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Observations on the Conglutinates of Ptychobranchus greeni (Conrad, 1834) (Mollusca: Bivalvia: Unionoidea)

ABSTRACT.—The conglutinates of *Ptychobranchus greeni*, a freshwater mussel, mimic aquatic dipteran larvae in shape, size, and coloration. They possess a terminal adhesive filament that may function as a holdfast to attach the conglutinate to rock or gravel substrata and maintain them in the riffle and shoal habitats of potential fish hosts. The decline and subsequent disappearance of *P. greeni* from much of its historic range may be partially explained by sedimentation and eutrophication within these habitats that limit the ability of the conglutinates to attach to the substratum.

INTRODUCTION

The larvae (glochidia) of most freshwater mussels in the Unionoidea are obligate parasites on fis before metamorphosing into juvenile mussels. Different species exhibit a variety of adaptations strategies to make contact with, and infect a suitable host. Among these are the packaging of multi glochidia into units called conglutinates that mimic fish food items. The glochidia of species that rele conglutinates are typically gill parasites. When a potential fish host consumes a conglutinate, the c glutinate breaks apart, and glochidia passing through the gill slits have the opportunity to attach the gill filaments.

We recently had the opportunity to examine a gravid female and conglutinates of the rare freshw mussel *Ptychobranchus greeni* (Conrad, 1834). *Ptychobranchus greeni*, an endangered species (Dep ment of Interior, 1993), was once common in streams and rivers of the Mobile River Basin above Fall Line, including the Black Warrior, Cahaba, and Coosa rivers and many of their tributaries (St bery, 1976). The species was associated with rapid currents over shoals, reefs, and riffles. *Ptychobranc greeni* has disappeared from most of its' former range (Stansbery, 1976; Department of Interior, 19 Today, it is present in low numbers in only two drainages: several tributaries in the Sipsey Fork drain of the Black Warrior River, Bankhead National Forest, Alabama; and the Conasauga River above Dal Georgia, Coosa River drainage.

The shells of *Ptychobranchus greeni* are highly variable, differing in shape, coloration, and marki between drainages and stream order, and at least six synonyms for the species are known (*see* Stansb 1976). The marsupia (modified brood pouches within the gills) and glochidia were briefly descri by Lea (1863). Ortmann (1923) commented on the anatomy and conglutinates (placentae). This pa describes the conglutinate of *P. greeni*, reports observations on the marsupia, other aspects of anato and conglutinate discharge in the species and comments on aspects of these observations that ma important in the recovery and management of this species.

METHODS

Several headwater tributaries of the Sipsey Fork, Black Warrior River drainage, originating and f ing through Bankhead National Forest (Winston County, Alabama) were carefully searched for mus between 14–17 March 1994. Of the mussels observed, two were identified as *Ptychobranchus greeni*, each from Flanagan and Brushy creeks. The Brushy Creek specimen was found wedged between large rocks on the bottom of a shoal in strong current. It appeared to be a gravid female, and collected for species verification and study. The live mussel was placed in a dry plastic bag, and st in a cooler for 3 days. In the laboratory, the specimen was placed in a shallow dish, covered with w and relaxed by adding a few drops of a dilute solution of pentobarbital sodium every few hours. A 48 h the specimen was fixed in 5% formalin for 24 h, rinsed, and preserved in 75% ethanol.

OBSERVATIONS

Approximately 14 h following initiation of the relaxation 17 conglutinates had been discharged the container. No additional conglutinates were released. When an attempt was made to remove conglutinates from the relaxing dish with an eyedropper, it was discovered that they were attache the bottom of the dish by one end. The attached conglutinate tip adhered tenaciously to a pin pa under it, to the point of breaking the surface tension of the water when the pin was removed.

