

SEM studies on the early larval development of *Triops cancriformis* (Bosc) (Crustacea: Branchiopoda, Notostraca)

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Abstract

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We investigated early larval development in the notostracan *Triops cancriformis* (Bosc, 1801–1802) raised from dried cysts under laboratory conditions. We document the five earliest stages using scanning electron microscopy. The stage I larva is a typical nauplius, lecithotropic and without trunk limbs. The stage II larva is feeding and has trunk limb precursors and a larger carapace. Stage III larvae have larger trunk limbs and a more adult shape. Stage IV larvae have well developed trunk limbs, and stage V larvae show atrophy of the antennae. We describe the ontogeny of selected features such as trunk limbs and carapace, discuss ontogeny and homologization of head appendages, follow the development of the feeding mechanism, and discuss trunk limb ontogeny.

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Introduction

Larval development is of increasing importance in our attempts to reconstruct crustacean phylogeny and how the stemline (stem species) radiated into the highly divergent forms displayed by both extant and extinct Crustacea (e.g. Walossek 1993). In such attempts the Branchiopoda have featured prominently for many years because they are argued to possess many plesiomorphic features and have even been argued to represent either ‘the most primitive’ Crustacea or the sister-group to all remaining Crustacea (Fryer 1992; Martin and Davis 2001).

The close morphological similarities among the ‘large branchiopods’ (i.e. the Anostraca, Notostraca, Laevicaudata, Spinicaudata and Cyclestherida) were established very early but works on ontogeny have been rare. The development of the Anostraca is probably the most studied, with a significant focus on *Artemia*, while the development of various ‘conchostracans’ is still severely understudied. The development of the Notostraca has been studied to some extent (see reviews in Fryer 1983, 1988; Walossek 1993; Olesen in press). Many previous studies have lacked detail and this has prompted the re-study of larval development in

some branchiopod taxa, e.g. Schrehardt (1987) (*Artemia*), Schlögl (1996) (*Branchipus*), Olesen (1999) (*Cyclestheria*), Eder (2002) and Olesen and Grygier (2003) (Spinicaudata).

In the Notostraca early works on development include Schäffer (1756) and Claus (1873). While interesting and well illustrated, these studies cannot be directly compared with recent studies using scanning electron microscopy (SEM). Fryer (1988) performed a thorough investigation on ontogeny and feeding in the Notostraca based mainly on light microscopy, but he also provided some SEM micrographs. However detailed, Fryer’s study was performed before the publication of Walossek (1993), which used SEM to describe the larval development of the Upper Cambrian *Rehbachella kinnekullensis* Müller. This, and other, accounts on microfossils from the remarkable ‘Orsten’ fauna have greatly stimulated the current debate on crustacean ontogeny and evolution (e.g. papers in Fortey and Thomas 1997; Edgecombe 1998; Martin and Davis 2001). An SEM-based description of larval development in Notostraca is therefore warranted.

We chose to investigate the early larval stages in *Triops cancriformis* (Bosc 1801–1802). We describe the external morphology and discuss the development and transition from a ‘true’ nauplius to a subadult form. Additionally, we

address questions related to trunk limb homologies in light of our findings.

Triops: taxonomic history

Although most research in the Branchiopoda was reviewed recently by Dumont and Negrea (2002), the story of the name *Triops* deserves a more penetrating account.

According to Longhurst (1955), the first officially recognized species description of *Triops cancriformis* was made by Bosc in 1801 under the name '*Apus cancriformis*'. Previous to this, the most important contribution to the knowledge of the Notostraca was by Schäffer (1756), whose excellent work even included colour drawings of several stages of the developing larva. Linné knew of Schäffer's work and quoted him in his section on the genus '*Monoculus*' (Linné 1758).

The genus name *Triops* was first used by Schrank (1803) in his work on the fauna of Bavaria. The 'Dreyauge' (English: Three-eye, Latin: *Triops*) he described was collected at the same locality near Regensburg (Germany) as Schäffer's specimens from the 1750s.

Zaddach (1841) continued the line of research by Schäffer (although he gave the wrong year of publication for Schäffer's work), and he produced detailed drawings of the larval and juvenile development. Later Grube (1853) gave an accurate review of many 'Phyllopora' and used the name '*Apus cancriformis* Schäffer' for that species.

Claus was among the first to give a detailed account of the earliest larval stages of what he called '*Apus cancriformis*' without stating the author. He has a reference to Brauer (1872), but in this work Brauer used the name '*Apus cancriformis* L.'. Claus (1873) included very precise, for the time, drawings covering the first five larval stages of '*A. cancriformis*'. Not as comprehensive, but still of value, are the works by Brauer (1872, 1877). Most notably, the 1872 work included a drawing of mating in *Triops* sp., a rarity in branchiopod literature in general and in particular in a species where males are very rare.

