The Life Cycle and Larval Development of *Neoechinorhynchus iraqensis* (Acanthocephala: Neoechinorhynchidae) in the Intermediate Host

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Abstract

For the first time, the life cycle of *Neoechinorhynchus iraqensis*, a parasite of *Liza abu* and other freshwater fishes in Iraq and its larval development in the intermediate host, the copepod *Cyclops hyalinus* was given. Hatching took place in the intestine of the copepod host within 10 minutes of exposure to the infective eggs. Motile acanthor penetrated the wall of the intestine within 2-4 hr. after exposure and was found free in the hemocoel where it metamorphoses through the acanthella stage. Acanthella stage revealed changes in the entoblast where the anterior section gave rise to brain, proboscis and proboscis receptacle. The posterior section differentiation gave rise to the genital system. By 12 days, development in the copepod apparently was completed and by 13-14 days, the juvenile was infective for the definitive host.

Introduction

The family Neoechinorhynchidae, order Eoacanthocephala includes eight species of the genus *Neoechinorhynchus* from freshwater fishes of Iraq (1). *N. iraqensis*, an intestinal parasite of the mugilid fish *Liza abu*, was described for the first time by Amin *et al*. (2). All acanthocephalans have the same fundamental life cycle and development stages. All require an arthropod as intermediate host for the larval development and all utilize a vertebrate definitive host (3). Species of the family Neoechinorhynchidae, whose life cycles had been elucidated, include *N. cylindratus* by Ward (4), *N. emydis* by Hopp (5), *N. rutili* by Merritt and Pratt (6), *Octospinifer macilentis* by Harms (7), *Paulisentis fractus* by Cable and Dill (8), *N. saginatus* by Uglem and Larson (9) and *N. cristatus* by Uglem (10). All these species use ostracods as intermediate hosts to complete their life cycles except *P. fractus* which uses a copepod. Al-Sady (11) used 13 species of crustaceans: one copepod (*Cyclops vernalis*), one amphipod (*Parhyla* sp.), one peracarid (*Mysis* sp.), two cladocerans (*Daphnia magna* and *Simocephalus vetulus*) and eight ostracods (*Stenocypris malcolmsoni*, *Potamocypris variegata*, *Cyprinotus putei*, *Cyprinotus salinus*, *Cyclocypris cruciata*, *Candona sigmoides*, *Cypricercus reticulates* and *Eucypris cisternina*) for experimental life cycle of *N. iraqensis*. None of them was an appropriate intermediate host in the life cycle.

Materials and Methods

Mugilid fishes (*Liza abu*) were brought to laboratory from a fish market at Baghdad city. Adult worms were obtained from the intestine of these fishes. Female worms were used as a source of eggs by rupturing the body in the posterior region. The eggs were removed from gravid females, washed with tap water and then were stored in the refrigerator at 4 ^{o}C in tap water. Copepods were collected from Al-Zawraa lake, Baghdad city, with the aid of zooplankton net (mesh size 335µ). Copepods were taken to the laboratory and kept in well-aerated aquaria at 25 ^{o}C. They were exposed to the infective eggs by allowing them to feed on a suspension of eggs for 1-2 hr. after being isolated from their source of food for one day. Several hundreds of copepods were removed with a pipette and examined for infection by putting them on a slide with a drop of 0.9% saline. Drawings were made with the aid of a camera lucida. All measurements were taken in micrometer unless otherwise indicated.

Results

Eggs and Acanthors:

Fresh infective eggs from adult females ranged from 38-42 × 17-20 wide (Fig. 1). Four envelops enclosed the acanthor. These are the inner membrane, the fertilization membrane, the inner shell membrane and the outer shell. The hatching of eggs occurred within 10 minutes after exposure. The hatching embryos (acanthors) rapidly increased in length to about 43-50 × 17-20 and showed contractile movement. After ingestion, the inner membrane ruptured near the anterior pole releasing the acanthor. In the anterior end, the tube-like structure observed, extended from the apex to the entoblast Fig. (2). The peripheral syncytium was granular and contained several large nuclei. After 2-4 hr. postfeeding, the acanthor was slightly large, 48-53 × 18-20. At this stage, the acanthor was able to penetrate the copepod gut wall. Following penetration, acanthors were unattached and immobiled in the hemocoel Fig. (3).