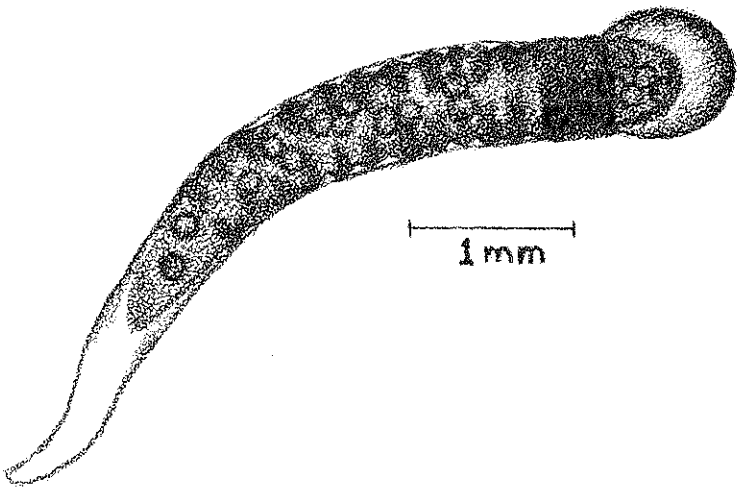

FIG. 1.—Conglutinate of *Ptychobranchus greeni*

The vermiform conglutinates measured approximately 5.5 mm in length and 0.75 mm in diam They were differentiated into three distinct regions (Fig. 1). The distal end (corresponding tc edge of the marsupium) was Ca. 1.0 mm long swollen, quadrate or oval in shape, surrounded translucent yellow-brown membrane, and enclosed a slightly smaller, reddish-pink oval structure. was followed by a swollen black collar about 0.25 mm long. The remainder of the conglutinate elongate (4.25 mm long), yellow-brown in color, and with regular constrictions giving the appear of segmentation. There was a narrow, darker lateral stripe along each side of this region. The terr end of this region of the conglutinate tapered to a short transparent filament or tube, visible under magnification. This filament had strong adhesive qualities. Fibers, dust particles and other d adhered tenaciously to the filaments. The filament of one conglutinate broke at its base when a fragment attached to it was removed. Overall, the conglutinates appeared to mimic dipteran la such as chironomids, in size, shape, and color. The regions corresponded to dipteran body segm including a reddish "head," black "thorax," and a tapering, yellow-brown "abdomen" (Fig. 2).

The membrane enclosing the conglutinate was durable, strong, and resisted handling. When pre was applied to the side of the "abdomen" of one conglutinate, however, it split along the dark la stripe releasing granular material and glochidia. The "head" was filled with red granular materia glochidia. The glochidia were small and suboval. The mean height and length of seven glochidia 0.23 mm and 0.20 mm, respectively.

The shell of the mussel studied was elliptical in shape, and measured 63 mm long, 38 mm high 25 mm deep (Fig. 3). Periostracum was yellow-brown, with no rays or other markings. The m relaxed well, with the foot extending for Ca. ½ of its length beyond the shell margin when prese Soft tissues were creamy white in coloration, with the exception of the gills, marsupia, apertures anus.

The marsupia encompassed the lower ½ of the outer gills and were characteristically strongly fc (Fig. 4). The ventral portion of the entire outer gills were used, with the exception of a few v tubes in the extreme posterior portion. The posterior quarter of both marsupia had been rec discharged, as evidenced by their distended and distinctive shapes. Coloration of the marsupia mat that of the conglutinates, *i.e.*, a red bead along ventral margin, a black stripe above this, and ye brown beyond. Approximately 160 conglutinates remained in the left gill, with about anothe distended, apparently recently discharged oviducts in the posterior quarter. Seven of the congluti in the posterior portion of the left gill were starting to ascend the oviducts toward the bran chamber. The inner gills and dorsal nonmarsupial portion of the outer gills were light brown.

