

Larval development and biometry of cysts in *Thamnocephalus venezuelensis* Belk & Pereira, 1982 (Anostraca)

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Abstract

The species *Thamnocephalus venezuelensis* is a native Venezuelan fairy shrimp that inhabits temporary ponds in semiarid zone in the north-western of the country. The complete larval development under laboratory conditions is described and illustrated; additionally the biometry of resting eggs (cysts) is characterized. Untreated and decapsulated cysts showed a mean diameter of 355 μm and 313 μm respectively. The first larval stage is a nauplius typical of the group that presented a mean length of 510 μm and 0.98 mg/egg of mean weight. These values have been the largest found in anostracan species until present. Larval development started with nauplii that gradually go through successive molts to reach the adult size. Females start producing eggs near twentieth day of development. Fairy shrimps have been considered for use as live food in larval fish rearing; therefore this information is of outstanding importance due to the potential value of *T. venezuelensis* for practical use in aquaculture or another practical purpose.

Key words: larval development, *Thamnocephalus*, Anostraca, fairy shrimps, resting eggs, biometry.

Introduction

The species of cosmopolitan genus *Artemia* (Anostraca), widely known as brine shrimps, represent a suitable food for many group of organisms of the animal kingdom, from invertebrates to fishes (Sorgeloos, 1980). As a consequence, an increasing interest in mass culturing of freshwater anostracan (fairy shrimps) for applied purpose took place in the last decades. Several fairy shrimps species have been used for different practical purpose, for instance: The species *Thamnocephalus platyurus* Packard, 1877 and *Streptocephalus sudanicus* Daday de Déès, 1910 were used as test organisms in aquatic toxicology (Van Sprang and Janssen, 1997; Lahr *et al.* 2001). The species *Streptocephalus macrourus* Daday, 1908 *S. proboscideus* (Frauenfeld), 1873 and *S. dichotomus* Baird, 1860 were employed as filter feeding stage in waste reclamation systems (Mitchell, 1991; Ali and Brendonck, 1995; Munuswamy *et al.* 1997). Several species of the genera *Chirocephalus*, *Branchipus*, *Branchinella* and *Tanytastix*, have been tested as live food in freshwater aquaculture in Italia (Mura, 1992), also the species *S. proboscideus* and *S. dichotomus* in India (Munuswamy *et al.* 1992; Prasath *et al.* 1994).

The fairy shrimp *Thamnocephalus venezuelensis* Belk and Pereira 1982, is the only species of this genus found in South America (Moore and Young, 1964); there is another record of this genus for Argentina, but the species has not been properly described yet (Cohen *et al.* 1999).

Thamnocephalus venezuelensis inhabits freshwater bodies of temporal nature in the semiarid region of Venezuela that includes Zulia, Falcón and Lara states in the northwest of the country. The taxonomic description is all we known about *T. venezuelensis*, remaining unknown other biological and ecological aspects. Therefore, the purpose of this paper is to describe the complete larval development of *T. venezuelensis* and characterize the biometry of the resting eggs (cysts), due mainly to its potential value as live food in aquaculture.

Materials and Methods

The samples were collected in temporary ponds in the area of Cerro Blanco (10° 8' 26"N; 69° 33' 53"W) in Lara state. *T. venezuelensis* is found typically in highly turbid pools devoid of any floating or submerged vegetation, accompanied by another anostracan species, *Dendrocephalus spartaenovae* Margalef 1961 (unpublished data).

Adult females were collected with a hand-net of 1 mm mesh sieve during the rain season in 1996, and carried to the laboratory for egg collection and storage. The cysts were incubated during two weeks in water from the natural habitat; afterwards they were cleaned removing the debris by filtration and washing them with distilled water; and dried to constant weight at 25°C and stored in darkness.

For biometric data the mean diameter of 250 randomly selected cysts were measured, in both hydrated and decapsulated cysts. Decapsulation was performed according to Bulkowski and Meade (1986). The following aspects were considered: outer diameter of hydrated cysts (1 hour in distilled water), decapsulated diameter, chorion thickness and instar I naupliar length. Chorion thickness was determined by the difference between outer and decapsulated diameter. Cysts and nauplii were observed and photographed using Scanning Electron Micrography (SEM). The mean weight was estimated by weighting five samples of 100 randomly selected cysts.

