SEM investigations of the larval development of *Imnadia yeyetta* and *Leptestheria dahalacensis* (Crustacea: Branchiopoda: Spinicaudata)

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Abstract

This paper presents a SEM documentation of the larval development of the two most abundant Austrian conchostracan species, *Imnadia yeyetta* (Limnadiidae) and *Leptestheria dahalacensis* (Leptestheriidae). As in several previously examined spinicaudatan species, five larval stages were documented: Nauplius 1, Nauplius 2, Metanauplius, Peltatulus, and Heilophore. Additionally, three postlarval stages of *L. dahalacensis* and the first larval instars of *Eoleptestheria ticinensis* and *Limnadia lenticularis* are shown and compared with the examined stages. Species identification of conchostracan larval stages is possible by using surface structures, and using SEM methods, except for *L. lenticularis* which can be identified more easily on the characteristic shape of the labrum.

Introduction

Conchostracan larval development has been previously described in a few species only. In most cases, incomplete descriptions or hand sketches of only a subset of the stages have been presented.

For European species, Lereboullet (1866) first described the larval development of a conchostracan, *Limnadia hermanni*, synonym of *Limnadia lenticularis* (Linné, 1761), with accurate artistic drawings providing detailed information, useful even now. *Limnadia lenticularis* larvae were also documented by Sars (1896a) and by Zaffagnini (1971), who published light microscopy photographs of one postlarval and three larval stages. Five larval stages and the first postlarval stage of *Eoleptestheria ticinensis* and *Limnadia lenticularis* are shown and compared with the examined stages. Species identification of conchostracan larval stages is possible by using surface structures, and using SEM methods, except for *L. lenticularis* which can be identified more easily on the characteristic shape of the labrum.

Described the larval stages of *Leptestheria dahalacensis* (Rüppell, 1837), and five stages of *Leptestheria saetosa* Marincek & Petrov, 1992 (most probably a synonym of *L. dahalacensis*) were described by Petrov (1992).

The first work on the development of a non-European conchostracan species was done by Sars (1887) on *Cyclestheria hislopi* (Baird, 1859), a species whose offspring develop within the brood chamber, an exception in conchostracans. Recently, the same species was studied by Olesen (1999) who presented the only systematic SEM documentation of conchostracan larval stages existing so far. Berry (1926) described larval stages of *Eulimnadia stoningtonensis*; within the same genus, Streth & Sissom (1975) described six larval stages of *E. texana*. The larval stages of *Limnadia stanleyana* were investigated by Anderson (1967) and Bishop (1968).

In Laevicaudata, Grube (1853) first described the metanauplius and the heliophore of *Lynceus brachyurus* O. F. Müller, 1776, which was presented together with the heliophore of *L. andronachensis* by Botnariuc (1947). Later, all larval stages of *L. brachyurus* were documented (Monakov & Dobrynina, 1977). The development of *Lynceus gouldi* was partly investigated by Gurney (1926).
This paper presents the first complete SEM documentation of the larval development of the two most abundant Austrian conchostracan species, *Imnadia yeyetta* Hertzog, 1935, and *Leptestheria dahalacensis*, belonging to the families Limnadiidae and Leptestheriidae, respectively. It is far from a complete description and intended as a starting point for further investigations.

**Materials and methods**

Adult egg-bearing females of *Imnadia yeyetta*, *Leptestheria dahalacensis*, *Limnadia lenticularis*, and *Eoleptestheria ticinensis* were collected in the flood plains of Morava and Danube rivers in eastern Austria (for distribution of species in Austria see Eder et al., 1997. A complete list of large branchiopod records is available at the ZOBODAT database in Linz, Upper Austria). They were kept in small aquaria until all eggs were discharged. Then, the aquaria were dried out naturally in the sun. After a drought period of at least 2 weeks, deionized water was added to the aquaria at room temperature and supplied with an air pump. Nauplii hatched after 8–48 h. Light was provided by a 60 W light bulb to induce algae production. Larvae were collected at intervals of 24 h and fixed in different ways (70% ethanol, glutaraldehyde with 2% osmium tetroxide re-fixation, osmium-microwave fixation, Bouin). Fixation with Bouin’s fixative led to the best results, with low shrinking effects and intact setae. The specimens were CP-dried (BAL-TEC CPD030), sputter coated with gold (BAL-TEC SCD 005) and observed with a JEOL JSM-35 CF scanning electron microscope.

Additionally, living larvae of *L. dahalacensis* were individually observed in a small vial throughout their larval development using a binocular, to check if any stages exist in addition to those observed morphologically.

