

## Observations on the immatures of *Dasyhelea necrophila* Spinelli & Rodríguez in laboratory (Diptera: Ceratopogonidae)

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### ABSTRACT

Data obtained from observations in the laboratory on the morphology and biology of immatures of *Dasyhelea necrophila* Spinelli & Rodríguez are provided. Eggs and larvae were collected from containers with rain water and organic matter in Gonnet, La Plata, Argentina. The C-shaped eggs were able to resist desiccations of approximately 48 hours. Hydrated eggs developed in 7-10 days and the emerging larvae (instar I) remained immobile during 30 minutes. The first molt took place inside the eggs exposed to drought periods. These eggs started to break when rehydrated and after 24 hours larvae II were born. Larva I showed positive phototropism and due to the fact that the processes of the last segment are still internal, it hardly moved. The pupa did not show any changes when placed under artificial light and remained immobile for short periods at the bottom of the glass tube. The larva in their different instars and the pupa increased mobility with the increase of temperature.

Key words: *Dasyhelea necrophila*, egg, larva, pupa, biology.

### INTRODUCTION.

The genus *Dasyhelea* Kieffer is well known because some of its species act as pollinators of cacao and other tropical cultures (Williams, 1964; Wirth *et al.*, 1968; Wirth & Waugh; 1976; Mullen & Hribar, 1988). Borkent & Spinelli (2000) recorded 61 species for the area south of the United States, although they omitted the inclusion of *D. necrophila* Spinelli & Rodríguez, 1999. Since then, only one species, *D. correntina* Ronderos & Diaz, was described from northeastern Argentina (Ronderos *et al.*, 2004).

Scientific knowledge of adults is well advanced in comparison with the knowledge of the immature stages, particularly of the egg and the initial larval instars. Most of the larvae have been described on the basis of the last instar, while for almost 500 named existing species, the eggs of only 8 are known, none of which inhabits the neotropical region (Borkent, pers. comm). As regards the breeding habitat and the relationship existing between the morphological structure and the type of feeding, these data are unknown or at best very poorly known for the majority of the species described so far. In this sense, Waugh & Wirth (1976) and Wirth (1978) studied the larval habitat of nearctic species, pointing to their occurrence in aquatic and semi aquatic environments. Mullen & Hribar (1988) studied breeding sites, also of nearctic species, and described types of feeding and locomotion. The larval habitats are generally associated with algae; they often breed in shallow water found in rocks, tree hollows or fitotelmic environments, while in

some cases breeding is carried out in rotten vegetal matter or in saline environments; some species can even resist the dessication of the habitat (Spinelli & Wirth, 1993).

*Dasyhelea necrophila* was described by Spinelli & Rodríguez (1999) on the basis of the adult, larva and pupa, although the description of the immature instars was incomplete. Ronderos *et al.* (2003) redescribed and illustrated the fourth instar larva and pupa, on the basis of ultrastructure observation under a phase contrast microscope and Scanning Electron Microscope (SEM). Detailed observations on the different field samplings carried out recently, as well as the breeding of eggs and larvae of this species in the laboratory, allowed us to obtain data on the morphology and biology of these stages.

### MATERIAL AND METHODS

Approximately 30 eggs and 10 larvae were captured from a plastic container 13.50 cm in diameter containing sand, organic matter and rain water in Gonnet, La Plata, Argentina during February 2005. These specimens were taken to the laboratory in their original medium (immersed in water rich in nutrients). A second sample was sucked with a pipette from a microcontainer 3 cm in diameter containing rain water and organic matter and were taken in tubes to the laboratory.

In the laboratory two masses of eggs, named Group A and Group B, were placed separately in Petri capsules inside telgopor boxes. The temperature oscillated between 20 and 25 °C and the humidity between 60-70%. Group A was hydrated daily while group B was exposed to dissection and rehydrated 48 hours later. The eggs of each group were observed daily under a stereoscopic microscope in order to recognize the different phases of development. They were photographed with a digital camera until the hatching of the larvae; the latter were fed daily with water with nutrients from its natural environment until they reached the pupal stage. These last were extracted with micropipettes and placed individually in glass tubes with 5 ml of water from the environment with loose cotton lids. These were periodically observed until the emergence of the adult which was specifically determined.

The material was separated for later study and prepared with different techniques for each step of the ontogeny. Eggs and larvae were mounted in Canada balsam according to Borkent (2000) techniques.

A fraction of eggs prepared with 10% glycolic acid during 30 minutes was mounted for observation under a Scanning Electron Microscope (SEM). Later, they were brushed to remove the gelatinous remains from the union of the egg mass; finally they were exposed to ultrasound for three 30 minute periods. Once cleaned they were placed in a saturated solution of phenol in alcohol and then passed through a battery of alcohols (100% - 70%). Finally, critical point was performed and gold metallized. Observations were carried out with a JSM6360LV Electron Microscope.

The terminology of the larva is the one used by Ronderos *et al.* (2003). The material examined was deposited in the collection of the Entomology Division of the Museo de La Plata, Argentina, preserved in alcohol 70% and in microscope slides in Canada balsam; the MEB tubes are also available.