Acanthellas:

One day after exposure, the parasite increased little in length to reach 50-60 × 40-42. Little changes occurred in the entoblast, but the giant nuclei become more distinct. This stage is called early acanthella Fig. 4). By 3-5 days, the acanthella began to be of a round shape, 75-100 × 69-75 Fig. (5). The giant nuclei were increased in size. These were four dorsals and one ventral. By 5-8 days, the larvae now 125-180 × 88-92. Five hypodermal nuclei were distinguished. The epidermis was apparent as a lighter area surrounding the internal organs which were as a number of small cells Fig. (6). At 8-10 days, the larvae reached 280 × 93. They were elongated and the proboscis, proboscis receptacle and brain had become well differentiated Fig.(7). The central nuclear mass was differentiated into the anterior primordial of the proboscis apparatus and the posterior primordium of the reproductive system. In this stage, the sex of a larva cannot be determined. The brain was then an evident as a group of small cells in the posterior end of the proboscis sheath. In 12 days, larvae are 0.5-1 mm (Fig. 8). Primordials of the lemnisci appeared, proboscis was not inverted and its hooks were sometime formed. Genital organs were differentiated. The two testes appeared in lateral

position of the body. In females of the same age, ligament sacs appeared with mass of cell to be the ovarian balls.

Juveniles

Larvae by 13 days of morphological differentiation which happened became anatomically adult-like. Females were slightly longer than males. The larval phase was completed and became infected to the definitive host.

Discussion

Neoechinorhynchus iraqensis has a wide spread among fishes in Iraq as 15 species of freshwater fishes were records as definitive hosts for this species in Iraq (12). In general, the development of this parasite in its intermediate host (*Cyclops hyalinus*) is similar to that of other neoechinorhynchids with minor variations. The acanthor of *N. saginatus* is similar to that of *N. iraqensis* in its increment in size and in showing contractile movement. Such increase in size seems to be characteristic for this group of worms, although Cable and Dill (8) noted the reduction in size in case of *P. fractus.* In the anterior end of the acanthor of *N. iraqensis*, the rostellar hooks appeared, such as in *P. fractus* (8) and *N. saginatus* (9). On the other side, the absence of these hooks has been noted in *N. cylindratus* (4) and *N. rutili* (6).

The infective stage of *N. iraqensis* was reached in about 13-14 days, in comparison with 21 days in case of *N. emydis*, a parasite of map turtle (5), 48-57 days in case of *N. rutili* (6), 30 days in case of *O. macilentis* (7), 13 days in case of *P. fractus* (8), 16 days in case of *N. saginatus* (9) and 20 days in case of *N. cristatus* (10). All these species used ostracods to complete their life cycles, except *P. fractus* which used a copepod. Al-Sady (11) used 13 species of crustaceans belonging to five orders to complete the life cycle of *N. iraqensis*. No evidence of penetration of gut wall was apparent. In the previous studies about the life cycles of the genus *Neoechinorhynchus*, the necessity of second intermediate host can be explained as an adaptation to the feeding habits of the definitive host. In her work with *N. cylindratus*, Ward (4) detected light infection in ostracods (*Cypria globula*), so she used small fishes (*Lepomis pallidus*) as a second intermediate host. *N. emydis* used ostracods (*Cypria maculata*) as the first host and snails (*Campeloma rufum*) as second intermediate host (5). Since *Liza abu* is relatively a small fish and feed on small crustaceans (13), this fish can get the infection with *N. iraqensis* directly from consuming crustaceans.