**Results**

Based on morphological features and individual observation for one of the species, both examined species show 5 larval stages that can be classified according to Botnariuc (1947, 1948) as follows: Nauplius 1 (stage 1), Nauplius 2 (stage 2), Metanauplius (stage 3), Peltatulus (stage 4), and Heilophore (stage 5).

*Imnadia yeyetta*

**Nauplius 1**

The first larval stage (1 in Fig. 1) is about 200 µm long and characterized by three pairs of limbs (antennulae, antennae, mandibulae) and an ovaly shaped, smooth and unsegmented postmandibular region of half the total body length ending in a terminal anal groove. The antennules are short processes, each carrying a terminal seta and a small, most probably sensory cavity with short finger-like sensitive organs. The antennae are the main swimming organs (naupliar locomotion does not differ from *Artemia* as described by Williams, 1994), biramous and show a characteristic setation: the basal endite bears a long terminal seta (‘gnathobase seta’), and more distally at the protopod, between the exopod and endopod, is a long seta. The exopod bears a row of one terminal and four swimming setae along the inner margin, and the endopod shows four terminal setae. This setation pattern does not change during the larval development. The mandibles are uniramous, bearing three terminal setae distally, one seta at the base of the third segment and two small setae medially on the second mandible segment. The mandibular coxa presents a proximal protuberance, the developing gnathobase (larval feeding and ingestive apparatus were described by Anderson, 1967). The labrum is oval, with a maximum width of 60 µm, posteriorly covering the mouth, and reaching the middle of the body. It is covered with groups of short (ca. 1 µm long), rudimentary setae. These structures can also be found on the antennae and on the postmandibular region, though less frequently. On the dorsal side of this stage the convex neck organ covers the naupliar region.

**Nauplius 2**

The second naupliar stage (2 in Fig. 1) is about 250 µm long and morphologically unchanged in the main characters. Besides its larger size, the main difference to the first instar is the prolonged postmandibular part of the body which has a conical shape and shows two slight dorsolateral humps, the developing bivalved carapace. The labrum has transformed to a trapeziform shape of approximately 80 µm width and has entirely lost its surface structure. The rest of the body is smooth, as well, except for a group of three small setae of approx. 10 µm at the base of the single median seta of the mandible. The tip of the enditic (coxal) ‘gnathobase seta’ of the antennae is setulated, as well as the median seta of the mandible. The pair of setae
near the mandibular base has brush setules. The shape of the dorsal organ is oval to rounded hexagonal; in specimens with shrinkage it shows three slight median humps.

**Metanauplius**
The third naupliar stage (3 in Fig. 1) is about 350 µm long. The mandibular gnathobase is functionally developed and bears setae approx. 5 µm long. For the first time, a differentiation of the postmandibular region can be seen externally. Dorsolaterally, the emerging bivalved carapace is visible under the cuticle, and ventrally, a weak segmentation is delineated externally at the future position of the trunk limbs. In light microscopy, the formation of zones of seven pairs of trunk limbs is visible, but still no segmentation is delineated externally. At the posterior end, next to the anal groove, two lateral humps delimit the telson. Most swimming setae have become longer and are now denticulated, with exception of the setulated setae mentioned above. The tip of the setulated ‘gnathobase seta’ of the antenna is bifid.

**Peltatulus**
The larva (4 in Fig. 1) has now doubled its hatching size, total length is approximately 420 µm, labrum width is 125 µm. The naupliar region of stage 4 has changed little, apart from a further increase in size. Most changes take place in the postmandibular region. The carapace emerges for the first time thereby giving the name to this stage (peltastes, gr. small leathern shield). Both shields are almost circular in shape, with a radius of almost 100 µm, and connect dorsally along a zone of more than 80 µm. Posterior to the carapace, the formation zones of six pairs of trunk limbs are clearly visible, the first four limbs showing a slight division into at least three limb portions. A seventh segment without externally visible limb rudiments is followed by a short unsegmented region leading to the telson, which ends in a pointed rudimentary furca.

**Heilophore**
The total length of stage 5 (5 in Fig. 1) is approximately 530 µm. The changes in the naupliar region are small, except for the first external outline of the com-
pound eyes, in front of the antennulae. The Heilophore is characterized by an enlarged bivalved carapace laterally covering the second pair of trunk limbs and a further development of the postmandibular region. Six pairs of limbs with a successive developmental delay are clearly differentiated, the first three trunk limbs show a beginning setation on the buds; additionally at least one internally undivided limb formation segment is visible. The telson has increased in length to more than 100 µm and is covered with rows of short setae.

