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The Freshwater Leech *Alboglossiphonia hyalina* (O. F. Müller, 1774) (Annelida: Hirudinea) from Tunisia: Life-Cyle Data and Embryonic Development

***R. Ben Ahmed,*[*1] *M. Gammoudi*[1]*, S. Tekaya*[1]**

[1] Faculté des Sciences de Tunis, Université Tunis El Manar, UR 11ES12 Biologie de la Reproduction et du Développement Animal, Campus universitaire 2092, Tunis, Tunisie; E-mails: raja_benahmed@yahoo.fr; mehrezgammoudi@yahoo.fr; saida.tekaya51@gmail.com

Abstract: The aim of the present study is to provide new data on the life cycle of the freshwater leech *Alboglossiphonia hyalina* (O. F. Müller, 1774) and to describe for the first time its egg-laying and development under laboratory conditions. The studied worm is semelparous, reproducing once and dies few weeks later. One leech deposited 30 to 50 cocoons in a plate with many rows. Each cocoon contained a single fertilised egg. The sequence of morphological changes in the developing eggs observed with light microscopy allowed us to identify five main embryonic stages. The first stage was characterised by aggregates of blastomeres, showing a spiral arrangement. Approximately 4 days after laying the cocoons (stage II), the archenteron and the body metamerisation took place. At stage III, the organogenesis of the definitive inner organs started. Between 11 and 12 days after laying the eggs, hatching occurred (stage IV). The newly hatched juveniles (stage V) remained attached to the parent's venter for 4-7 days. They consumed their yolk supply during a post-embryonic brooding period. It seems that the temperature increased the number of deposited eggs.

Keywords: *Alboglossiphonia hyalina*, Hirudinea, life cycle, embryonic development, ecology

Introduction

Freshwater leeches inhabit streams, spring ponds and lakes in temperate and tropical regions. Some are predators on invertebrates, whereas others are sanguivorous ectoparasites of invertebrates, fishes, amphibians, reptiles, water birds or mammals (Elliott, Mann 1979).

Hirudinida show complex female gonads, *i.e.*, ovaries composed by an outer wall, the so-called ovisac and two polarised, long and convoluted structures termed as ovary cords that float freely within the ovisac (Ben Ahmed *et al.* 2010). After fertilisation, eggs are enveloped with a thin egg capsule called cocoon (Sawyer 1986). Following fertilisation, cocoons are laid through female gonopore. Within the Hirudinea, only members of the family Glossiphoniidae show extensive parental care. Following Sawyer (1971), the degree of protection given to the offspring can be arranged in a series of increasing complexity: (A) species attach the cocoons to the substrate and cover them with their body until the young hatching; (B) species attach the cocoons directly to their ventral surface; in both (A) and (B) the newly hatched young cling to the ventral surface of the parent and are carried around by it. The species of another group (C) carry their eggs and young in an internal brood pouch (Kutschera, Wirtz 1986, 2001).

Like all annelids and similarly to flatworms, ribbon worms and mollusks, leeches exhibit a clear spiral cleavage and are therefore important models for studying embryonic development. They are char-

*Corresponding author

acterised by their direct development, which involves no metamorphosis of the hatched juveniles, while in other annelids (*e.g*. Polychaeta) the development is indirect and includes trochophore larvae.

No studies have been conducted on the embryonic development and the life history of freshwater leeches of Tunisia by now, except that producing some preliminary data published by Ben Ahmed *et al.* (2008). Information on life history is important in many types of ecological studies on freshwater invertebrates (Erba *et al*. 2003). In fact, changes in the life history descriptors (*e.g.*, life cycle, survival or mortality, fecundity, and growth rate) of species are used in studies of ecological assessment as an indicator of the environmental stress (Raddum, Fjellheim 1993, Metcalfe *et al.*, 1988).

The present study aims to determine the life cycle of the glossiphoniid *Alboglossiphonia hyalina* under laboratory conditions. In addition, its embryonic development is analysed for the first time, by means of light microscopy, in order to describe the sequence of the main morphological changes occurring between laying the cocoons and hatching. Furthermore, the influence of water temperature on the reproduction of this species is suggested.

Material and Methods

The leeches were collected monthly during one year (2006-2007) from Lebna Reservoir in the northeast of Tunisia (36° 42'N 10° 56'E), using a timed handcount. The procedure involved picking up stones from the bottom of the water within an area of 5x15 m for approximately one hour and removing the attached leeches. Field water temperature was recorded monthly during the period of study.

The identification of the species was made by live observations of morphological characteristics, such as colouration, body shape and size, number and arrangement of eyes and location of gonopores.