In a field identification key of the freshwater fauna of Germany, Keilhack (1909) used the family name Triopsidae for all extant Notostraca, taking up the name proposed by Schrank. Furthermore Keilhack used the correct (see later) names *Triops cancriformis* (Bosc) and *Lepidurus apus* (L.).

The works of Gurney (1923, 1924) contributed to the taxonomic knowledge of '*Apus cancriformis* Schäffer' and raised the question of which genus name was correct. Keilhack had already suggested that the genus name *Apus* be replaced by *Triops* Schrank since an avian genus had been described under the name *Apus* Scopoli (see Gurney 1923 for additional references). Gurney preferred the name *Apus* Schäffer and suggested that the name '... *Triops* Schrank, may be returned to the obscurity from which it was unearthed'. Linder (1947, 1952) used the genus name '*Apus*'. Based on more comprehensive material from North America, Linder (1952) expanded on his 1947 work on body rings and spi-

ralling. In addition to a thorough morphometric investigation, he included a taxonomic review of both notostracan genera.

A true landmark work was the taxonomic revision of Longhurst (1955). He also provided the clue to the problem of the correct genus name. Longhurst supported Keilhack's genus name '*Triops*' instead of '*Apus*' and provided the historical evidence in support thereof. He also provided the original author of *T. cancriformis* as *Triops cancriformis* (Bosc, 1801) with a full history of synonymy to support it.

The International Commission on Zoological Nomenclature (ICZN) followed Longhurst (1955) in their ruling on the usage and origin of the names *Apus* and *Triops* in 1958 (Hemming 1958). ICZN rejected the genus name *Apus* in favour of *Triops* Schrank (1803) (ICZN name no. 1246). They also recognized the name *Triops cancriformis* (Bosc, 1801–1802) (ICZN name no. 1476) as officially the oldest, thus following Longhurst (1955). Finally, ICZN recognized Triopsidae Keilhack (1909) (Name no. 207) as the official family name (Hemming 1958).

Materials and Methods

Dry cysts were obtained from Dr E. Eder (University of Vienna, Austria) and hatched in distilled water in aerated glass containers at ambient room temperature. Larvae were regularly inspected under a standard dissection microscope. The larvae were fixed in 2.5% glutaraldehyde or Bouin's fixative, brought through a graded alcohol series and critical-point-dried in acetone in a Bal-Tec 030 CPD. The dry specimens were mounted on SEM stubs, sputter coated with gold, and observed in JEOL JSM-840 or JEOL JSM-6335-F SEM. All images were saved and processed digitally.

Classification (following Martin and Davis 2001)

Subphylum	Crustacea Brünnich, 1772
Class	Branchiopoda Latreille, 1817
Subclass	Sarsostraca Tasch, 1969
Order	Anostraca Sars, 1867
Subclass	Phyllopora Preuss, 1951
Order	Notostraca Sars, 1867
Family	Triopsidae Keilhack, 1909
Genus	Triops Schrank, 1803
Order	Diplostraca Gerstaecker, 1866
Suborder	Laevicaudata Linder, 1945
	Spinicaudata Linder, 1945
	Cyclestherida Sars, 1899
	Cladocera Latreille, 1829

Results

Description of the larval stages

The first five larval stages (stages I–V) show the most significant morphological changes during development. Following