For hatching, a sample of 600 cysts was placed in a 1000 ml beaker with artificial freshwater, prepared following Peltiers and Weber (1985) methodology. The culture medium was previously aerated until saturation, under constant light (4000 Lux) and room temperature (25±2°C). After hatching, a sample of 10 nauplii was collected every hour for 12 hours, and then every 6 hours for the subsequent 5 days; finally, every 12 hours for the next 21 days. All samples were stored in formalin 5%. The description of the larval development is based in changes that occur through time following the indications given by Bernice (1972) and Pereira and González (1994). Each larva was measured (total length) with an ocular micrometer. Drawings of whole larvae were made with the aid of a drawing tube mounted on a binocular microscope. The larvae of fairy shrimps were kept under artificial light and fed daily with a mixture of the algae *Selenastrum capricornutum* (Printz) and *Scenedesmus* sp. (Chlorophyceae) grown in the laboratory according to Wayne-Nichols (1975). The water was aerated by means of an electric pump and replaced every two days.

Results

The eggs have a polyhedral shape showing a surface of rough aspect, ornamented with borders that form polygonal surfaces (Figure 1A). Results of the biometrical characteristics for the cysts and nauplii are shown in the Table I. The cysts present a mean diameter (hydrated) of 355 µm and a mean weight (desiccated) of 0.98 mg/egg. Hatching occurs 12 hours after incubation in two phases: Pre-eclosion and eclosion. During the pre-eclosion the egg is hydrated until the eggshell is broken, while the nauplius remains in the eclosion membrane. Subsequently, the nauplius breaks the membrane of eclosion and starts the free swim.

The first larval stage is a nauplius typical of the group with a total length of 0.51 mm (0.35-0.62 mm) (Figure 1B). It presents head and thorax, a central naupliar eye, labrum and three pairs of cephalic appendages. An incipient thoracic segmentation can be observed. The pair of uniramous antennules has three distal setae (Figure 2A). The pair of biramous antennae has the exopodite with 14 to 17 setae, and the endopodite bisegmented, with three distal setae; and a protopodite divided in basipodite and coxopodite, both with one spine (Figure 2B). Mandibles biramous composed by four segments, a proximal coxopodite that possesses a lateral spine and the basipodite with two lateral setae, followed by an endopodite with two lateral setae and finally an exopodite with three distal setae (Figure 2C). The size of the antennae and the number of post-mandibular segments increase during the 6-12 hours interval (Figure 2D).

In the stage at 24 hours a pair of compound eyes starts developing, and begins the differentiation of the ocular peduncles and the maxilla. There are 10 post-mandibular segments, the first segments

present exopodite and endopodite. There are two pairs of caudal setae at the posterior end at each side of the anus. The mean total length of the larvae in this stage is 1.2 mm (1.1-1.2 mm) (Figure 2E).

The stage, 48 hours after hatching, presents pedunculated eyes and the maxillas II and I are more differentiated. The antennae II are directed anteriorly. There are eleven thoracic segments; of those the first six have associated appendages. The differentiation of abdominal segments begins. There are between 9-11 caudal setae at each side of the anus. The mean total length of this larval stage is 1.23 mm (1.2-1.3 mm) (Figure 2F).