**Leptestheria dahalacensis**

**Nauplius 1**
The first instar of *L. dahalacensis* (1 in Fig. 2) is about 165 µm long and characterized by the three naupliar pairs of limbs and an oval-shaped, unsegmented postmandibular region less than half of the total body length. The antennules are short processes, each carrying a terminal seta and a small, most probably sensory cavity with short finger-like sensory organs. The antennae are biramous and show a setation similar to the larvae of *I. yeyetta*: the basal endite bears a long terminal seta (‘gnathobase seta’), the protopod bears a long seta distally between the exopod and the endopod. The exopod bears a row of two terminal and three median swimming setae, and the endopod shows three terminal setae. The mandibles are uniramous, three-segmented, bear three terminal setae, one median seta and two small setae medially near the base. The mandibular coxa presents a proximal protuberance, the developing gnathobase. The labrum is spoonlike, long, oval, with a maximum width of 50 µm, posteriorly covering the mouth, and reaching in length more than two thirds of the body. It is covered with rows of 3–7 short (ca. 1 µm), rudimentary setae. These structures can also be seen at a lesser extent on the antennae and on the postmandibular region.

**Nauplius 2**
The stage after the first moult (2 in Fig. 2) is about 230 µm long. Besides its larger size, the main difference to the first instar is the elongated postmandibular region. In contrast to *I. yeyetta*, no developing bivalved carapace can be seen externally in this stage. The labrum is spoonlike, long, oval, with a maximum width of 50 µm, posteriorly covering the mouth, and reaching in length more than two thirds of the body. It is covered with rows of 3–7 short (ca. 1 µm), rudimentary setae. These structures can also be seen at a lesser extent on the antennae and on the postmandibular region.

**Metanauplius**
The third naupliar stage (3 in Fig. 2) is about 260 µm long. The mandibular gnathobase has developed and bears 5 µm long setae. For the first time, slight dorsolateral humps of the developing bivalved carapace are visible in the postmandibular region. In light microscopy, the formation zones of seven pairs of trunk limbs are visible, but still no segmentation is delineated externally. At the posterior end, beside the anal groove, two lateral humps delimit the telson. Most swimming setae have become longer and are now denticulated, except the setulated setae mentioned above. The tip of the setulated enditic ‘gnathobase setae’ of the antenna has become bifid.

**Peltatulus**
Total length is approximately 360 µm. The naupliar region of stage 4 (4 in Fig. 2) shows only small changes from the previous stage, apart from a further size increase and the further development of the mandibular gnathobase. Most changes have taken place in the postmandibular region. Both carapace shields are visible. Posteriorly, the formation zones of seven pairs of trunk limbs are clearly visible. At least one additional segment without externally visible limb rudiments is followed by a short unsegmented region leading to the telson, which terminates in a pointed rudimentary furca covered with rows of short setae.

**Heilophore**
The total length of stage 5 (5 in Fig. 2) is approximately 520 µm, the labrum shows a rounded triangular shape of 170 µm maximum width. Its setation covers the distal and the lateral parts. The carapace is further enlarged, each valve now about 180 µm long, and laterally covering the body until the fifth trunk limb. Seven pairs of limbs with a successive developmental delay are clearly differentiated in the postmandibular region. The first five trunk limbs show an incipient setation on the primordial endites as well as on the distal limb portions. Additionally, at least two internally undivided limb formation segments are visible. Caudally, a more or less undifferentiated segment formation...
Figure 2. Ventral view of the larval stages of *Leptestheria dahalacensis*. 1, Nauplius 1; 2, Nauplius 2; 3, Metanauplius; 4, Peltatulus; 5, Heilophore. Scale bar = 100 µm.

zone delimits the telson which is approximately 120 µm long and covered with rows of short setules.

**Postlarval stages**

The first postlarval stage of *L. dahalacensis* has a total length of approximately 600 µm and an expansion and folding of the carapace into a bivalved shield covering all developed limbs. The naupliar region has remained almost unchanged during larval development, but it changes significantly between the heilophore and the first juvenile stage. The labrum is short, enditic processes and median setae of antennae are reduced and the mandibles persist only as enlarged gnathobases. Seven pairs of legs are developed with successive delay, two to three primordial limbs show outlined portions, followed by at least two visible rudiments in the segment formation zone which is known from adult conchostracans.

The (most probably) second postlarval instar (2 in Fig. 3) has a total length of almost 900 µm. The bivalved carapace now covers the entire body. Thirteen pairs of limbs are developed with successive delay, the first five legs possessing an adult-like epipod, followed by the formation zone. The telson and furcae closely resemble those of adult individuals.