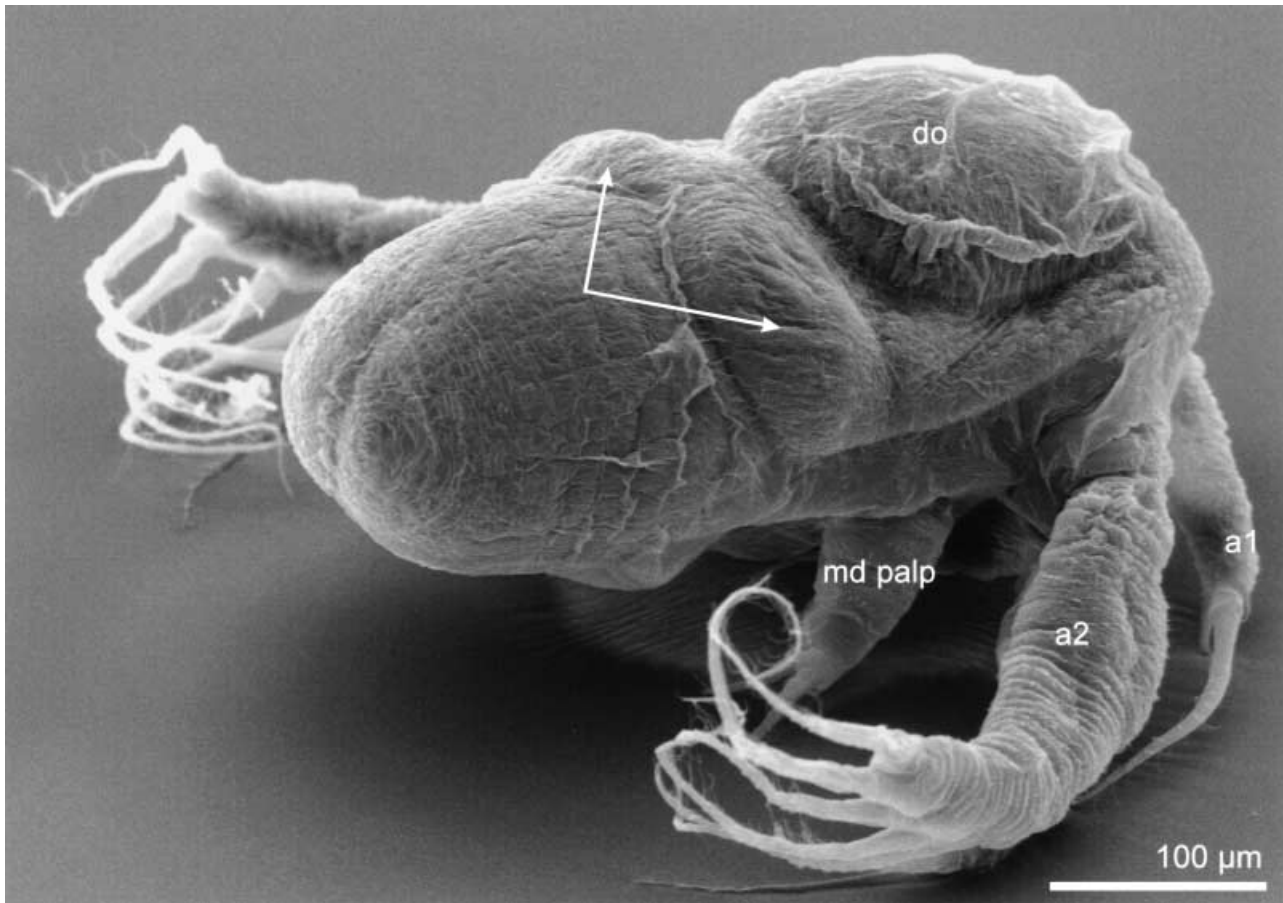


Fig. 1—*Triops cancriformis*, larval stage I, postero-lateral view of nauplius showing the postero-lateral protrusions of the carapace (arrows) and the large dorsal organ. a1, antennule; a2, antenna; do, dorsal organ; md, palp mandibular palp.

stage V there is little change between individual stages and the larvae develop rather gradually into the adult shape.

Stage I (Figs 1–3)

Defining characteristics Length approx. 450 μm . Nauplius with only the naupliar set of appendages, namely the antennule, the antenna, and the mandible. General lecithotropic appearance. Carapace present only as a pair of small humps behind the dorsal organ. Caudal end with no furcal protrusions.

Description The antennule is a tubular and uniramous appendage carrying two setae on its tip. The appendage appears multiannulated with no apparent evidence of true segmentation. The antenna is large and biramous and armed with large setae on the distal parts; the coxal endite bears a robust masticatory seta while a smaller seta arms the basis (Fig. 3B,C). The endopod bears two long setulated setae and one short and naked seta. The exopod carries five long setae along its length. These are articulated, armed with long setules and primarily used for propulsion of the larva.

The mandibular coxa is present but weakly developed, and it cannot meet its counterpart in the midline (Fig. 3A). It carries a seta on its posterior face, which in this stage is small, unsetulated and orientated almost directly posteriorly. The remaining (distal) part of the mandible (the mandibular palp) is uniramous and divided into three segments: a basis and two endopodal segments (Figs 2A and 3D). The basis and the first endopodal segments each have two medially orientated setae, whilst the second endopodal segment has three terminal setae.

The labrum is irregularly triangular with a broad base and appears thickened and without marginal setation (Fig. 3A).

No trunk limbs have developed, and no limb primordia are visible externally. Two or three weak lines can be seen on the ventral side of the trunk region; these are the precursors of the endite rows of the appendages. The larva is rounded posteriorly with two slight protrusions. These are the precursors of the furcal rami that will appear later.