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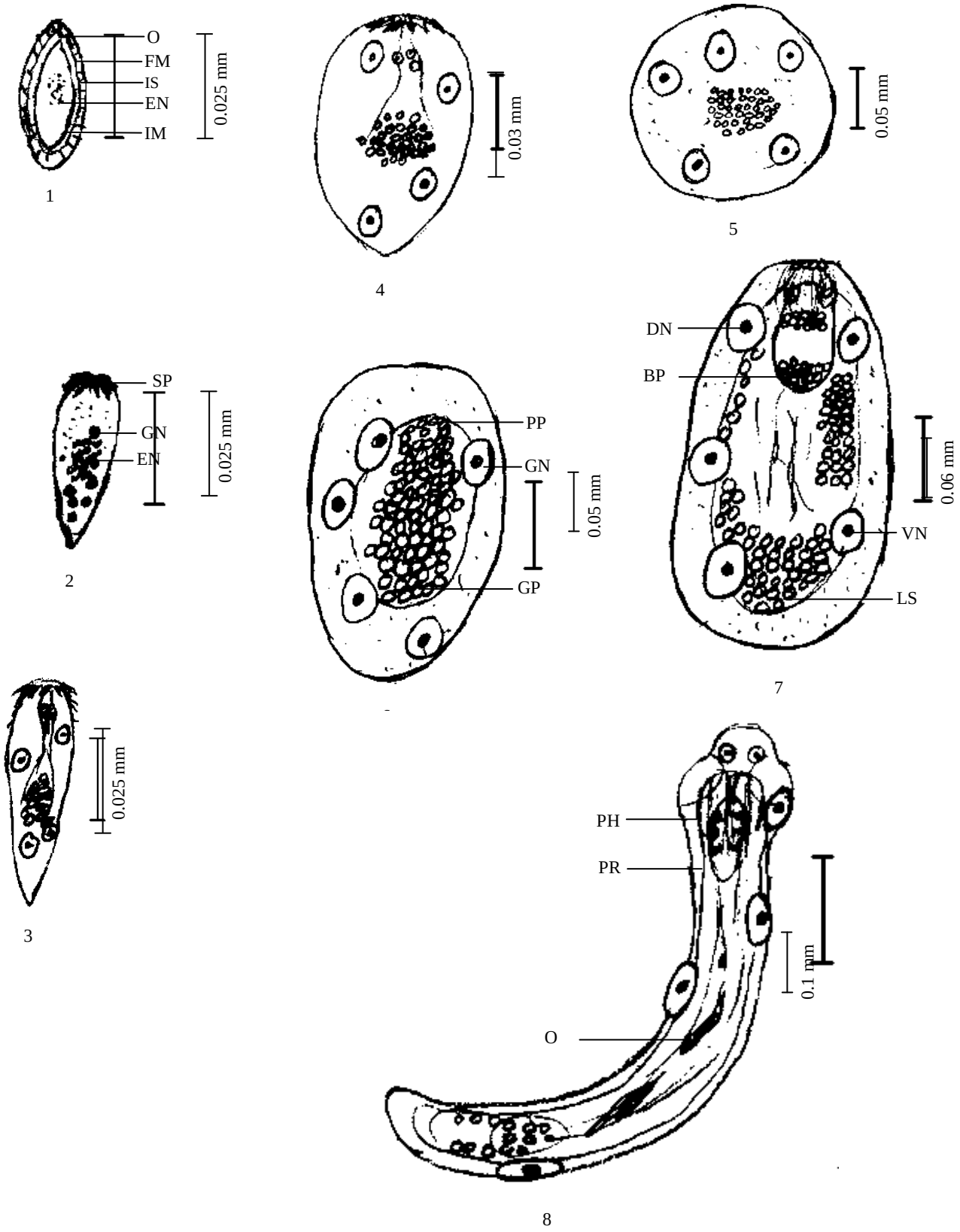

Fig. (1-8): Life cycle and larval development of *N. iraqensis*. 1. Egg. 2. Ten minutes after exposure. 3. Acanthor, 2-4 hours after exposure. 4. Early acanthella (1 day). 5. Acanthella (3-5 days). 6. Acanthella (5-8 days). 7. Acanthalla (8-10 days). 8. Female acanthella (12 days). Abbreviations: BP: Brain primordium, DN: Dorsal giant nucleus, EN: Entoblast, FM: Fertilization membrane, GN: Giant nucleus, GP: Genital primordium, IM: Inner membrane, ISM: Inner shell membrane, LS: Ligament sac, OS: Outer shell, OV: Ovarian sphere, PH: Proboscis hooks, PP: Proboscis primordium, PR: Proboscis receptacle, SP: Spine, VN: Ventral giant nucleus.

دورة الحياة والتطور اليرقي للدودة شوكية الرأس *Neoechinorhynchus iraqensis* في المضيف الوسطي

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الخلاصة

شملت الدراسة الحالية لأول مرة وصف دورة الحياة والتطور اليرقي للدودة شوكية الرأس *Neoechinorhynchus iraqensis* في المضيف الوسطي، وهو طفيلي يصيب أسماك الخشني *Liza abu* وأنواع أخرى من أسماك المياه العذبة في العراق. المضيف الوسطي المستعمل هو أحد أنواع القشريات وهو مجذافي الأقدام *Cyclops hyalinus*. فقست البيوض داخل أمعاء مجذافي الأقدام بعد10 دقائق من تناولها عند تعريضها لبيوض الطفيلي المخصبة.الجنين الفاقس (يسمى أكانثور) Acanthor يخترق جدران أمعاء القشري بعد مرور ٢-٤ ساعات من الفقس وبعدها يتوافر حراً في الجوف الجسمي وهنا يعاني من تغيرات مظهرية وصولاً إلى المرحلة الثانية وهي المشوكة الصغيرة Acanthella . تحصل تغيرات في الكتلة النووية ،اذ يتمايز الجزء الأمامي منها ليكوّن الدماغ والخطم وغمد الخطم. أما الجزء الخلفي فيتمايز إلى أجزاء الجهاز التناسلي. بعد مرور ١٢ يوماً من إصابة مجذافي الأقدام تكتمل دورة حياة الطفيلي في المضيف الوسطي وخلال اليومين ١٣و١٤ من الإصابة يصل الطفيلي إلى مرحلة المشوكة المتكيسة أو اليافعة Juvenile وهذه المرحلة هي الطور المعدي للمضيف النهائي.