

Description of the glochidium of *Margaritifera auricularia* (Spengler 1793) (Bivalvia, Unionoidea)

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The glochidium of *Margaritifera auricularia* is described for the first time by using light microscopy, scanning electron microscopy and histological techniques. The larval mantle is formed by only two layers of cells; the inner one being much thicker, with microvilli. All cell masses of the glochidium are temporary aggregations that are the rudiments of organs of the subsequent juveniles which will be released after metamorphosis in the host tissues. In the glochidium there are three main masses of cells: (i) the muscle, which is in an anterior position; (ii) the oral plate in the centre of the larva; and (iii) the more ventrally and posteriorly situated ventral plate, or foot rudiment, flanged with lateral pits all bearing dense cilia. No rudimentary organs such as the pericardium, the kidney, the heart or nerve ganglia have developed. There are no visible hooks in the valve margins, but by using light microscopy we observed minute teeth covered by a rim of the periostracum. Near the margin of the shell there are two pairs of sensory hair tufts only observable by scanning electron microscopy. The glochidium of *M. auricularia* is the largest of the family Margaritiferidae and intermediate between the glochidium of the known species of this family and those of Unionoidea.

Keywords: *Margaritifera auricularia*; Unionoidea; glochidium; anatomy; Spain

1. INTRODUCTION

Freshwater bivalves of the superfamily Unionoidea contain a parasitic stage in the reproductive cycle that typically includes a fish host and a modified larva, the glochidium. The glochidia develop from fertilized eggs that are maintained in the gills of the female or the hermaphrodite parents and are released into the water where they must attach to the gills or fins of, in some cases, specific fishes to develop a parasitic stage. Ortmann (1911) and Lefevre & Curtis (1912) were pioneers in the study of larvae of North American species, whereas the glochidia of most European unionid species were described by Pekkarinen & Englund (1995a,b), Harms (1907, 1909), Smith (1976), Young & Williams (1984) and recently Nezlin *et al.* (1994) and Pekkarinen & Valovirta (1996) described the glochidium of *Margaritifera margaritifera* (Linnaeus 1758), a Holarctic species and one of the few representatives of the old (Upper Cretaceous) genus *Margaritifera*. With the exception of *M. margaritifera* and the vanishing Irish population of the putative species *Margaritifera durrovensis* (Phillips 1928), there have been no records of living specimens of the other European species *Margaritifera auricularia* since 1917 (Haas 1917), and no data are available on its host fish, reproduction, development or the morphology of its glochidium. As was supposed by Lefevre & Curtis (1912), with only one exception (see Bauer 1994), the type of glochidium is constant for each genus (Pekkarinen & Englund 1995a) and therefore may be useful for species identification and classification (Giusti 1973). However, it

has not been described in all species of the genus *Margaritifera*. In their recent paper, Pekkarinen & Valovirta (1996) stated differences between descriptions of the glochidium of different populations of *M. margaritifera*, mostly whether there were teeth present (Harms 1909; Smith 1976) or absent (Young & Williams 1984; Nezlin *et al.* 1994; Pekkarinen & Valovirta 1996). This indicated either that glochidia of the same species may present morphological differences among different geographical locations or that these microscopic structures have been understood in different ways by different authors.

The discovery of a relict population of *M. auricularia* (Araujo & Ramos 1996) has allowed us to study the reproductive cycle of this species. By using optical and electronic microscopy and histological techniques, we describe here the anatomy and morphology of the glochidium of *M. auricularia*. The specialized structures of this larva are described and compared with those of other unionacean species.

2. MATERIALS AND METHODS

Specimens of *Margaritifera auricularia* were collected in February 1996 in an irrigation channel of the Ebro River in Zaragoza, Spain. Animals were transported live to the laboratory in a net inside a portable refrigerator with ice. They were kept in an aquarium at 19 °C.