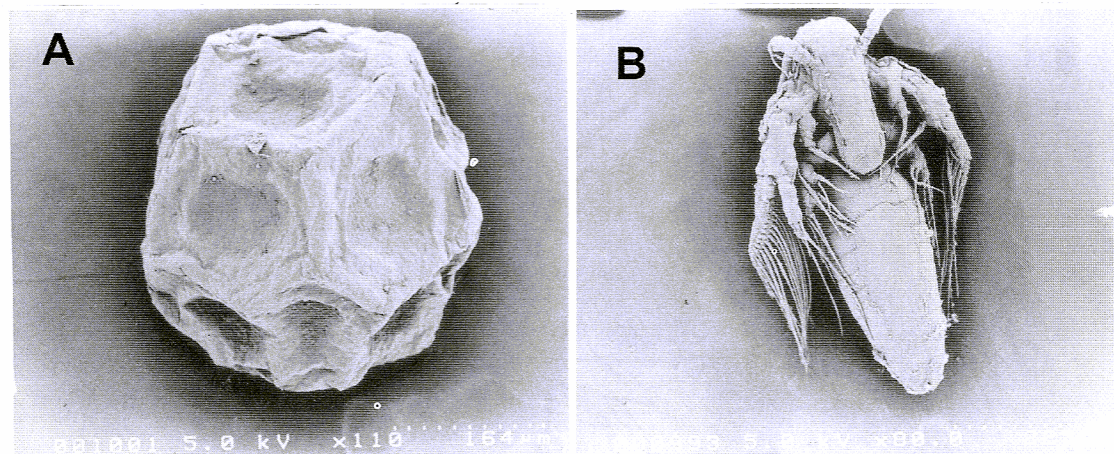


Figure 1: Scanning electron microscopy micrographs of an egg and a nauplius of *T. venezuelensis*. A. Complete egg; B. Nauplius at zero hours after hatching when the larva begins the free swim.

Table I: Biometrical characteristics of cysts and nauplii of *T. venezuelensis*.

Biometrical characteristics	n	mean	range
Diameter of not decapsulated cysts (μm)	250	355	(320-380)
Diameter of decapsulated cysts (μm)	250	313	(260-350)
Corion thickness (μm)	250	43	(32-64)
Nauplii sizes (mm)	100	0.51	(0.35-0.62)

The stage at 72 hours shows complete development of the ocular peduncles. The antennae II and mandibles show a considerable reduction. The setae of the basipodite have disappeared in the antennae II, and the endopodite and coxopodite are reduced. The mandibles present a reduction of the basipodite, endopodite and exopodite to a small mandible palp (Figure 3A). There are 7 abdominal segments. The cercopods begin to differentiate, each one possess 15-19 caudal setae (Figure 3B). The mean larval length is 1.5 mm (1.3-1.9 mm).

At 108 hours the cephalic region is similar to the previous stages, the 11 thoracic segments have associated appendages. There are 8 abdominal segments well differentiated. Both cercopods have 21 caudal setae (Figure 3C).

In the stage at 120 hours, the endopodites and coxopodites of the antennae II have disappeared. The males show two segmented pair of antennae, which bear a small frontal outgrowth on the basal segment, that will form the frontal appendage (Figure 3D). The antennae II in females show a total reduction of the endopodites and coxopodites, whereas the exopodites becomes wider and longer. The mean larval length is 2.6 mm (1.7-3.1).

In the stage at 144 hours, the abdomen of both sexes presents incipient sexual appendages on the first segments. In the females there is a small ovisac, and two incipient penis in males (Figure 4A; B). Each cercopods have between 30-35 caudal setae.

In the stage at 192 hours (8 days), the males have 2 separate outgrowths on the distal border of their head, each with two distal ramifications; females have well developed antennae II, similar to the adult stages. The mandibles are represented only by a sclerotized coxopodite (Figure 4C).