The carapace extends over approximately two-thirds of the larva's length and is dominated by the circular dorsal organ (Fig. 2B,C). It consists anteriorly of a weakly developed lobe

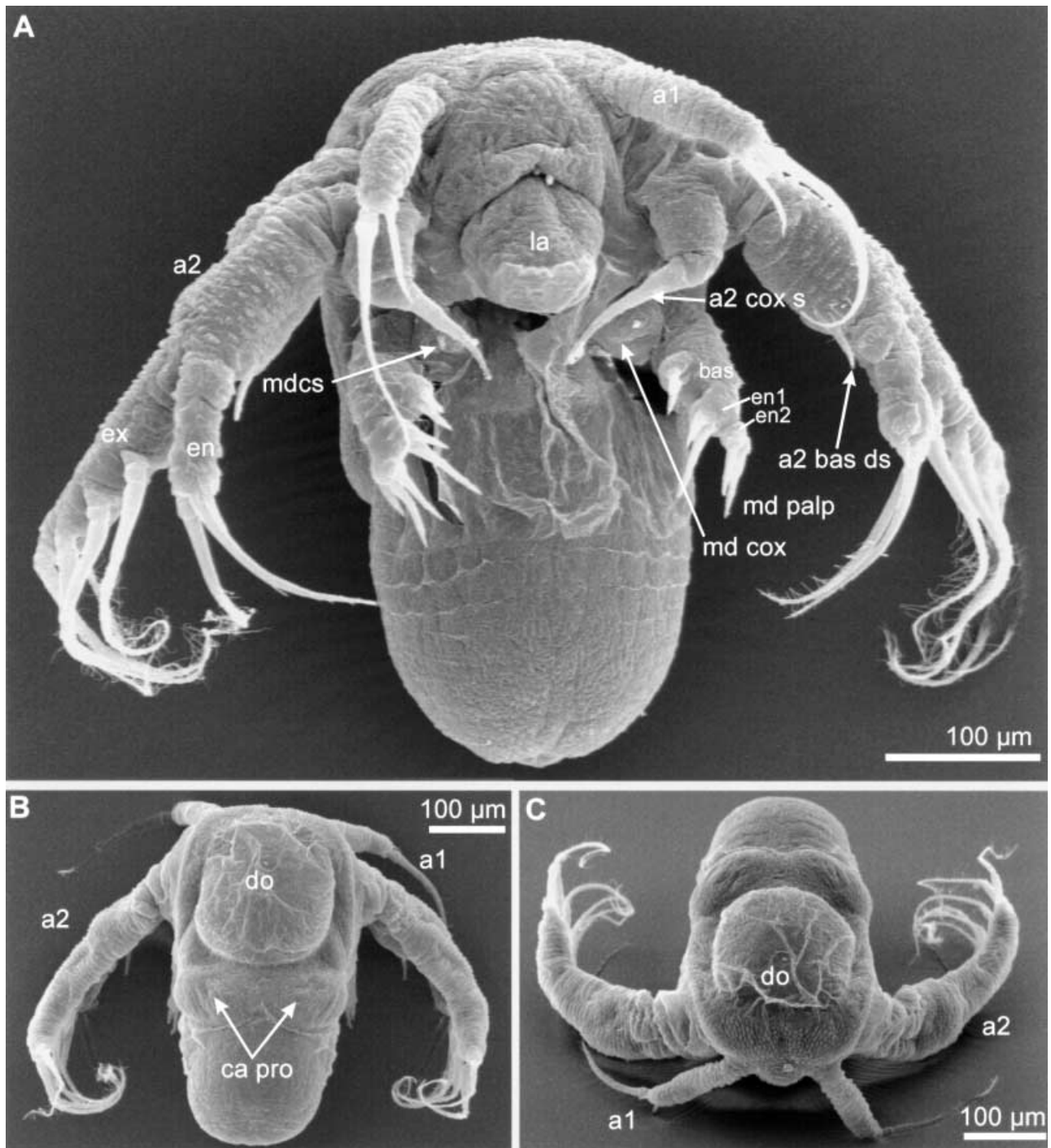


Fig. 2—*Triops cancriformis*, larval stage I. —**A**. Ventral view of nauplius larva. —**B**. Dorsal view. —**C**. Frontal, dorsal view. a1, antennule; a2, antenna; a2 bas ds, antennal basis distal seta; a2 cox s, antennal coxal endite seta; bas, basis; ca pro, carapace

protrusion; do, dorsal organ; en, endopod; en1–2, endopodal segment one and two; ex, exopod; la, labrum; md cox, mandibular coxa; mdcs, mandibular coxal seta; md palp, mandibular palp.

on each side of the huge dorsal organ and posteriorly of two dorso-lateral protrusions (or a pair of ‘humps’), immediately behind the dorsal organ (Figs 1, 3F and 9A). The lateral margins of the carapace are small, revealing the more lateral

position of the antenna limb origin relative to the median position of the mandible. Areas with cuticle of a special appearance with rhomboid shapes or ‘islands’ are found on the anterior rim (Fig. 3G). Weak lines or folds can also be