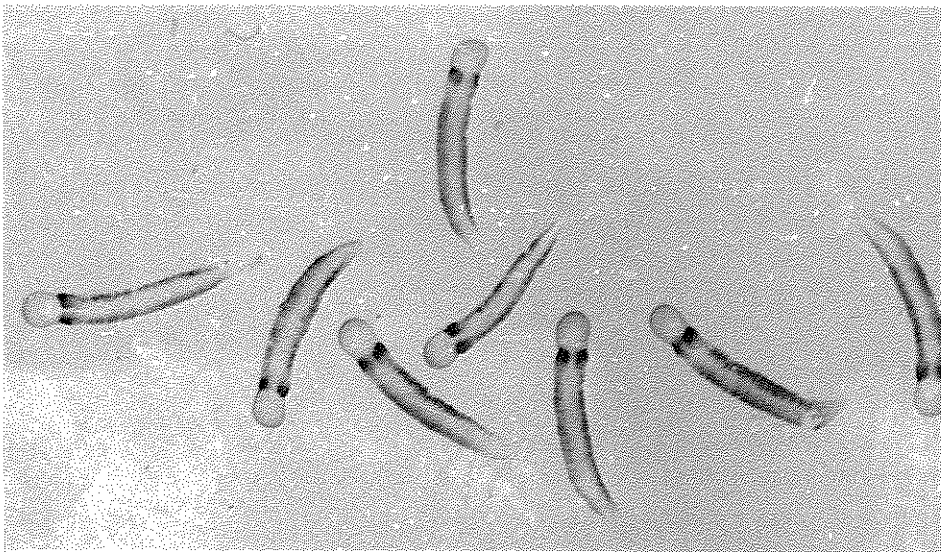

FIG. 2.—Conglutinates of *Ptychobranchus greeni* mimic dipteran larvae, such as chironomids

The apertures were yellow-brown in coloration. Papillae of the incurrent aperture were small a slender. An obsolete mantle flap anterior to the apertures appeared serrate along its margin due the presence of minute papillae, and was lightly colored with black pigment. The anus was stron produced into a slender sharp point, white along the anal margin and blackish on the sides and abo

DISCUSSION

Ortmann (1910, 1911, 1912) reported the discharge of conglutinates in *Ptychobranchus* through t edge of the marsupium. His observations, however, were based on conglutinates protruding from t marsupium in alcohol preserved specimens of *P. fasciolaris* (Rafinesque, 1820) (Ortmann 1910). O observations indicate the conglutinates of *P. greeni* are discharged via the oviducts, branchial chamb and excurrent aperture. The conglutinates reported here were discharged while the specimen w alive. The animal was fixed in a relaxed state, and the preserved specimen shows several conglutina ascending the oviducts toward the branchial chamber. The margins of the empty oviducts showed openings and were entire. Ortmann's observations (1910, 1911, 1912) may have been an artifact preservation, and/or handling. Further observations on *P. fasciolaris* are needed to verify Ortmann observations.

Luo (1993) noted that conglutinates of *Ptychobranchus subtentum* (Say, 1825) from the Tenness River drainage resembled larval aquatic insects, and that the method of release was unknown. Ho identified for this species were shoal-dwelling fish, including four darter species and one sculpin (Lu 1993). Chironomid larvae are a primary food for such fishes, and are commonly found clinging rock, gravel or bedrock within riffle and shoal habitats. As noted above, *P. greeni* conglutinates min chironomid larvae in size, shape, coloration and overall appearance. It is likely that the primary h fish used by this species are also shoal-dwelling, benthic insectivores such as darters.