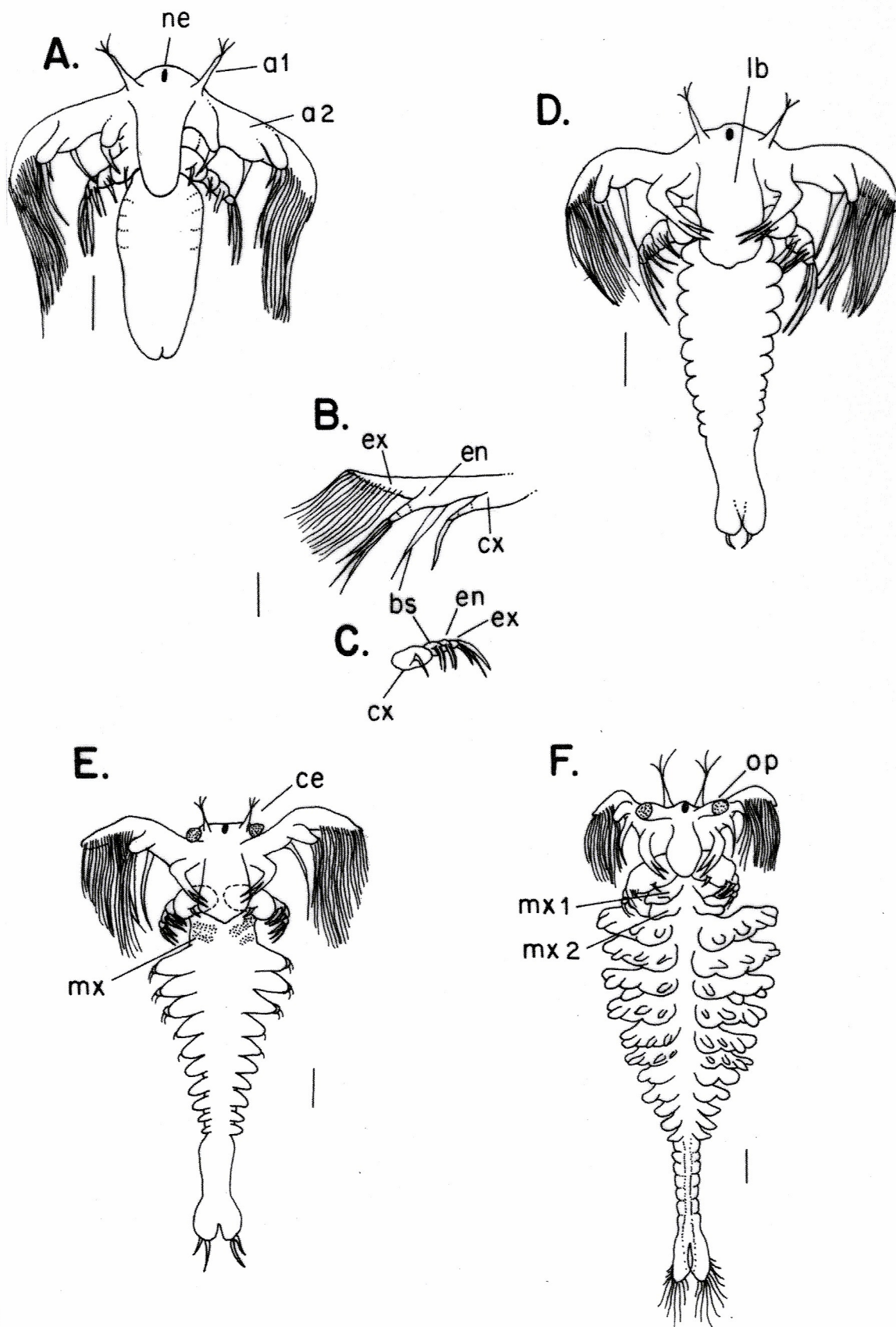


Figure 2: Larval development of *T. venezuelensis*. A. First instar nauplius, a1. antennula; a2. antenna; B. Detail of antenna II, ex. exopodite; en. endopodite; bs. basipodite; cx. coxopodite; C. Detail of mandible; D. Metanauplius 12 h after hatching, lb. labrum; E. Metanauplius 24 h after hatching, ce. compound eyes; mx. maxillas; F. Metanauplius 48 h after hatching, op. ocular penducle; mx1. first maxilla; mx2. second maxilla. Scale 0.1 mm.

