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VERSITA

Larval survival strategy during winter of *Chordodes nobilii* Camerano, 1901 (Gordiida, Nematomorpha)

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Abstract

There are numerous open questions concerning the life cycle of Gordiida (Nematomorpha), especially about egg development and viability of larvae during winter, when the temperature of freshwater environments where they inhabit is low. On the basis of experimental studies we demonstrate that egg development of *Chordodes nobilii* takes 20–25 days at 22°C and 45–55 days at 5°C. We also observed that larvae of *C. nobilii* obtained from egg strings at 5°C and maintained at that temperature during six months remained inside their egg shells as a survival strategy.

Keywords

Nematomorpha, *Chordodes nobilii*, temperature, larvae survival

Introduction

Gordiida, a metazoan group hardly studied in its ecological aspects, has recently been the subject of large investigations regarding its morphology and taxonomy (see Schmidt-Rhaesa 1997; De Villalobos *et al*. 2005, 2006), its life cycle (see De Villalobos and Ronderos 2003; De Villalobos *et al*. 2003; Hanelt and Janovy 1999, 2003, 2004; Schmidt-Rhaesa and Ehrmann 2001; Schmidt-Rhaesa *et al*. 2005) and its distribution (Miralles and De Villalobos 1993, Schmidt-Rhaesa and Menzel 2005).

The life cycle of the species of Gordiida consists of two free living phases, adult and larva, in aquatic environments (streams, lakes, ponds and rivers) and a parasitic phase, as juveniles that are parasitic in arthropods. According to Bolek and Coggins (2002), adults appear in spring and disappear in autumn, this contradicts our field observations (not published), as specimens of *Chordodes nobilii* and *C. brasiliensis* have been collected in different regions of Argentina during winter. Nevertheless, and in agreement with Dorier (1930) adults are rarely able to release eggs in winter. Likewise, Thorne (1940) observes that the adults of gordiids can hibernate.

Mainly during spring, summer and autumn (De Villalobos *et al*. 2000, 2003) worms mate and produce eggs. The gordiid larvae, which emerge from the eggs are completely different from the worm-like adults and have hooks and stylets adapted to start the parasitic phase of the life cycle (Zapotosky 1974, Müller *et al*. 2004).

In spite of the studies carried out in gordiids on the aquatic free-living phases of their life cycle, numerous aspects of the egg development and larval longevity remain poorly documented. For example, it is not known whether the eggs laid by the end of autumn are able to survive and continue their development during winter when the temperature of the water of the streams is low (5–7°C) (in Argentina in some areas a thin layer of frost can be observed on the streams surface). Some authors (see Villot 1874, Dorier 1930) observed that larvae form a cyst outside the host, surviving for several months. So far, free living cysts have not been reported again (Hanelt *et al*. 2005). Whittaker and Barker (1983) consider that the larvae of *Paragordius* sp. probably retard their hatching as a survival strategy until the hosts become available.

The purpose of this investigation is to experimentally analyze the time required for the eggs of *C. nobilii* Camerano, 1901 to complete their development at 22°C and at 5°C. Likewise, to evaluate if the larvae obtained from the eggs at 5°C, maintained at low temperature during several months, remain included within the egg membrane or hatch or form cysts. Through experimental infections we aim to evaluate whether the larvae remain able to infest a host.

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Materials and methods

Collection and maintenance of *Chordodes nobilii*

At the end of the autumn, in the southern hemisphere (4th April 2006) 15 males and 6 females of *Chordodes nobilii* were collected from El Negro stream (38°07′28″S, 61°45′32″W), Sierra de La Ventana, Argentina. Copulating pairs of adult worms were isolated, and placed in 1000 ml plastic containers with aerated well water. They mated and released egg strings into the water. Immediately after oviposition, 16 pieces of egg strings were separated each 4 mm in length with approximately 5400 eggs (see Hanelt and Janovy 1999) and used as experimental units.

In order to test the time required for egg development at 22°C (± 1°C), 3 of these pieces of egg strings were stored in 50 ml glass jars in well water and were observed every 5 days under the light microscope (LM).

The other 13 pieces of egg strings were stored in water at 5°C (± 1°C), 3 of which were used to evaluate the time required for egg development (at 5°C) and were observed at 20, 35, 45 and 55 days under the light microscope.

