the native erpobdellid *Mooreobdella tetragon* Sawyer and Shelley, 1976, and the alien salifid *B. weberi*, to trace the distribution of the alien species in the area of study since adult individuals are scarcely available. Owing to its incomparable reproductive success, from egg to egg under constant conditions, *B. weberi* may make a useful laboratory research tool, especially for developmental studies.

Key words: Albemarle-Pamlico, introduced species, *Mooreobdella*, leech cocoon, model organism

Introduction

In an ongoing eco-systematic study of the Hirudinea in the Albemarle-Pamlico region of northeastern North Carolina, we routinely collected specimens of Erpobdellidae. Most of these were tentatively identified as the native species *Mooreobdella tetragon* Sawyer and Shelley, 1976, originally described from coastal South and North Carolina. Upon closer microscopic examination, we were surprised to discover amongst these was the Asian species *Barbronia weberi* (Blanchard, 1897).

Barbronia weberi is a native of south Asia (Sawyer 1986, pp 746, 769), but in the last two decades it has been reported in the wild in various countries, including Italy, Spain, South Africa, Australia, Argentina, Brazil, Costa Rica and Mexico (e.g., Pamplin and Rocha 2000; Govedich et al. 2003; Genoni and Fazzone 2008; Pavluk et al. 2011; Garduño-Montes

et al. 2016; Iwama and Arruda 2016), presumably introduced via the aquarium trade. In the United States it has been reported previously from a home aquarium in south Florida (Rutter and Klemm 2001).

Material and methods

In this study, erpobdelliform leeches and cocoons were hand collected by examining submerged plants, plastic, glass and similar surfaces in selected localities in northeastern North Carolina. Individual leeches were relaxed by slowly adding 70% ethanol, and then fixed and stored in 5% formalin. Cocoons were exposed only to 5% formalin because ethanol coagulates the albumen. Specimens were examined with a Wild M7A stereo microscope with Volpi Intralux 4000 light source, as well as a Lapsun Spot Point LED lamp. Images were taken with a digital camera (Conrad Electrics, Model DP-M14). All specimens are retained under the respective deposition numbers

(BW-1, BW-2, MT-1) in the collection of the Medical Leech Museum, Swansea, UK.

Results

The alien *Barbronia weberi* was found in two geographically separate localities in northeastern North Carolina: Pasquotank County (BW-1), near Mill Dam Creek, Esclip Road (36.154832; -76.170233), 3 May 2017; and Craven County (BW-2), northwest New Bern, near junction NC State Highways 43 and 55, in spillover pond of a roadside service station (35.148612; -77.106459), 29 April 2016. The two localities are similar in being man-made with muddy bottoms and fully exposed to the hot Carolina sun, but differ significantly in other respects.

The Pasquotank County location (BW-1) is a remote rural roadside drainage ditch at the edge of a large field of recently planted maize. Cocoons were numerous, and found almost exclusively on the stems and leaves of the nonindigenous alligatorweed (*Alternanthera philoxeroides*) which was introduced into the southern United States in the late 1800s (Alligatorweed Fact Sheet 2017). Physid snails were also abundant, but there was no evidence of snails eating the cocoons, as observed for a native American erpobdellid *Erpobdella punctata* (Sawyer 1970; see also Kutschera 2003). The cocoons varied somewhat in size, with a typical large cocoon measuring about 5.0×2.0 mm. Eleven cocoons were collected, and of those with contents and undamaged, the number of embryos per cocoon varied from 3 to 6 (mean 4.75; $N = 8$), and only one appeared to have hatched naturally prior to collection. Four of the cocoons bore vorticella-like ciliates.

Although cocoons were abundant at this site, only one individual erpobdelliform leech was collected, in a clump of aggregate at the water's edge. Fortunately, this individual was fully mature and displayed all the key taxonomic characters of *B. weberi*, including the unique accessory pores (Figure 1). The relaxed dimensions of this individual were: length, 41.5 mm; width at male gonopore, 3.0 mm; caudal sucker width, 2.0 mm; distance from tip of head to clitellum and male gonopore, 6.0 mm and 9.5 mm, respectively; length of clitellum, 5.0 mm.