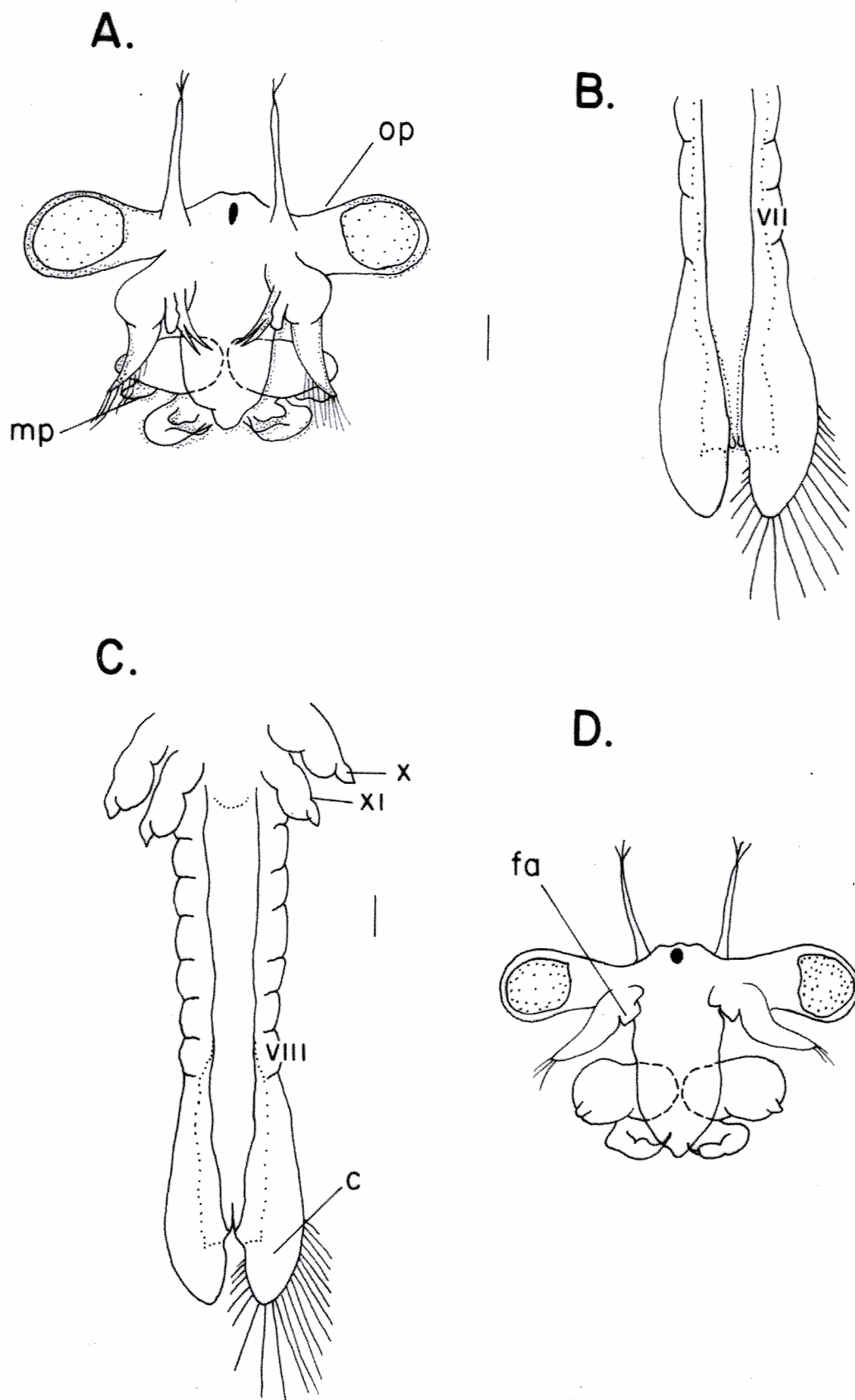


Figure 3: Larval development of *T. venezuelensis*. A. Metanauplius 72 h after hatching, op. ocular peduncle; mp. mandible palp; B. Detail of cercopods 72 h after hatching, C. Metanauplius 108 h after hatching, detail of the X, XI thoracic segments and abdomen; c. cercopods; D. Metanauplius 120 h after hatching, fa. outgrowth of future frontal appendage. Scale 0.1 mm.