## RESULTS AND DISCUSSION

The observations carried out on the egg development demonstrated the ability of eggs to resist desiccations of approximately 48 hours. The eggs are found on a gelatinous mass in groups of 10-12 (Fig. 1) although not sticking to each other. Each egg is 150 (n=3) milimicrons in length and 72.3 milimicrons (n=3) in maximum width. Under the SEM the surface of the corion is smooth (Fig. 9), its internal face clearer with visible dark interweaved lines (Fig. 10). The color varies during its development, from very light brown at the moment of laying to dark brown when the larva matures. It is C-shaped with one of the ends sharp and the opposite, where the cephalic capsule is placed, rounded. Before maturity their ends almost touch (Fig. 2) beginning to separate and to turn to one side previous to the moment of eclosion. At the moment of eclosion the egg starts opening by means of an internal longitudinal rupture (Figs. 3-4) made by the action of a small tooth placed dorsally in the cephalic capsule (Fig. 7).

Larva I (Figs. 6-8) is thin, hypognathous, with the body slightly curved and lacking setae or important processes to be described. The cephalic capsule (Fig. 7) is yellowish brown, short, HL 0.23 (0.20-0.23, n = 3) mm and wide 0.13 (0.10-0.13, n=3) mm. In the developing pharynx skeleton the combs are not observed yet. The posterior end of the body carries the retractile pseudopods still internal and developing (Fig. 8).

The development of larva I from hydrated eggs shows differences in comparison with the eggs exposed to drought periods. In group A (Figs. 5-6) the larva hatches from the egg and remains immobile for approximately 30 minutes (Fig. 6), then starts moving; and after 24 hours it feeds for the first time. In group B (Figs. 3-4) instar I develops inside the egg (Fig. 4) being instar II the emerging free larva which starts moving immediately and starts feeding after a short while.

Development time to reach the adult stage is short (3-7 days) in comparison to the time taken for the development of the egg. The latter developed in approximately 7-10 days in Group A, while in group B the egg started to break when rehydrated, and after 24 hours larvae II were hatched, as the first molt took place within the egg.

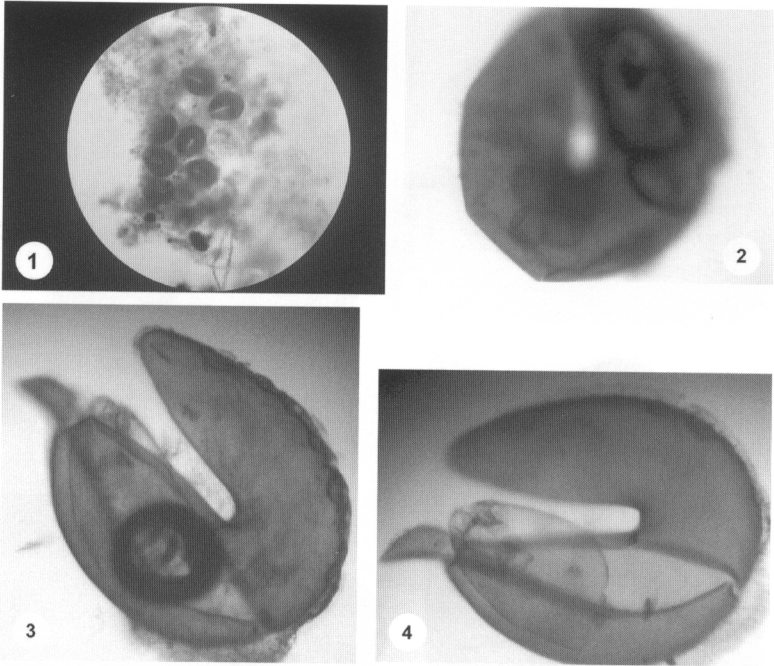
Even though the mature larva of *Dasyhelea necrophila* observed by Ronderos and collaborators in previous captures shows a remarkable activity and leaping with the hooks on the caudal segment, instar I hardly moves and does not leap because the processes of the caudal segment are still internal. It shows a positive phototropism because its activity increases much under light. Instars II and III are more active and instar IV starts to decrease the activity as it matures, remaining immobile at the moment of reaching the pupal stage, at which time it develops movements of lateral flexion when stimulated with a microneedle.

On the other hand the pupa does not show any changes when placed under artificial light and remains immobile for short periods at the bottom of the hemolysis tube. Likewise, the larvae in their different instars, as well as the pupa, show an important change of activity when the temperature of the water varies, increasing mobility with an increase in temperature.