The (most probably) third postlarval stage (2 in Fig. 3) has a total length of approximately 1.5 mm and does not differ significantly from the second postlarval stage. Seventeen to 18 trunk segments bear limbs, nine of them being fully developed. Postlarval ontogeny of *L. dahalacensis* has not been followed beyond this stage, the number of subsequent juvenile stages until sexual maturity is therefore unknown.

**Discussion**

The larval development of both examined species goes through the following stages: Nauplius 1, Nauplius 2, Metanauplius, Peltatulus, and Heilophore. After the fifth moult, the main characters of the naupliar region are reduced, mainly due to the change of feeding mode.

Strenth & Sissom (1975) described six larval stages for *Eulimnadia texana*, as well as Sars (1896a)
Figure 3. Postlarval (juvenile) stages 1–3 of *Leptestheria dahalacensis* in lateral view. In 2 and 3, the left carapace shield was removed. Scale bar = 100 µm.

For *L. lenticularis*, which led to a general uncertainty regarding the frequently found number of five larval stages in spinicaudatans (C. Sassaman, pers. com.) My individual observations on living *Leptestheria dahalacensis* larvae confirmed the number of five distinct stages found morphologically. Additionally, in both examined species, the whole number of formation zones of the trunk limbs can be seen in light microscopy at the Metanauplius stage, whereas in *E. texana* the trunk limbs emerge in two steps, the fourth larval stage showing only three pairs of limbs (Strenth & Sissom, 1975). Therefore, until further evidence, the number of larval stages is supposed to be five for *Imnadia yeyetta*, as well, although no individual observations on living larvae were made for this species. In comparison to the development of *Rehbachiella* and *Artemia*, spinicaudatans show an accelerated development (Fig. 5) which is obviously an adaptation to the extreme habitat conditions of temporary water bodies (Walossek, 1993, 1995). The slightly longer larval de-
development of *E. texana* may therefore be interpreted as a plesiomorphy.

The total sizes of *L. dahalacensis* larval stages in this study differ significantly from the measurements given by Monakov et al. (1980). Differences in size up to almost 100% could also be observed when comparing material of *L. dahalacensis* larvae from Austria to equivalent larval stages from Italy; so the size differences appear to be true and not caused by an artifact or different investigation methods. It would be interesting to compare whether branchiopod larvae in northern regions are generally larger than those of the same species in southern regions.

Identification of conchostracan larvae present in Austria is only possible using SEM methods. In two species, *Limnadia lenticularis* (1 in Fig. 4) and *Cy- zicus tetracerus* (cf. heilophore given in Botnariuc, 1947) the labrum shape presents a good indicator for identification. The three other species present in Austria, *L. dahalacensis*, *I. yeyetta*, and *Eoleptestheria ticinensis* (2–4 in Fig. 4) have a more or less oval labrum, which allows a specific determination only in combination with surface structures. Most of the setation seems to be almost identical in all spinicaudatan larvae, as far as can be seen from literature. However, individual differences could be observed. In one case, the first instar of *L. dahalacensis* showed two terminal plus three median swimming setae on the exopod of the left antenna, but three terminal plus two median setae on the right exopod. Regarding this individual variation, the differences discussed by Petrov (1992) between ontogeny of *Leppestheria saetosa* and *L. da- halacensis* (using the descriptions by Monakov et al., 1980) seem rather insignificant to me, although larval development may not be sufficient evidence to decide on the validity of a possible synonymy of the two species.

One of the most significant differences between the two examined species is the number of developed limbs after the fourth moult. The fifth stage of *Imnadia yeyetta* shows six limbs with clearly differentiated portions, whereas *Lepestheria dahalacensis* heilophore shows seven primordial limbs. The heilophore stage has often been suggested as a possible origin of devel-

![Figure 4. Comparison of the first larval stage of four Austrian conchostracans (left side: Limnadiidae, right side: Leptestheriidae). 1, *Limnadia lenticularis*; 2, *Lepestheria dahalacensis*; 3, *Imnadia yeyetta*; 4, *Eoleptestheria ticinensis*. Scale bar = 100 µm.](image-url)
development of Cladocera by neoteny (Claus, 1876; summarized by Schminke 1981), as this stage is similar to certain cladocerans, especially the ctenopods, due to the six pairs of developed trunk limbs. At least for Leptestheriidae, this comparison does not apply: the *L. dahalacensis* heilophore presents seven limbs with outlined limb portions plus two additional pairs of limbs without externally developed portions. Recently, *Clypestrheria* has been regarded as a possible sister group of Cladocera (Olesen, 1999) representing a separated branchiopod order (Negrea et al., 1999; Spears & Abele, 2000). Olesen (1999) proposed a simple reduction of body segments in the branch leading to Cladocera instead of classical neoteny.

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