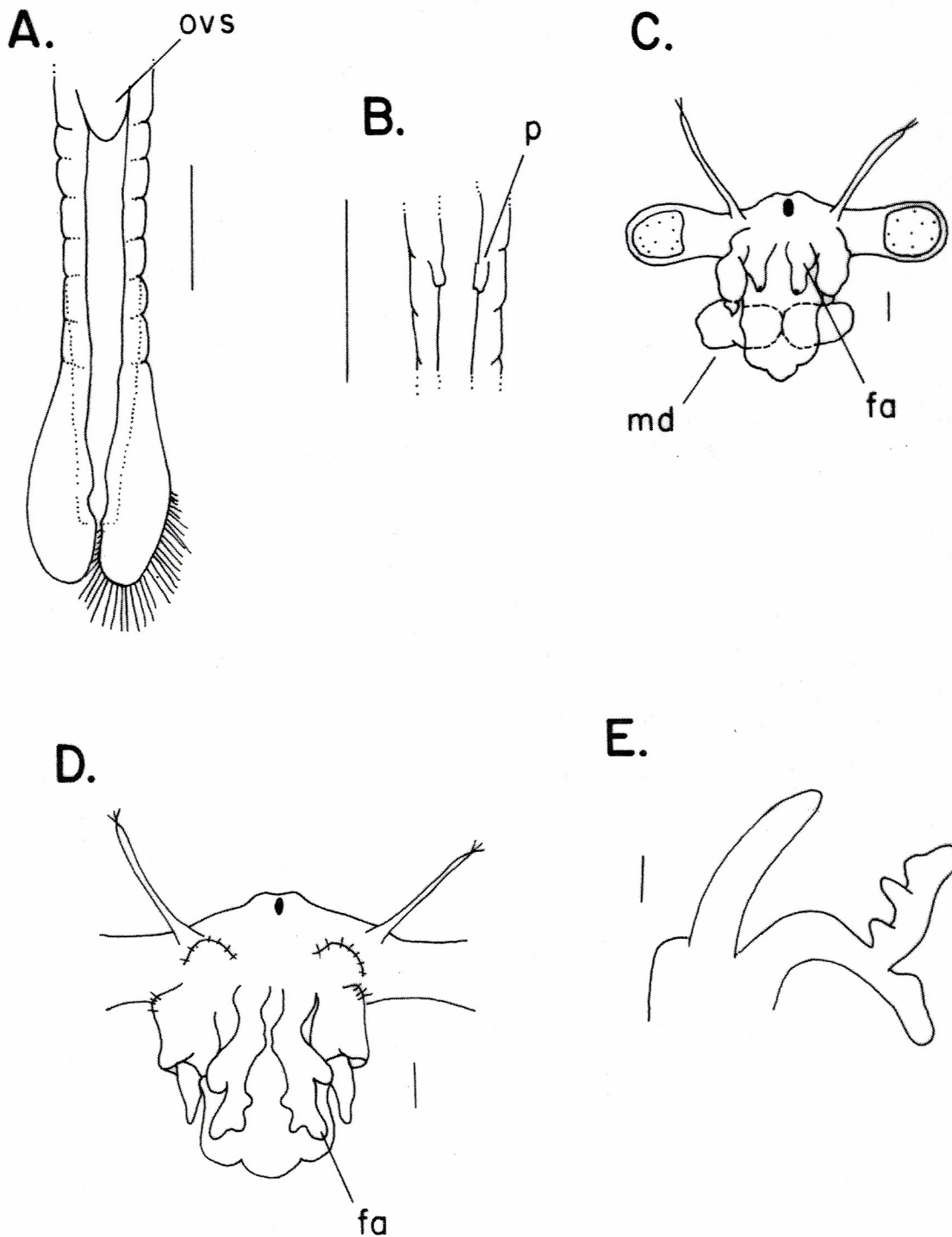


Figure 4. Larval development of *T. venezuelensis*. A. Abdomen of metanauplius 144 h after hatching, ovs. incipient ovisac, females; p. incipient penis, males; Scale 0.5 mm; B. Metanauplius 192 h after hatching, fa. outgrowth of future frontal appendage; md. mandible; C. Metanauplius 312 h after hatching, fa. frontal appendage; D. Metanauplius 360 h after hatching, detail of frontal appendage. Scale 0.1 mm

At 312 hours (13 days) each outgrowth of the males presents four ramifications (Figure 4D), and the cercopods have more than 50 setae around their margin.

In the stage at 360 hours (15 days), the outgrowths in males have six distal ramifications (Figure 4E). The cercopods show total differentiation conforming to the caudal furca (Figure 5A). The ovisac of females and the penis of males are in an advanced stage of development (Figure 5A, B). The total length at this stage is 9.8 mm (7.5-11.1).