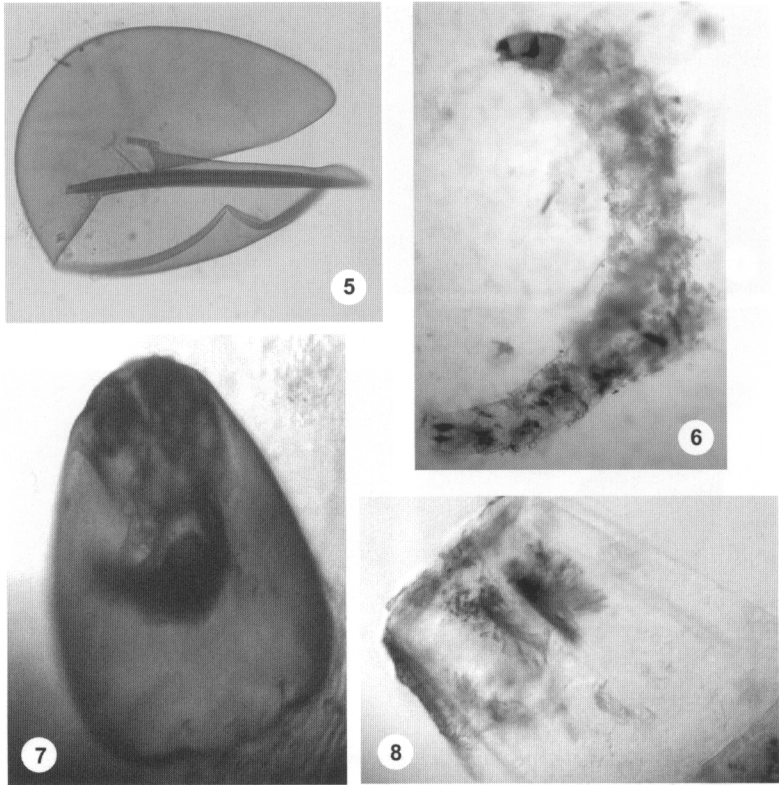
Further studies about the most important events taking place during the ontogenesis of ceratopogonids are needed, especially considering the adoption of control measures of relevant species from the economic and/or sanitary point of view.

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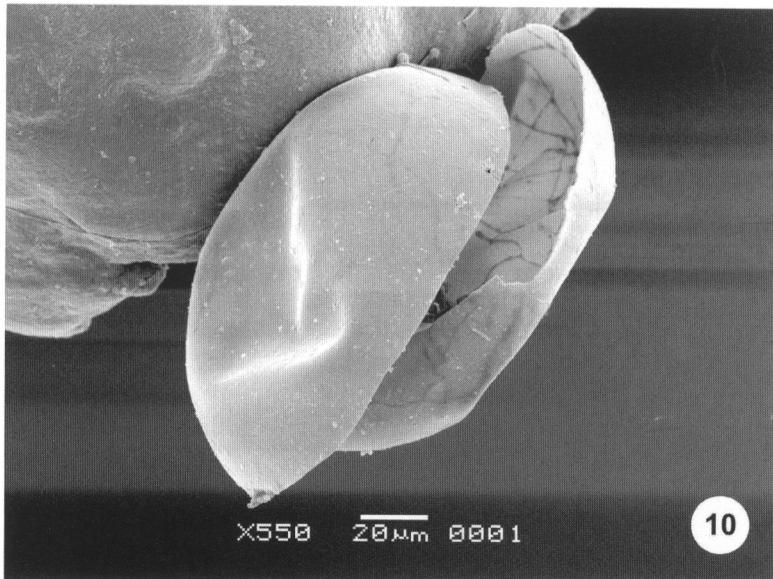
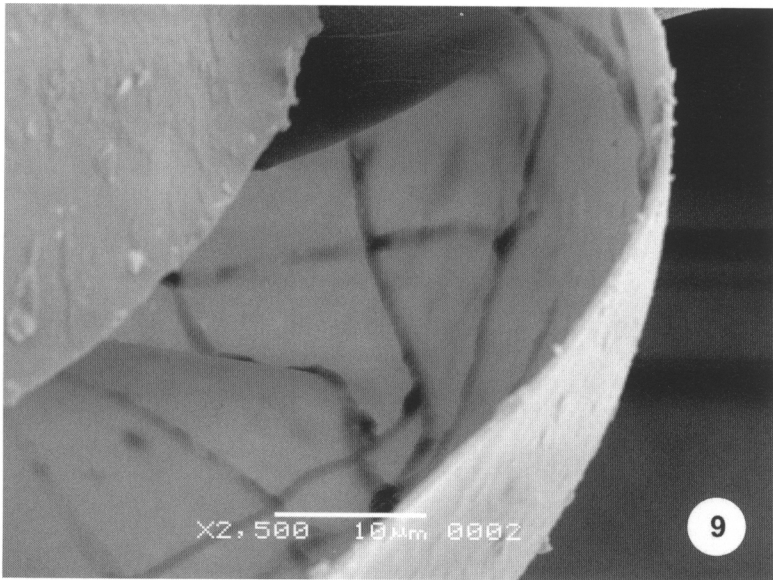
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Figs 1-4. *Dasyhelea necrophila* 1, egg mass. 2, immature egg. 3, broken egg . 4, egg with first molt (group B).



Figs 5-8. *Dasyhelea necrophila* 5, broken egg. 6, larval instar I. 7, cephalic capsule, larval instar I. 8, caudal segment, larval instar I (group A).



Figs 9-10. *Dasyhelea necrophila* 9, ultrastructure of egg, internal face (550 X). 10, ultrastructure of egg, external face (550 X).