Ptychobranchus greeni is currently found in high quality, clear streams originating and flowing throu Bankhead National Forest. It is not found in similar sized streams originating and flowing throu private lands in the vicinity. Streams on private lands in this area are variously affected by high lev of sedimentation, and/or eutrophication caused by leaky septic systems, chicken farm and cattle feed runoff, cultivation, surface mine runoff, *etc.* (pers. observ.). The species is also found on private lar in the Conasauga River immediately downstream of Cherokee National Forest. It declines in a dov

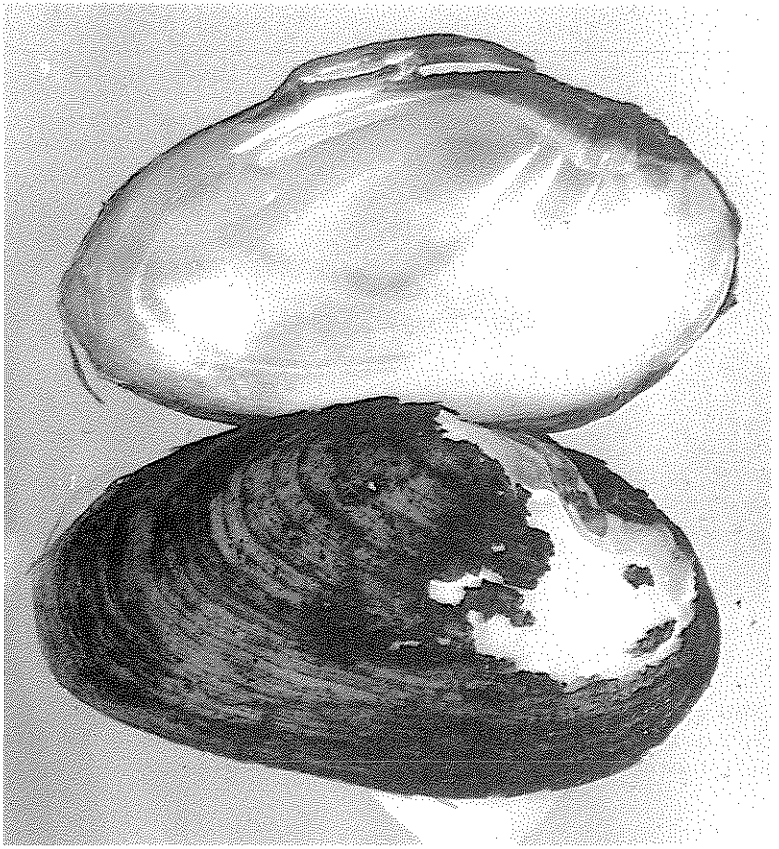

FIG. 3.—Shell of *Ptychobranchus greeni*

stream direction as turbidity and eutrophic conditions increase due to the accumulating effe
private home runoff and drainage, agriculture, and instream cattle watering.

The adhesive nature of the conglutinate terminal filament could serve to maintain the con
nate within shoal and riffle habitats by adhering to gravel, rocks, or other hard substrata as it tur
along the stream bottom following release. If correct, this aspect of the species' natural histor
explain, at least in part, the decline and disappearance of *Ptychobranchus greeni* from most
historically occupied range. It is likely that under optimum conditions only a small percenta
the conglutinates released within a shoal would successfully attach to rocks. The covering of s
by fine sediments, an environmental problem currently common in many southeastern str
would reduce the likelihood that conglutinates would remain in these habitats. Algal growth on
and gravel substratum as a result of eutrophic conditions might also reduce attachment su
Conglutinates released under such conditions would be swept into downstream pools, where s
dwelling fish hosts would be few, and where the conglutinates would be less obvious. Land-us
terns, stream conditions and the current distribution of *P. greeni* discussed in the preceding
graph appear to support this hypothesis.

The high level of mimicry exhibited by *Ptychobranchus greeni* conglutinates, and an unusual r
anism for maintaining conglutinates within host fish habitat, are selective traits to facilitate para
and survival of offspring. Physical habitat changes are affecting this aspect of the species reprod