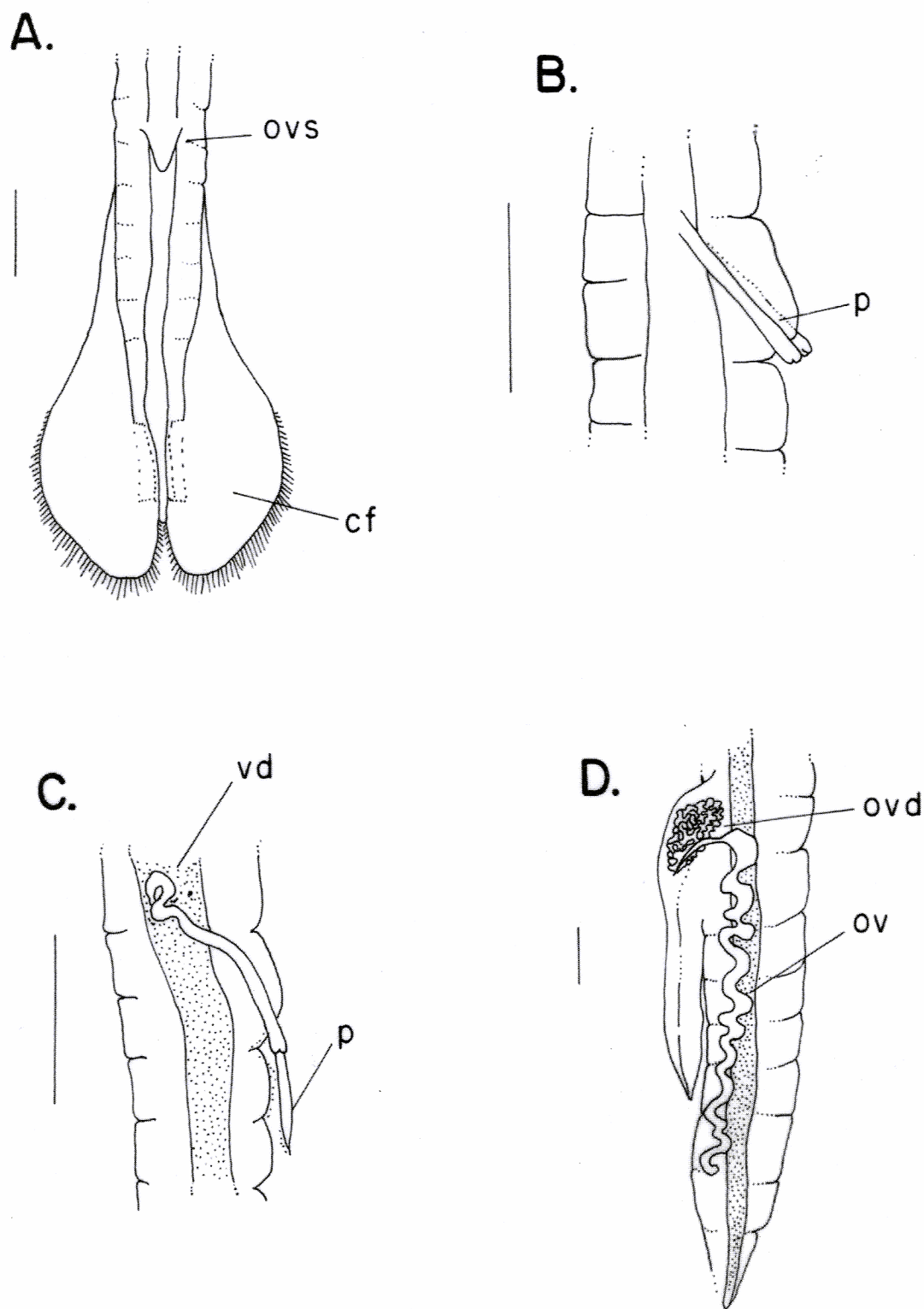


Figure 5: Juvenile of *T. venezuelensis* 360 h after hatching. A. Abdomen of female, ovs. future ovisac; cf. caudal furca; B. Abdomen of male, p. pair of incipient penis. C. Stage at 384 h (16 days) after hatching, detail of abdomen of males; vd. vas deferens; p. penis; D. Stage at 432 h (18 days), abdomen of females, ovd. oviduct; ov. ovary. Scale 1 mm.