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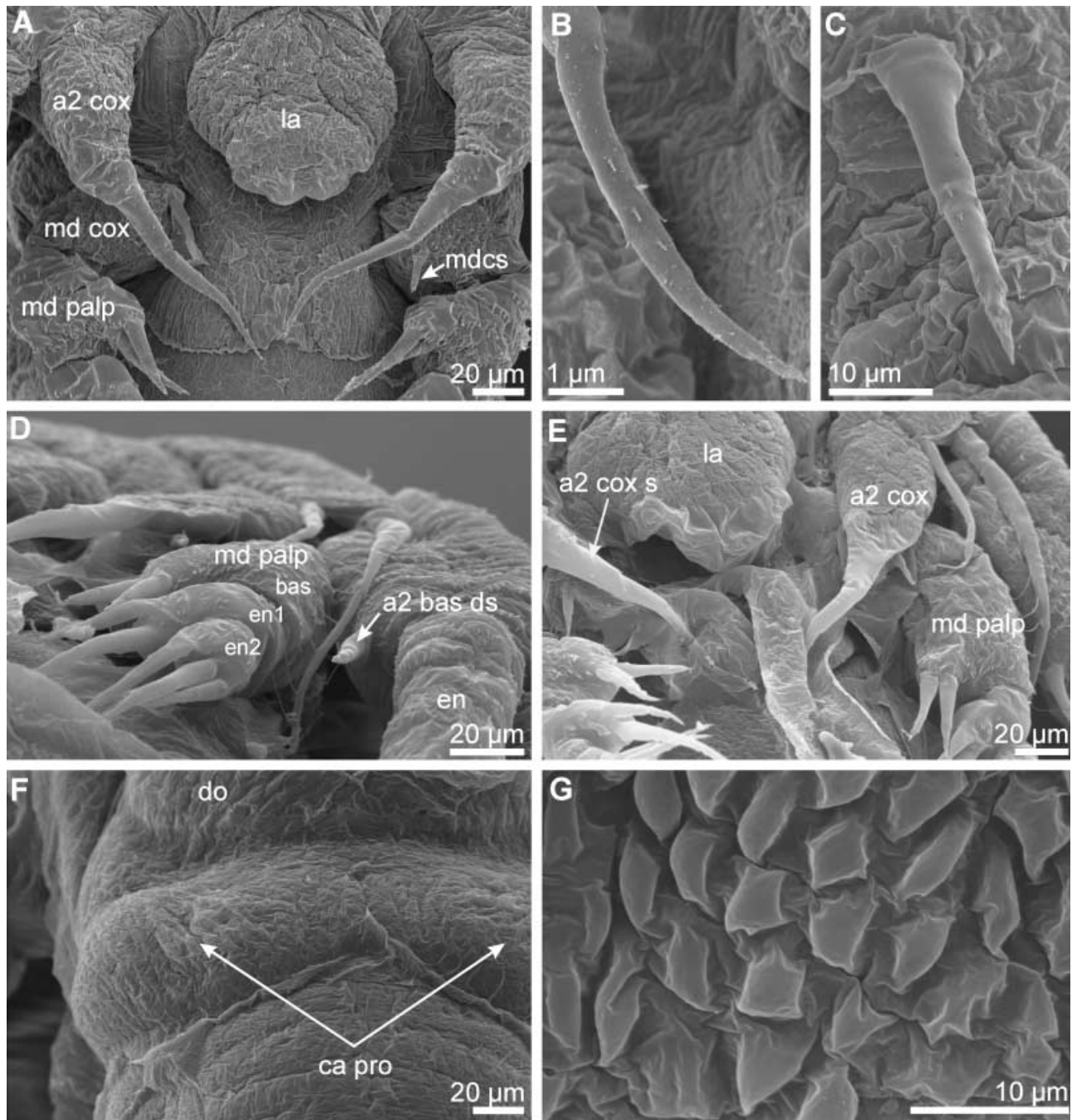


Fig. 3—*Triops cancriformis*, larval stage I. —**A**. Mouth area with nonfunctional mandibles. —**B**. Close-up of antennal coxal endite seta. —**C**. Close-up of antennal basis distal seta. —**D**. Mandibular palp and antenna, posterior view. —**E**. Mouth area of specimen with folded cuticle in ventral midline, atrium oris. —**F**. Postero-lateral protrusions of carapace. —**G**. Cuticular surface with rhomboid

shapes, from antero-dorsal edge of carapace. a2 cox, antennal coxal endite; a2 bas ds, antennal basis distal seta; a2 cox s, antennal coxal endite seta; bas, basis; ca pro, carapace protrusion; do, dorsal organ; en, endopod; en1–2, endopodal segment one and two; ex, exopod; la, labrum; md cox, mandibular coxa; mdcs, mandibular coxal seta; md palp, mandibular palp.

found on the lateral rims of the carapace. They run from a point approximately in line with the centre of the dorsal organ and curve backwards towards the posterior rim of the carapace.