The remaining ten egg strings were stored in water at 5°C (± 1°C), during 6 months. After that period, the 10 pieces of egg strings, were rinsed in 1:250 Clorox bleach well water solution (Hanelt and Janovy 1999) and exposed at 22°C (± 1°C) for 1 hour. In order to test whether larvae remain included within the egg membrane or hatch or form cysts, 200 eggs from each piece were observed under the light microscope and 3 developmental stages, gastrula (G), larva included within the egg membrane (EL) and free larva (FL) were considered.

Evaluation of larval survival obtained from eggs at 5°C (± 1°C) during 6 months of *Chordodes nobilii*

Eggs of *Aedes aegypti* (Diptera) were placed in a 500 ml glass container. After hatching, 300 of 2nd and 3rd *A. aegypti* larvae were distributed in 10 glass containers and fed with wheatgerm. Each treatment group was exposed to 400–500 larvae (EL, FL) of *C. nobilii*, after analyzing the egg strings, maintained at 5°C during 6 month, by LM. After 1 hour of exposure, eight hourly observations under LM were performed, in order to record the amount of parasitic larvae within *A. aegypti* larvae. Afterwards, two observations after 24 and 48 hours were performed.

Results

The eggs of *C. nobilii*, maintained at 22°C (± 1°C) completed their development and free larvae were observed within 20–25 days (Fig. 1).

The egg strings exposed at 5°C (± 1°C) continued their embryonic development and after 55 days 80% of the larvae, completely formed, remained within the egg membrane (Table I).

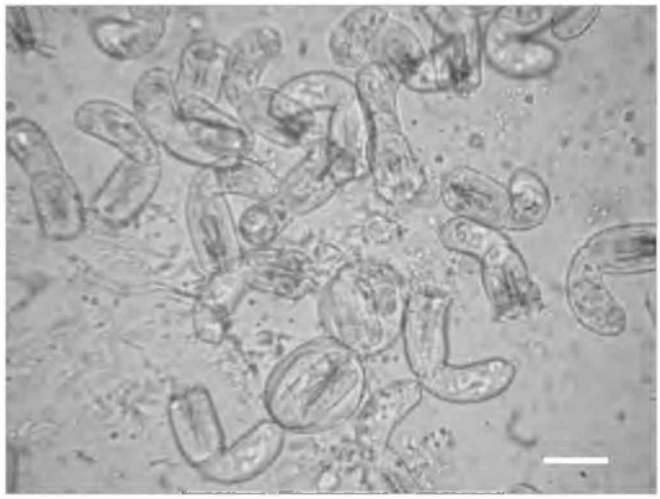

Fig. 1. Free larvae of *Chordodes nobilii* obtained from egg strings maintained at 22°C during 25 days. Scale bar = 15 μm

Table I. Number of developmental stages of egg strings of *Chordodes nobilii* maintained at 5°C in the different times of observation (days)

Days	B	G	EL
20	122	78	0
35	72	126	2
45	20	84	96
55	4	36	160

For explanations: B – blastula, G – gastrula, EL – larvae inside the eggs.

The egg strings stored at 5°C (± 1°C) during 6 months, when exposed at 22°C (±1°C), most of the eggs were found with the complete larva inside (87.85%); others, in less quantity, in the gastrula stage (9.15%), and few with free larvae (3%) (Table II, Fig. 2).

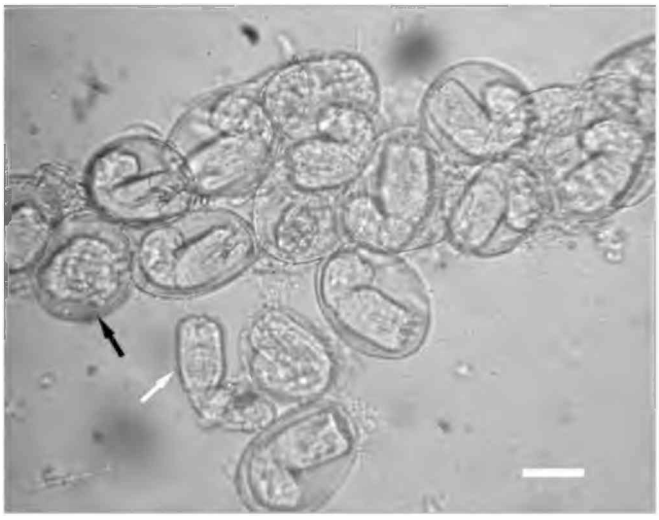

Fig. 2. Egg strings of *Chordodes nobilii* maintained at 5°C during 6 months. Gastrula stage (black arrow), free larva (white arrow). Scale bar = 15 μm