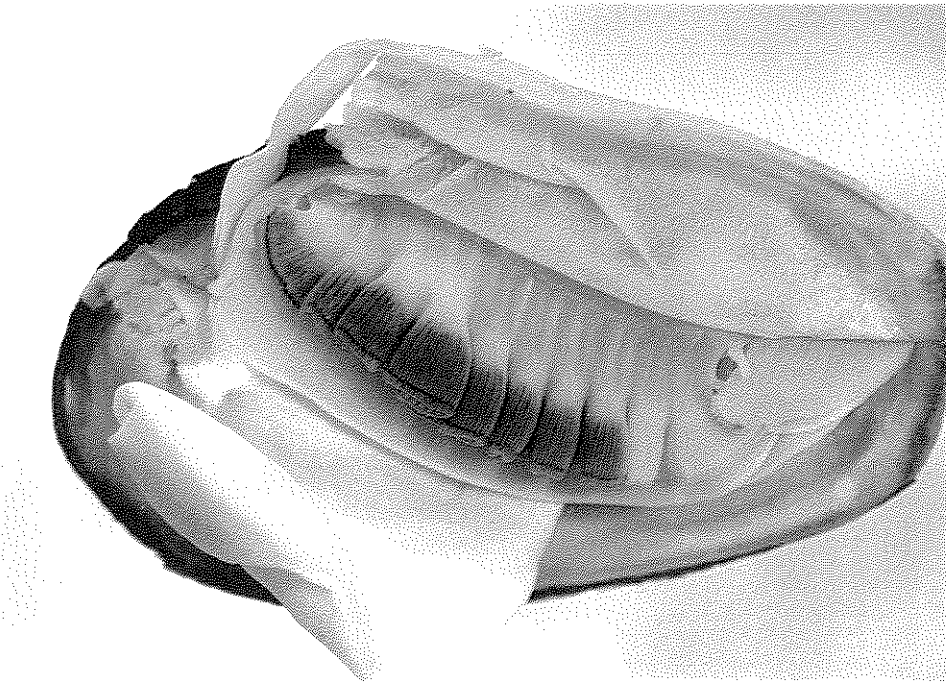

FIG. 4.—Marsupialized outer left gill of *Ptychobranchus greeni*, showing the orientation of the congl tinates in the marsupium. Note the discharged oviducts in the posterior fold, and the bead along th ventral margin of the marsupium produced by the "heads" of the conglutinates

strategy, and the long-term survival and recovery of *P. greeni* may depend on controlling sedimentatic and eutrophication of occupied and potential stream habitat.

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LITERATURE CITED

DEPARTMENT OF INTERIOR. U.S. FISH AND WILDLIFE SERVICE. 1993. Endangered and threatened wildli and plants: endangered status for eight freshwater mussels and threatened status for thr freshwater mussels in the Mobile River drainage. *Federal Register,* **58**:50. p. 14330–14340.

LEA, I. 1863. Descriptions of the soft parts of one hundred and forty-three species and some embryon forms of Unionidae of the United States. *Proc. acad Nat. Sci. Phila. Ser.* **2**:401–456.

LUO, M. 1993. Host fishes of four species of freshwater mussels and development of an immur response. M.S. Thesis, Tennessee Technological University. Cookeville. 32 p.

ORTMANN, A. E. 1910. The discharge of the glochidia in the Unionidae. *Nautilus,* **24**:94–95.

———. 1911. A monograph of the naiades of Pennsylvania. *Mem. Carnegie Mus.,* **4**:307.

———. 1912. Notes upon the families and genera of the najades. *Ann. Carnegie Mus.,* **8**:305–309.

———. 1923. Notes on the anatomy and taxonomy of certain Lampsilinae from the Gulf Coast
age. *Nautilus,* **37**(2):56–58.

STANSBERY, D. H. 1976. Naiad mollusks, p. 49. *In:* H. Boschung, (ed.). Endangered and threa
plants and animals of Alabama. The University of Alabama, University, Alabama.

PAUL HARTFIELD, U.S. Fish and Wildlife Service, Jackson, Mississippi 39213 AND ELIZABETH H
FIELD, Mississippi Museum of Natural Science, Jackson 39213. *Submitted 6 April 1995; accept
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