The cocoons were obtained from a laboratory culture of 42 adult specimens and kept at 20 ± 1°C in petri dishes. Water was changed daily or at least several times a week. The cocoons were examined immediately after laying and then at 2, 4, 6, 12, 18 and 24 hours during the first day. Subsequently, the transparent cocoons were observed daily in order to follow the developmental stages. For documenting the egg plates, embryonic development and juveniles, photographs were taken with a Nikon Coolpix digital camera.

In the statistical analysis, all values are given as average and standard deviation, followed by the number of the examined specimens.

Results

Life cycle and breeding season

In the laboratory, *Alboglossiphonia hyalina* was found to be semelparous. These leeches reproduced only once and then senesced and died few weeks after hatching of the eggs.

In the field, the specimens were usually found only during the winter/ spring seasons. Many of the leeches carried either embryos or hatchlings during the four months: January, March, April and May which corresponded to their breeding season. Mature adults (8 to 15 mm length) with attached eggs appeared at the beginning of January. This suggests that the fertilisation had taken place before (probably in December). In February/ April, adults brooding either eggs or juveniles were collected, while only brooding of juveniles was observed in May. In the rest of the year this leech was almost absent in our collections.

Young free-living *A. hyalina* were never recorded in the field. We suggest that the newly hatched leeches spend most of their life cycle as endoparasitic in the mantle cavity of snails and leave the snail as adults before the laying of eggs. However, more field studies are required to support this hypothesis.

Copulation

A. hyalina is a glossiphoniid leech, in which a penis is absent, and thus indirect or hypodermic insemination occurs. In this case, during copulation, spermatophores are usually released in the clitellar region of the concopulant; however, the spermatophores can also be implanted anywhere in the posterior part of the leech body (Mann 1962). After injection into the body wall, the spermatozoa are released from the spermatophore and have to reach the ovaries. In our laboratory population, no spermatophores were observed attached to leeches; groups of mature specimens were aggregated together, with the dorsal body surface of one leech being covered by the ventral surface of the other.

Laying of eggs

In the laboratory, leeches that could lay eggs had a length of 8 to 15 mm. The eggs were laid inside transparent cocoons formed by secretion from the clitellar glands. The specimens that had come near to cocoon production were recognised by their considerably enlarged white ovaries containing numerous yellowish yolky eggs visible through the transparent body wall (Fig. 1a, b, c). Before the laying of eggs, *A. hyalina* moved to the wall of the jar and attached to it by its anterior and posterior suckers. The eggs were pressed out of the common gonopore and fixed