Stage II (Figs 4, 5)

Defining characteristics Length 550–600 μm. Easily recognizable by the presence of two small conical furcal rami, the

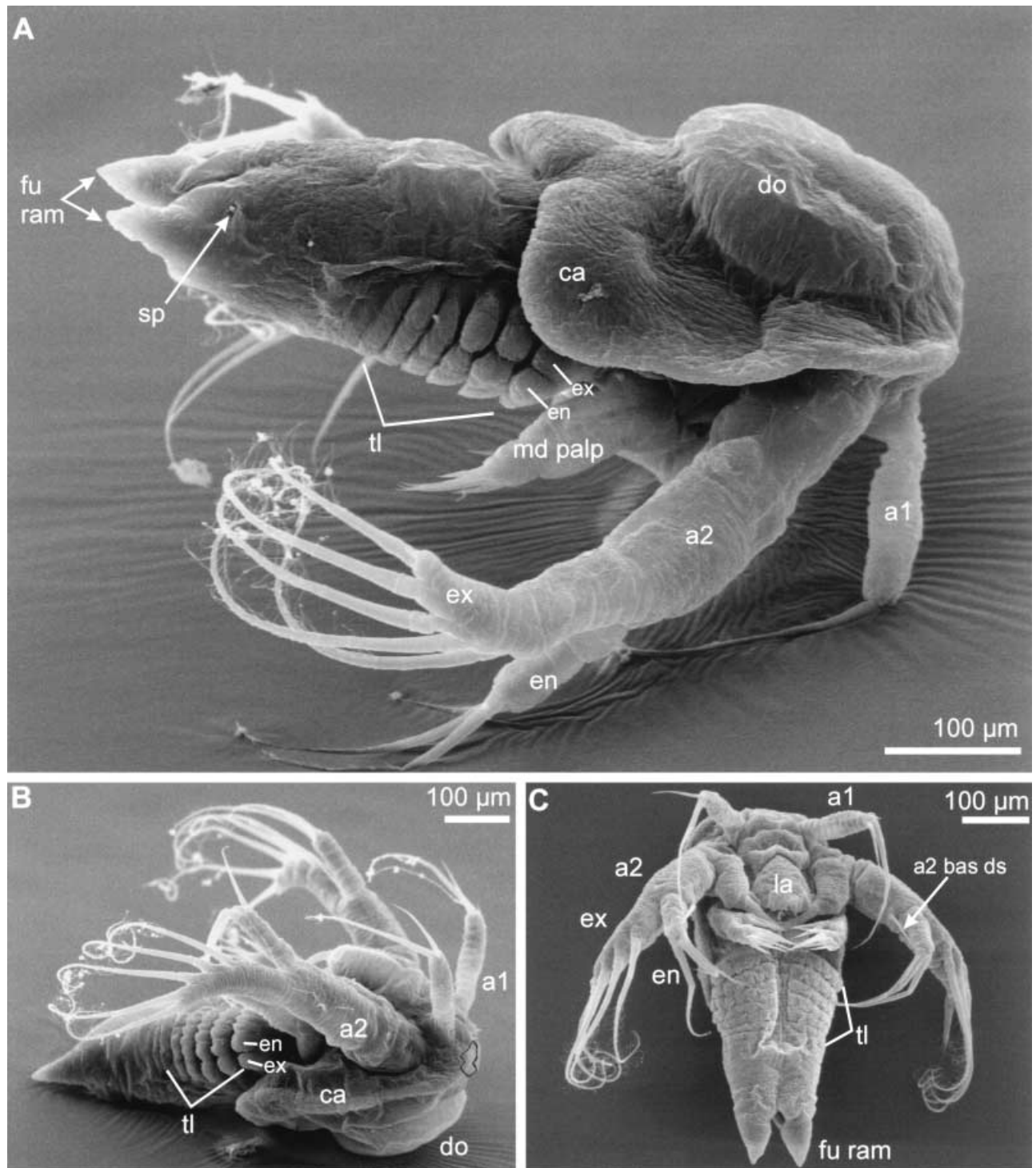


Fig. 4—*Triops cancriformis*, larval stage II. —**A.** Latero-dorsal view showing trunk limb precursors and extension of carapace. —**B.** Oblique lateral view, ventral side up. —**C.** Full ventral view. a1, antennule; a2, antenna; a2 bas ds, antennal basis

distal seta; ca pro, carapace protrusion; do, dorsal organ; en, endopod; ex, exopod; fu ram, furcal rami; la, labrum; md palp, mandibular palp; sp., setal precursor; tl, trunk limbs.