Table II. Number of developmental stages of *Chordodes nobilii* 6 months after being maintained at 5°C

Sample	G	EL	FL
1	15	176	9
2	3	162	35
3	66	134	0
4	40	160	0
5	13	187	0
6	27	173	0
7	17	181	2
8	0	189	11
9	2	196	2
10	0	199	1

For explanation: G – gastrula, EL – larvae inside the egg and FL – free larvae, in each sample (n = 200).

Evaluation of the *C. nobilii* larval survival was estimated according to their capacity of passing through the intestinal walls and remaining in the body cavity of *A. aegypti* larvae. Although an increase in the quantity of parasitic larvae was observed during the test, the average of larvae observed per hour during the first 8 hours was higher than the observed at 24 hours and at 48 hours (Table III). As observed by De Villalobos *et al.* (2006), parasitic larvae do not form cysts inside the body cavity of *A. aegypti* larvae.

Table III. Number of parasitic larvae of *Chordodes nobilii* found in *Aedes aegypti* larvae per sample (host, n = 30) in the different times of observation (hours)

Hours	Mean abundance	SD
1	0.13	0.43
2	0.2	0.55
3	0.2	0.41
4	0.33	0.5
5	1.07	1.44
6	1.6	1.67
7	2.63	2.51
8	3.1	1.56
24	4.28	2.45
48	8.31	4.75

Discussion

Our observations on the embryonic development of *Chordodes nobilii* allow us to agree with the studies carried out by Dorier (1925, 1930), Baer (1951) and Cappucci (1976) about the temperature being a principal factor influencing egg development in gordiids and about the time required for the eggs to incubate increasing as the temperature of the water was reduced. Dorier (1925, 1930) observed that the embryonic development in *Gordius aquaticus* takes 23 days at 20°C and 74 days at 10°C. Baer (1951) for *G. aquaticus* observed that when the temperature is 13°C incubation lasts 35 days and when it is only 10°C it lasts 74 days. Cappucci (1976) observed that larvae may hatch from eggs within 30–33 days at an aquatic temperature of 20°C while at 10°C it takes 60–63 days in *Gordius robustus*. In this study we demonstrate that in *C. nobilii* the egg development takes between 20–25 days at 22°C and 45–55 days at 5°C.

It is also important to note that our results about the larvae remaining included within the egg membrane at low temperatures, are coincident with the studies of Cappucci (1976) who observed that no hatch resulted when the water was kept at 4°C.

There are previous and many times contradicting reports on the longevity of post-hatching larvae. According to Dorier (1930) the *G. aquaticus* larvae maintained at 12–18°C were able to survive 7 months and became active when the temperature rose to 20°C. Schmidt-Rhaesa (1997) pointed out that the nematomorph larvae do not survive longer than one week, while Hanelt and Janovy (2004) observed that *Paragordius varius* larvae remained active for 10 days and their infectivity decreased to zero after 30 days. Taking into account that the free larvae are not agile swimmers, the best method to evaluate their survival is through analyzing their infectious capacity as carried out by De Villalobos *et al.* (2003) as well as in the present research.

In coincidence with Hanelt *et al.* (2005) so far, post hatching larvae forming cysts as described by Villot (1874) and Dorier (1930) were not observed. In agreement with Whittaker and Barker (1983) Gordiida larvae retard their hatching, as a survival strategy, until the hosts become available. This last observation would explain the possible coordination between the life cycles of the Gordiida and their hosts (paratenic or definitive). This is based on the fact that the nematomorph larvae can survive the winter cold inside their egg shells and therefore be capable of infesting the new born or hibernating hosts in spring.

Conclusions

In this study we can prove for the first time that the egg strings maintained at low temperatures (5°C) during a long period of time (6 months) were capable of continuing their embryonic development and that most larvae, once formed, remained inside the egg shell as a survival strategy. We can also confirm that these larvae survived and were able to infect *Aedes aegypti* larvae.

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