Between 384 hours (16 days) and 432 hours (18 days), the males show vas deferens (Figure 5C) and the females show ovary and oviduct well differentiated (Figure 5D). Finally, the first eggs in the ovisac are observed after 480 hours (20 days).

Discussion

The biometry of the cysts shows that despite the relative large size of decapsulated cysts, the nauplii sizes is only slightly larger than those values observed in other anostracan species (Table II). This characteristic makes this species potentially usable as live food in the aquaculture industry.

There are no other publications on the life cycle of species of the genus *Thamnocephalus*. Within the Streptocephalidae, Baqai (1963) described the larval development of *Streptocephalus seali* Ryder, 1879, and Bernice (1972) of *S. dichotomus* Baird, 1852. Anderson (1967) and Baid (1967) described the development of *A. salina*; and finally Pereira and González (1994) reported the larval development of *Dendrocephalus geayi* Daday, 1908 within the Thamnocephalidae. The instars I or nauplius of *Thamnocephalus venezuelensis* presents a similar morphology to that observed in other naupliar descriptions for anostracan species; however, it shows a higher number of spines on the exopodites from second antennae than in the other anostracan species (Table III). Additionally, it is observed a slight segmentation in the post cephalic region, which is not mentioned in the description of other anostracan species. The sexual differentiation, based on the start of frontal appendage developed in the second antennae, in male begins at 120 hours after hatching, similar to that observed in *Dendrocephalus geayi* (Pereira and González, 1994). The caudal furca begins differentiation at 120 hours after hatching and it is thoroughly developed at 360 hours (15 days). The genitalia segments begin their differentiation at 144 hours (6 days) in males and females, and the first eggs appear in the ovisac of female about 480 hours (20 days) under these laboratory conditions.

Fairy shrimps have been considered for use as live food in larval fish rearing, due to their suitable size and nutritional value; therefore the present information is of outstanding importance for practical use of *T. venezuelensis* in aquaculture, or another practical purpose such as, toxicology or aquariophily.

Table II: Mean values and range of biometrical characteristics of cysts and instar I (nauplii) from *T. venezuelensis*, 1982, compared with data from other anostracan species.

Species	Diameter of not decapsulated cysts (μm)	Diameter of decapsulated cysts (μm)	Corion thickness (μm)	Nauplii sizes (mm)	References
<i>A. salina</i>	244 (225-260)	228 (210-243)	8 (7-11)	0.46 (0.43-0.52)	Vanhaecke and Sorgeloos, 1980
<i>D. geayi</i>	271 (160-346)	191 (144-224)	40 (29-150)	0.41 (0.20-0.60)	García <i>et al.</i> , 2000
<i>D. spartaenovae</i>	211 (176-240)	157 (128-240)	60 (48-112)	0.31 (0.27-0.38)	García <i>et al.</i> , 2000
<i>T. platyurus</i>	278 (208-320)	240 (176-288)	19 (16-56)	0.44 (0.35-0.51)	Mura, 1995
<i>T. venezuelensis</i>	355 (320-380)	313 (260-350)	43 (32-64)	0.51 (0.35-0.62)	Present work
Other anostracan species	287 (247-430)	242 (197-355)	23 (8-36)	0.43 (0.34-0.48)	Mura, 1992

Table III: *Thamnocephalus venezuelensis* Belk & Pereira, 1982, spines number on exopodite from second antenna, compared with data for other known naupliar descriptions of anostracan species.

Species	Spines on exopodite, second antenna	References
<i>S. seali</i>	13	Baqai (1967)
<i>S. dichotomus</i>	10	Bernice (1972)
<i>D. geayi</i>	12	Pereira and González (1994)
<i>A. salina</i>	9	Baid (1967)
<i>T. venezuelensis</i>	14-17	Present work

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