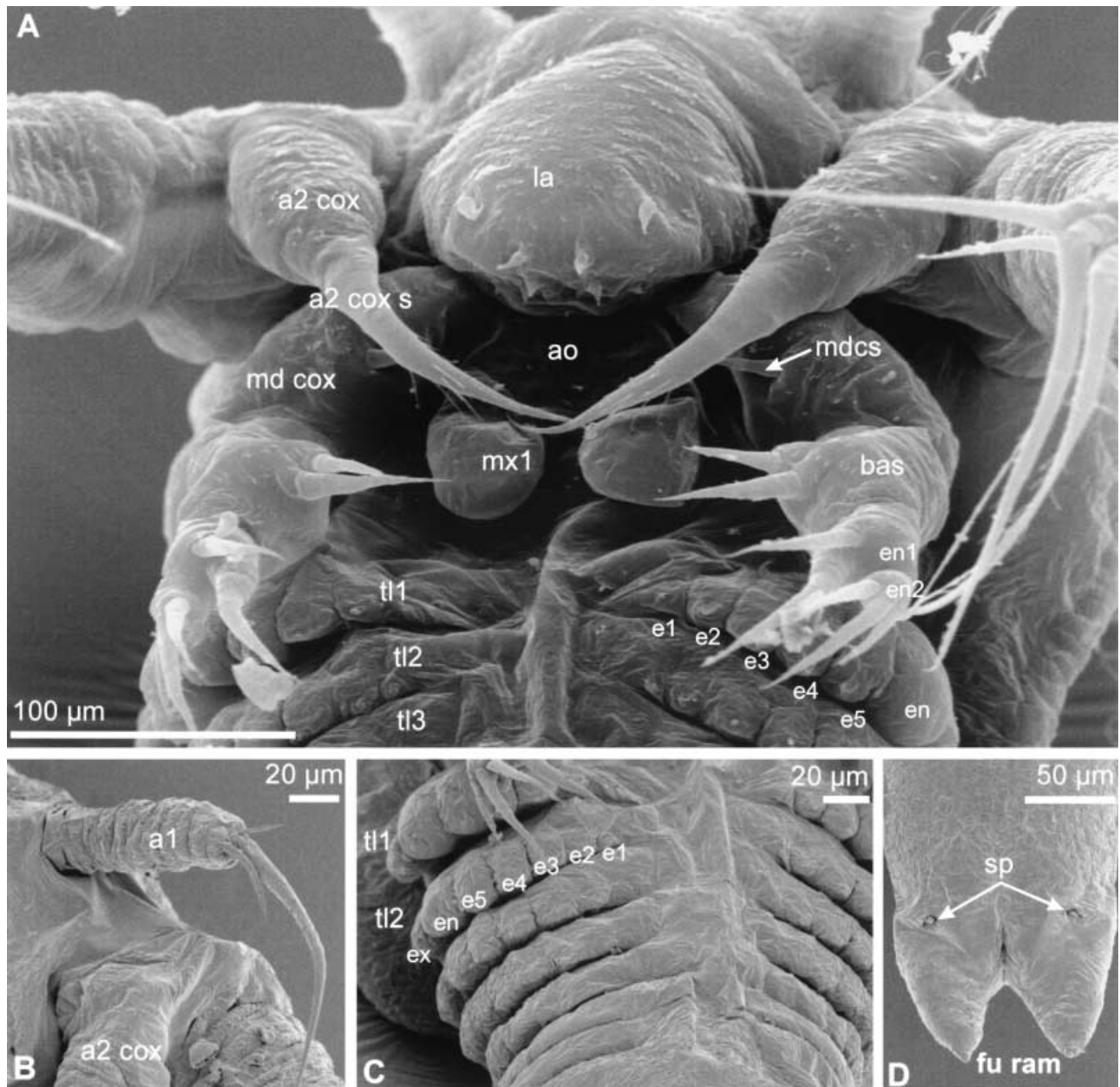


Fig. 5—*Triops cancriformis*, larval stage II. —**A.** Mouth region of larva, ventral view. —**B.** Antennule, ventral view. —**C.** Anterior trunk limb precursors showing endites, ventral view. —**D.** Telson with furcal rami, dorsal view. ao, atrium oris; a1, antennule; a2 cox, antennal coxal endite; a2 cox s, antennal coxal endite

seta; bas, basis; e1–5, endites one to five; en, endopod; en1–2, endopodal segments one and two; ex, exopod; la, labrum; md cox, mandibular coxa; mdcs, mandibular coxal seta; mx1, maxillules; sp, setal precursor; tl1–3, trunk limbs one to three.

presence of trunk limbs, the free posterior rim of the carapace and the generally more elongate shape than stage I larvae.

Description The antennules now carry three setae on their tips (Fig. 5B). The antennae have become larger and are very prominent. The endopod bears three long setulated setae. The large setae on the coxal endite seem fully functional and

the distally placed setae on the basis have become more prominent (Fig. 4C).