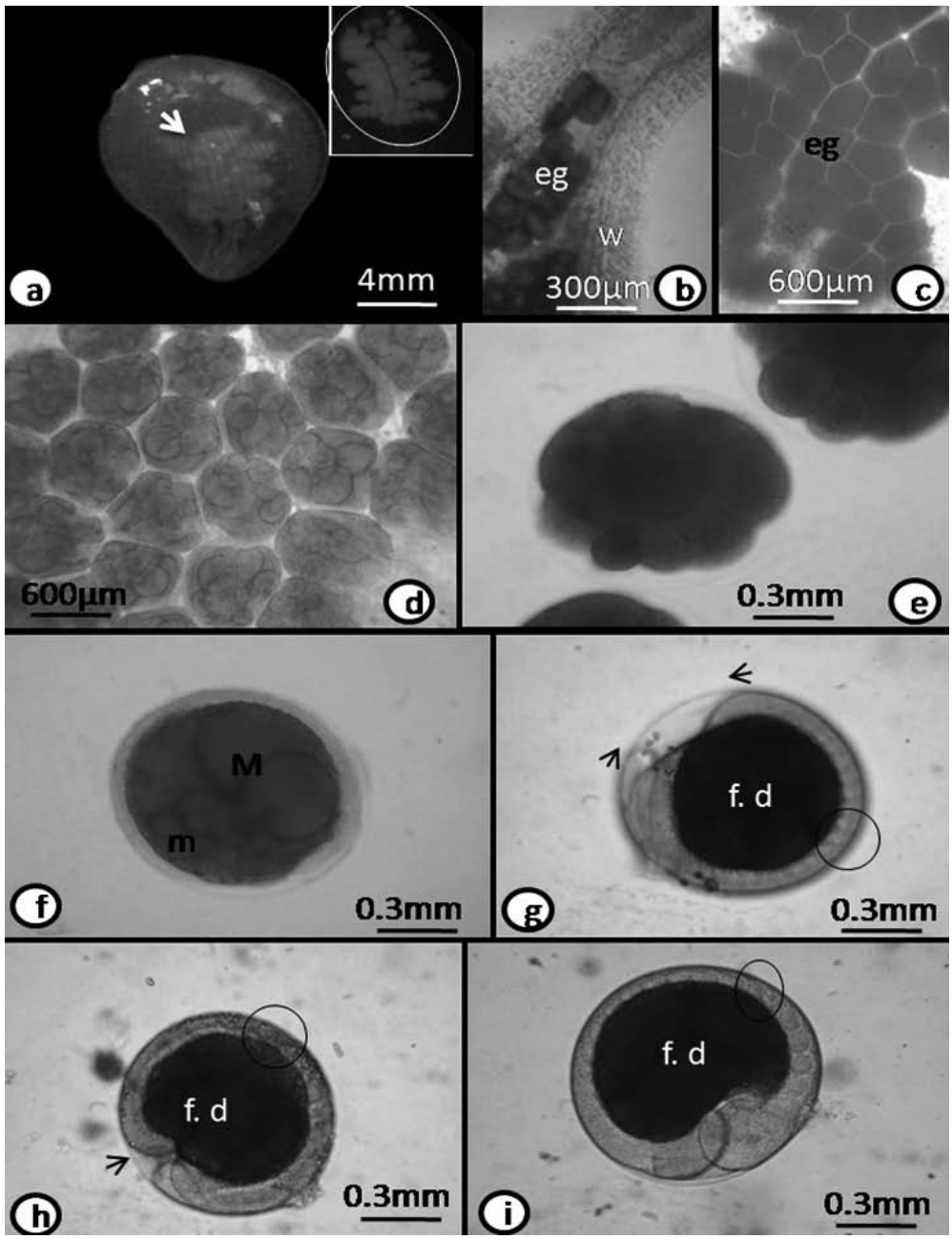

Fig. 1. Early stages of embryonic development in *Alboglossiphonia hyalina.*
a : A photograph of an adult *A. hyalina* ; ***in set*** : plate of eggs inside the body wall. **b, c:** eggs (eg) of *A. hyalina* visible through the body wall (w). The eggs (eg) are arranged in several rows. **d, e, f:** cliveage of eggs. Micromeres (m), macromers (M). **g, h, i :** 5 days old embryo after oviposition. Future digestive system (f.d) is visible. The metamerism (circle) is defined. Arows in **g** show the ectodermic mouvement. Arrow in **h** mark the blastopore

to the ventral side of the parent. They were arranged on the body region behind the common gonopore and in front of the posterior sucker. The flexible egg sacks were carried around by the brooding leech, which fans the cocoons with continuous waves of lateral contractions of its body flanks. The freshly laid cocoons were deposited in a plate with many rows (Fig. 1a, b, c). A single worm deposited 30-50 cocoons, each one containing one fertilised egg. The average diameter of the transparent ovoid cocoon was about 0.65±0.05 mm (n = 20).

Embryonic development and hatching

In this study, *A. hyalina* needed 11 to 12 days to complete its embryonic development at 20 ± 1°C. All embryos were observed to pass synchronously