The clitellum is prominent, opaque and comprised of 15 distinct annuli. The conspicuous male gonopore lies in a mid-ventral furrow located between the 8th and 9th annuli of the clitellum, whereas the female gonopore is on a ring located $4\frac{1}{2}$ annuli posterior to the male. The anterior accessory pore lies within the clitellum in a furrow located between the 6th and 7th annuli anterior to the male gonopore. The posterior accessory pore is more conspicuous and lies outside

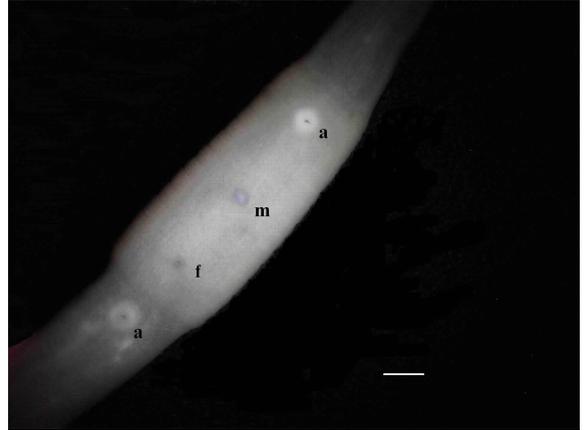


Figure 1. Ventral view of specimen BW-1 of *Barbronia weberi* from Pasquotank County, North Carolina, showing the characteristic positions of the male (m) and female (f) gonopores, and the two accessory pores (a). Scale bar, 1.0 mm.

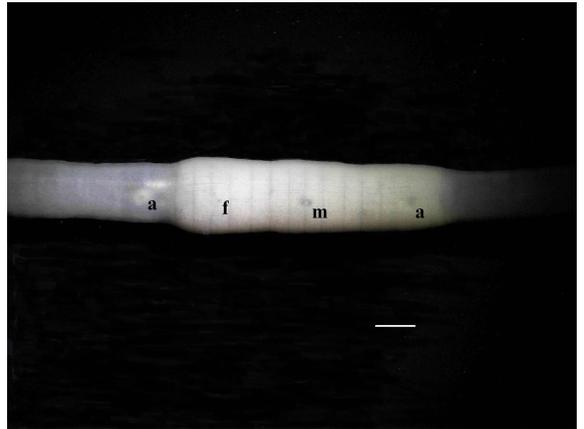


Figure 2. Ventral view of specimen BW-2c of *Barbronia weberi* from Craven County, North Carolina, showing the characteristic positions of the male (m) and female (f) gonopores, and the two accessory pores (a). Scale bar, 1.0 mm.

the clitellum in a furrow $4\frac{1}{2}$ annuli posterior to the female gonopore. This individual possessed three pairs of eyespots characteristic of the species, one labial and two buccals. These eyespots are sometimes evident in advanced embryos while still in the cocoon.

The Craven County site (BW-2) is an urban artificial pond which receives spillover from an asphalt forecourt of a busy service station. Eight mature specimens were collected on discarded plastic and glass. Two hatchlings were collected but no cocoons were noted. The relaxed length of the adults varied from 29.8 mm to 42.0 mm (mean 33.9 mm). Each had a prominently swollen opaque clitellum comprised of 15 annuli. In each case the male gonopore lay in a furrow between the 8th and 9th annuli of the

clitellum, and the female gonopore on a ring, 4½ annuli posterior to the male (Figure 2).

The anterior accessory pore was located in a mid-ventral furrow between the 6th and 7th annuli anterior to the male gonopore, whereas the posterior accessory pore was in a furrow 4½ annuli posterior to the female gonopore. Of taxonomic significance, the accessory pores were not detectable in all specimens of this series. In two individuals the anterior pore could not be detected, whereas in another two individuals both the anterior and posterior accessory pores appeared to be lacking. This apparent absence of one or both accessory pores in some individuals was generally found in the smaller specimens, suggesting a developmental dimension such that the posterior accessory pore develops first. Each of the eight specimens (BW-2) possessed three pairs of eyespots, one labial and two buccals. However, three individuals also possessed an extraneous labial or buccal eyespot of little taxonomic significance.

Internally, a dissection of the foregut of a large specimen (BW-2c) revealed three longitudinal pharyngeal ridges which terminated anteriorly as unpigmented swollen tissue (myognaths) in mid-dorsal and ventro-lateral positions, respectively, all anterior to the circum-pharyngeal nerve ring. Each myognath bore two easily overlooked, but distinct, white stylets even after 18 months in an acidic preservative. They are similar to those illustrated by Genoni and Fazzone (2008, Figure 2) and Pavluk et al. (2011, Figures 2, 3). Parenthetically, the foregut of the specimen from Pasquotank County (BW-1) was anatomically comparable in detail, except that stylets could not be verified on the myognaths with full confidence. The anterior gut of the latter specimen contained a long tubular cuticle reminiscent of a fully digested oligochaete.