Glochidia were collected in the aquarium with a pipette directly from the mussels' exhalant apertures. The study was made with live and fixed glochidia by using stereomicroscope, light microscope and scanning electron microscope (SEM) techniques. Images of live glochidia

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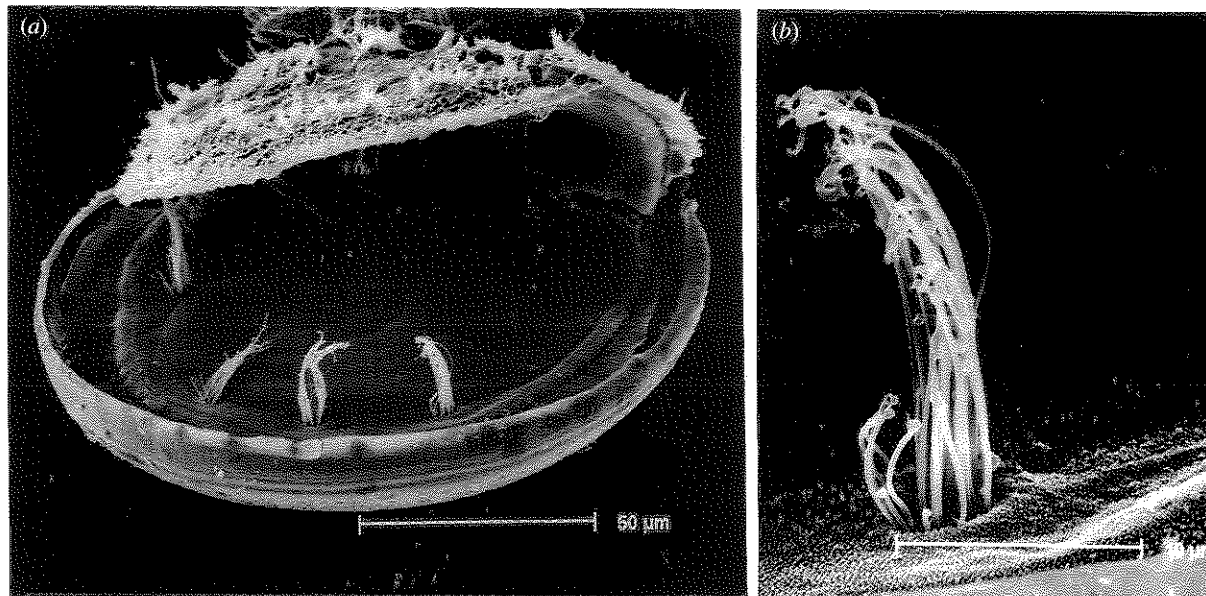


Figure 9. (a) Opened glochidium with the two pairs of sensory tufts. (b) Part of one tuft.

developed at this stage (Harms 1909). Owing to the absence of a nervous system the contraction of the adductor muscle may be explained as a tactile response transmitted by the hair tufts, as Pekkarinen & Valovirta (1996) suggested for the glochidium of *M. margaritifera*.

Our observation of a lack of any kind of larval thread in the mature glochidium agrees with Lefevre & Curtis (1912), who, in a study of hookless glochidia, only found larval threads in species of *Unio*: 'we have never seen any sign of such a structure in the ripe glochidia of the other genera which possess hookless glochidia'. Other authors (Schierholz 1889; Conner 1907) have also reported glochidial threads in species of *Unio*. There is no sign of this thread in the glochidium of *M. margaritifera*; however, Harms (1907, 1909) cited a very long filament when the glochidia are surrounded by the vitelline membrane, which is lost in the mature glochidium. As in *M. margaritifera* (Pekkarinen & Valovirta 1996), *M. auricularia* has only two pairs of sensory hair tufts instead of the four pairs of other unionoid glochidia (Lefevre & Curtis 1912; Pekkarinen & Englund 1995b).

Regarding the behaviour and reactions of the glochidia of *M. auricularia*, recorded on more than 10 h of videotape which was then studied, very little may be added to the results of Lefevre & Curtis (1912) concerning hookless glochidia. Glochidia are incapable of locomotion by the spasmodic contractions of the adductor muscle. The shell is so delicate that this contraction causes a depression in the larval shell immediately inside the area of the muscle insertion.

The presence of many glochidia of *M. auricularia* attached to the gill filaments of sturgeon and its absence on tail and fins (Araujo & Ramos 1996) indicates that this glochidium, like that of *M. margaritifera*, is exclusively a gill parasite. The host of the glochidium of *M. auricularia* is unknown. The decline of this species in an area with abundant specimens of *Unio elongatulus* (C. Pfeiffer 1825), *Anodonta cygnea* (Linnaeus 1758) and *Psilunio littoralis* indicates either a high degree of fish specificity, as in North

American unionids (Zale & Neves 1982) and *M. margaritifera* (Ziuganov *et al.* 1994), and/or a greater sensitivity to water quality.

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