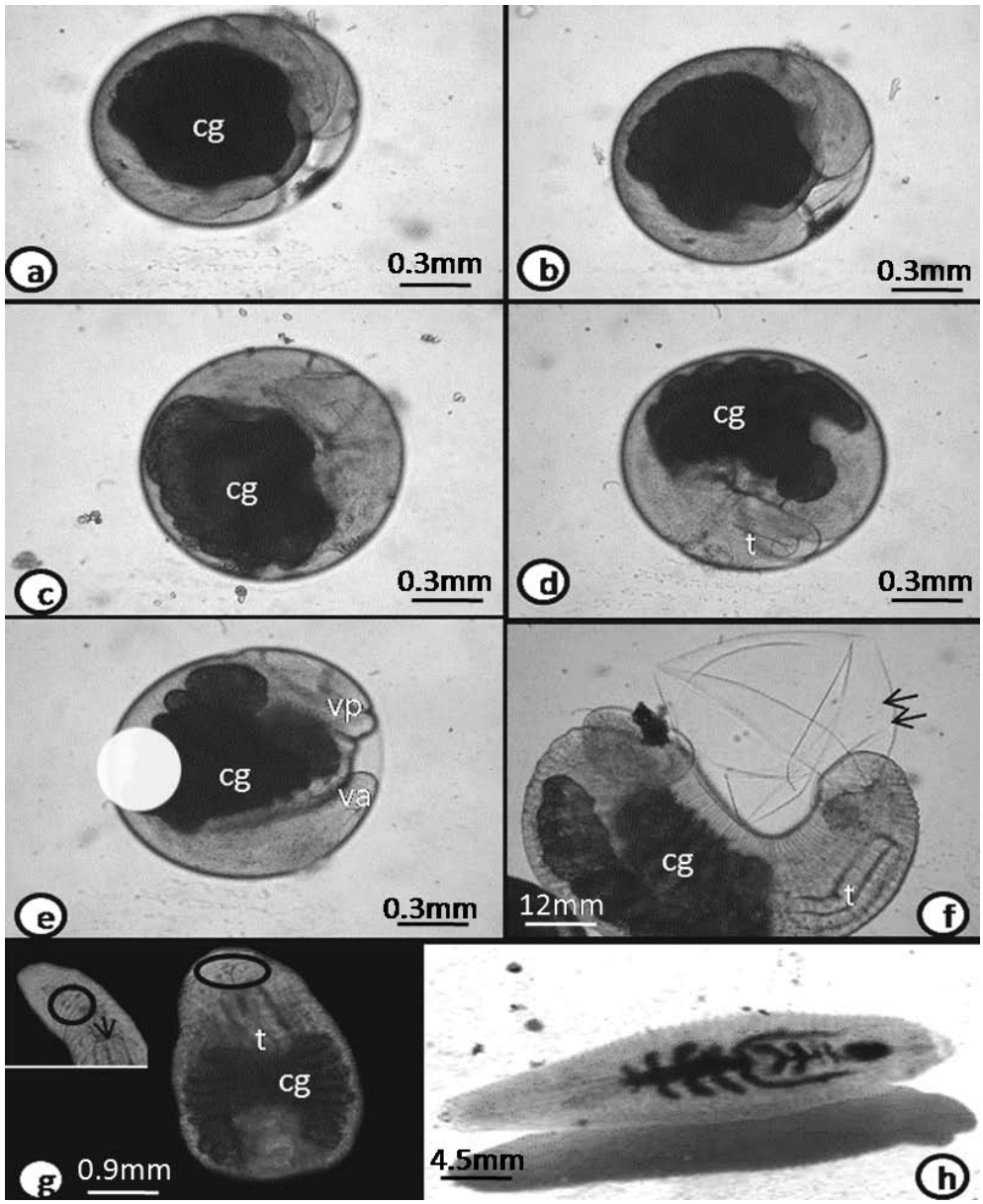

Fig. 2. Late stages of embryonic development in *Alboglossiphonia hyalina* and hatching.
a - e: gastric caeca (cg), proboscis (p), draft of the posterior sucker (ps) and the anterior sucker (as) are visible. **f:** hatching occur after 11 days. The young shows the gastric caeca (cg) which is filled with yolk. The proboscis (p) is also visible. The thin wall of the cocoon is marked (double arrow). **g:** Juvenile provided with three pairs of eyes of reddish color (ellipse). The proboscis (p) and the gastric caeca (cg) are defined ***in set*** : details of the anterior sucker showing three pairs of eyes (ellipse) and proboscis (arrow). Note that metamerisation is clearly visible. **h :** specimen collected in the field showing blakish fluid within the gut

through a number of embryonic stages. The five main stages were as follows:

-Stage I: Cleavage, between 0 and 3 days after oviposition, resulted from mitotic divisions of the egg and the cells showed a varied number of blastomeres. The cleavage progressed in the typical spiral pattern (Fig. 1d, e, f). The eggs were divided unequally and consequently, the blastomeres were unequal in size and separated into macromers and micromeres arranged along a spiral type (Fig. 1f).

-Stage II: Gastrulation and early organogenesis: four to five days after the cocoon deposition the embryo formed an early gastrula (Fig. 1g). A few hours later we noted the appearance of the archenteron (Fig. 1h), which would be later the intestine, while the blastopore would turn into a mouth situated in the anterior sucker after ontogeny. Thereafter, the body elongated and bent as a result of the dorsal metamerisation (Fig. 1g, h, i).

-Stage III: Late morphogenesis: some great

modifications took place, which prepared the embryo for hatching. In fact, the segmentation, both suckers, developing midgut (crop caeca), which presented lateral diverticula, and hindgut (intestine and rectum), appeared and they were clearly defined by photonic microscopy (Fig. 2c, d, e). These two later were still filled with yolk, forming a dark amorphous mass (Fig. 2a, b, c d, e).The proboscis was visible, being located in the anterior half of the body (Fig. 2d).

- **Stage IV:** Hatching stage: eleven days after the oviposition, the posterior end of the juvenile leech was seen emerging first via holes in the cocoon membrane (Fig. 2f), followed by the anterior end. The juveniles adhered to the belly of the parent and remained there, digesting their reserves of yolk. Then they developed into hatchlings attached to the parent's venter by their posterior sucker, until their final liberation. The anterior ends were extended free, possibly for searching food.