Discussion

These two populations of *B. weberi* are separated geographically by more than 150 km by air and 220 km by road. This raises the question of whether they represent one unique introduction or are the result of two independent events. The Pasquotank County locality is particularly perplexing in that it is very isolated, and far from human habitation. One possibility is that this alien species is far more established in coastal North Carolina than currently thought, i.e. it has a more or less continuous distribution resulting from a single introductory event some considerable time in the past. In this context, in a survey of the leeches of North and South Carolina roughly 40 years ago, this species was not recorded (Sawyer and Shelley 1976).



Figure 3. Comparison of the cocoons of the alien salifid *Barbronia weberi* (left two, Bw) and the native erpobdellid *Mooreobdella tetragon* (right two, Mt). The former were collected in Pasquotank County, North Carolina (BW-1) and the latter in nearby Tyrrell County, North Carolina (MT-1). Scale bar, 1.0 mm.

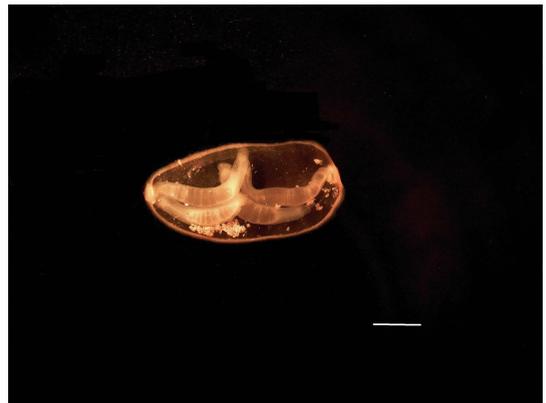


Figure 4. Cocoon of *Barbronia weberi* containing four advanced embryos within a matrix of albumen. This cocoon was collected in Pasquotank County, North Carolina (BW-1). Scale bar, 1.0 mm.

In the current ongoing survey of the leeches of coastal North Carolina, the most common erpobdelliform species in this region is the aforementioned *Mooreobdella tetragon* which superficially resembles *B. weberi*. Unfortunately, under field conditions in this swampy region erpobdelliform leeches are far more evident as isolated cocoons, the adults generally being very elusive indeed. Toward a practical understanding of whether *B. weberi* in this region has a continuous distribution, it is proposed herein that *B. weberi* can be largely distinguished from *M. tetragon* (and possibly other Erpobdellidae) based on their respective cocoons (Figure 3). These differences can be used to trace the distribution of the alien species in the study area since adult individuals are scarcely available.

The cocoons of both species are of similar size and overall appearance. However, those of *B. weberi* are generally more squat in shape, being disproportionately broad in outline, as in a rounded rectangle (Figure 3, left). Its surface is not smooth, being perceptibly rough and wrinkled, especially evident at either end. Furthermore, the walls of the cocoon typically contain numerous, irregular brown flecks, at least this is the case for cocoons from the Pasquotank County locality (BW-1). In contrast, the cocoons of *M. tetragon* are domed and elongated in shape, with both ends slightly drawn to a point (Figure 3, right). Its surface is sleek and smooth without wrinkles, and generally more reflective of light.

Finally, why has *B. weberi* become so successful as an introduced species in various parts of the world? One reason is that it is reproductively unusual in being able to procreate from egg to egg under constant warm conditions (Govedich et al. 2003). In contrast, erpobdellids and many other temperate leech species generally lose fecundity in the laboratory (Sawyer 1986, p 526; see also Kutschera 2003), presumably requiring poorly understood environmental cues.

Ironically, this unusual capability to breed under laboratory conditions from generation to generation makes *B. weberi* highly desirable for research purposes. Furthermore, as an erpobdelliform leech *B. weberi* has other unrecognized attributes as a laboratory animal, at least in principle. For example, with adroit handling a gravid individual can be coaxed to deposit a single cocoon onto a microscope slide, a cocoon which is resistant to dehydration and is transparent under transmitted lighting (Figure 4). Furthermore, in theory recently laid eggs can be removed and a single one re-inserted in the same or another cocoon. Similarly, the chemical composition of the albumen presumably can be altered.

In this context, over recent decades a few glossiphoniid (yolky egg) leech species with similar generational fecundity have been used successfully for studies of early development, especially of the nervous system (e.g., Weisblat and Huang 2001; Kutschera and Weisblat 2015). Likewise, *B. weberi* may be ideal for later developmental studies, not only of the nervous system but also investigations into cellular differentiation, gene expression and pluripotency, as well as pharmacology and teratology.

Acknowledgements

We are grateful to A. Christopher Meekins, North Carolina Department of Cultural Resources, Raleigh, NC, for field and other assistance during the ongoing study of the leeches of North Carolina. We also acknowledge the invaluable technical assistance by Carl Peters-Bond, Biopharm (UK) Ltd, Swansea, UK. All photos were taken by the senior author. The authors are very grateful for the constructive comments by the Reviewers.

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