- **Stage V:** Post-embryonic stage: the juvenile body, with a sucker at each end, was translucent, showing yolk-filled digestive branches (Fig. 2f, g). The anterior sucker displayed a proboscis, which protruded through the mouth pore. At this stage, symmetrical eyes appeared in the dorsal region and became visible owing to their reddish pigmentation (Fig. 2g). During this post-embryonic brooding period and over the next days, the young consumed their yolk supply and developed into juvenile leeches. In the laboratory, these juveniles later could not be successfully feed on any offered type of food; they survived in petri dishes at 20°C for up to 3 weeks before dying of starvation.

Albglossiphonia hyalina is a glossiphoniid leech with direct development. The newly hatched juveniles, which are dorsoventrally flattened, have an average length of 0.3 mm.

Parental care

The parent incubated the fertilised ova within its trough-like venter until their hatching. During this period the brooding parent stopped its feeding and movements, attached to the substrate and remained quiescent. It was observed that the young remained attached by their posterior suckers to the parent's venter for 4-7 days, while the rest of their bodies moved freely.

Ecological observations

The mean number of eggs carried by *A. hyalina* ranged from 30 to 50. It seems that variation in the number of eggs was correlated with water temperature in the different months. In fact, in the field, the specimens with the greater number of eggs were collected in April at 14.7° (46.4 ± 1.5, n = 19), while those with the lesser number (32.8 ± 1.6, n = 23) were recorded in February at 9°. Furthermore, we observed that *A. hyalina* had a considerable number in spring, which might be explained by the abundance of gastropods in the reservoir and the increase in temperature during this period. Specimens with blackish fluid within the gut were often observed in the same period (Fig. 2h).

Discussion

The embryonic development of the glossiphiniid leeches varies greatly in its duration: between 6 and 30 days after cocoon deposition (Grassé 1959). In this study, the hatching of *A. hyalina* occurred 11 days after laying the cocoons (at 20 ± 1°C). At the same time, Kutschera, Writz (1986), mentioned that in *Helobdella striata* the hatching takes place 6 to 12 days after the production of cocoons. The brooding period in *Glossiphonia complanata* is over 3-4 weeks after the cocoon deposition (Kutschera, Writz 2001). The eggs of *Oligobdella biannulata* hatch within 10–20 days after the oviposition (William *et al.* 2005). The brooding period takes 2 to 3 weeks in *Theromyzon tessulatum* (Tarin *et al.* 2005). Hatching in *Alboglossiphonia polypompholyx* lasts 15 days (El-Shimy, Davies 1991). Furthermore, leeches produce cocoons that contain a variable number of eggs (Nagao 1958, Van der Lande, Tinsley 1976, Kutschera 1983). A total of 3 to 7, frequently 4 or 6, cocoons are attached to the ventral side of *Helobdella striata*, each cocoon containing 2-20 eggs rich in yolk (Kutschera, Writz 1986). In *Helobdella stagnalis,* between 2 and 6 thin-walled, transparent cocoons, each containing 6 to15 eggs, are produced (Kutschera, Writz 1986). *Theromyzon tessulatum* deposited their clutches of eggs into 3-8 cocoons, each containing 30-50 fertilised eggs (Tarin *et al.* 2005). The species in this study deposited 30-50 cocoons, which contained only one egg each.

On comparing the egg-laying seasons of glossiphoniid leeches, we came to the conclusion that most species prefer laying the eggs in the spring months. It is noted that *Glossiphonia heteroclita* reaches maturity in April and breeding starts in May (Hatto 1986). *Alboglossiphonia polypompholyx* produces cocoons twice per year: in October/November and in February/March (El Shimy, Davies 1991). The snail leech *Glossiphonia camplanata* reproduces only once per year, usually during the spring (March/April). We suggest that water temperature is the major factor that controls the breeding in some

leeches, and the rising temperature in spring may be a stimulus for copulation and egg production. It seems that in our study, temperature had an influence on the number of the produced eggs. The same dependence was recorded within Glossiphoniidae (Moore 1966, Sawyer 1972).

Alboglossiphonia hyalina is semelparous, reproducing once and then dying. It is similar in this to other African leeches, such as: *Alboglossiphonia polypompholyx* (El Shimy & Davies, 1991), *Marsupiobdella africana* (Van der Lande & Tinsley, 1976), the duck leeches *Theromyzonn cooperei* (Oosthuizen & Fourie, 1985) *Theromyzon rude,* and *Theromyzon tessulatum* (Wilkialis & Davies, 1980).

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