The mandibular coxa has enlarged and changed its orientation. The single seta on its posterior face is now larger and directed medially, bringing the coxa and its seta into a 'functional' orientation. The mandibular palp is larger and more elongate. It extends over several thoracic limb primordia

while retaining the setation pattern of the previous stage. The basis and two endopodal subdivisions are more evident at this stage.

The maxillules are now present close together in the ventral mid-line as knob-like protrusions without setae (Fig. 5A). The proximal (anterior) part of the labrum has narrowed with its distal part (posterior) now clearly covering the coxae of the mandibles. The labrum now carries a seemingly irregular number of short spines on the posterior margin continuing on the underside, i.e. the roof of the atrium oris.

On each side of the ventral midline six or seven rows of trunk limb primordia can be seen (Figs 4 and 5A,C). The anteriormost rows are the most developed and show clear lobation, heralding the onset of differentiation into all five endites and the endo- and exopods. The setae are only poorly developed and can be seen as short primordia at the tips of the lobes. A small space can be seen between the pair of incipient proximal endites, which is where the future food groove and ventral food handling space will develop. The more posterior rows of limb primordia are progressively smaller and less developed. The precise number of discernible rows can vary due to shrinkage during SEM preparation. The furcal rami are now well developed and of conical shape and give the hindmost third of the larva a generally cone-shaped appearance. A pair of small setal precursors can be seen dorsolaterally on what is probably the telson (Figs 4A and 5D).

The carapace has increased in size and the lateral and posterior margins have now become free. The posterior margin now reaches the second or third trunk limb, and the lateral margins cover the proximal parts of the antennae. The protrusions on the posterior rim have almost disappeared, and the carapace has a more adult shape with a small median sulcus. The carapace remains dominated by the large dorsal organ (Fig. 4A).

Stage III (Fig. 6)

Defining characteristics Length 800–825 µm. More cylindrical abdomen than stage I and II, trunk appendages more developed, longer furcal rami.

Description The antennules and antennae retain their features from the previous stages. The tips of the large setae on the coxal endites of the antennae are now bifid with a strong outer branch (Fig. 6B). The mandibular coxa is clearly able to meet its counterpart medially, and is covered by the labrum (Fig. 6C). The mandibular palp retains its form from stage II. The maxillules are sufficiently developed to be armed with 'teeth' or 'stout setae' to macerate food. The labrum still has a narrowing of its proximal part and a generally round shape.

The thorax carries 10–12 rows of trunk limb primordia with the most developed anteriorly. The first to third trunk limbs now have long, slender and generally tubular endites,

but the transition to the less developed broad-based, comb-like limb primordia is gradual when the rows are followed posteriorly. The tips of the endites have only simple setae, but they are larger and more numerous than in the previous stage. Ventrally, the proximal endites of the thoracic limbs are separated by 40–50 µm, and the sternal plates show signs of the food groove (Fig. 6D).

The telsonic region has an almost completely cylindrical shape with a furrow in the ventral midline. The furcal rami are slender and tubular, ending with a seta approximately one-quarter of the total body length (Fig. 9F). The paired setae on the dorsal side of the telson are now readily visible (not shown).

Posteriorly the carapace reaches the fourth or fifth trunk limb. The anterior margin reaches well beyond the antennules along the anteroposterior axis of the larva. The lateral margins cover the coxal endite of the antennae, but the primary growth of the carapace seems to be posteriorly orientated. The median sulcus is present posteriorly as well as a short carina, and no traces of the posterior protrusions remain. The dorsal organ is still prominent but is now relatively smaller.

Stage IV (Fig. 7)

Defining characteristics Significantly longer furcal rami, and more developed anterior trunk limbs with long and distinct endites.

Description The antennules and antennae have approximately the same size as in stage III, which make them proportionally smaller relative to the overall larval length. Both the distal and proximal parts of the antennae more or less retain their appearance. The outer branch of the bifid seta on the antennal coxal endite is now thinner (Fig. 7C).

The coxae of the mandibles have attained adult shape, bringing the palps further apart. The mandibular palp has begun to atrophy (Fig. 7B,D). A pair of paragnaths is discernible, though small. The maxillules are still not of adult shape but have larger setae. The proximal part of the labrum is similar to the previous stage but the lateral and posterior edge has a somewhat thickened appearance.

The number of visible trunk limbs has increased to 15–16, including well-developed limbs anteriorly and rows of limb buds posteriorly. The first to fifth trunk limbs are the most developed, giving this region of the trunk a subadult, layered appearance (Fig. 7E). A limb from this region has a well-developed basis, and all the endites have reached a semitubular form with the endopods and exopods clearly visible and armed with setae. The setal armature is now well-developed on all endites of the first to fifth trunk limbs. The remaining trunk limbs display the characteristic primordial crest-like appearance as in previous stages.

The telson has a clear furrow continuing the food groove of the trunk segments in the ventral midline. Several spines

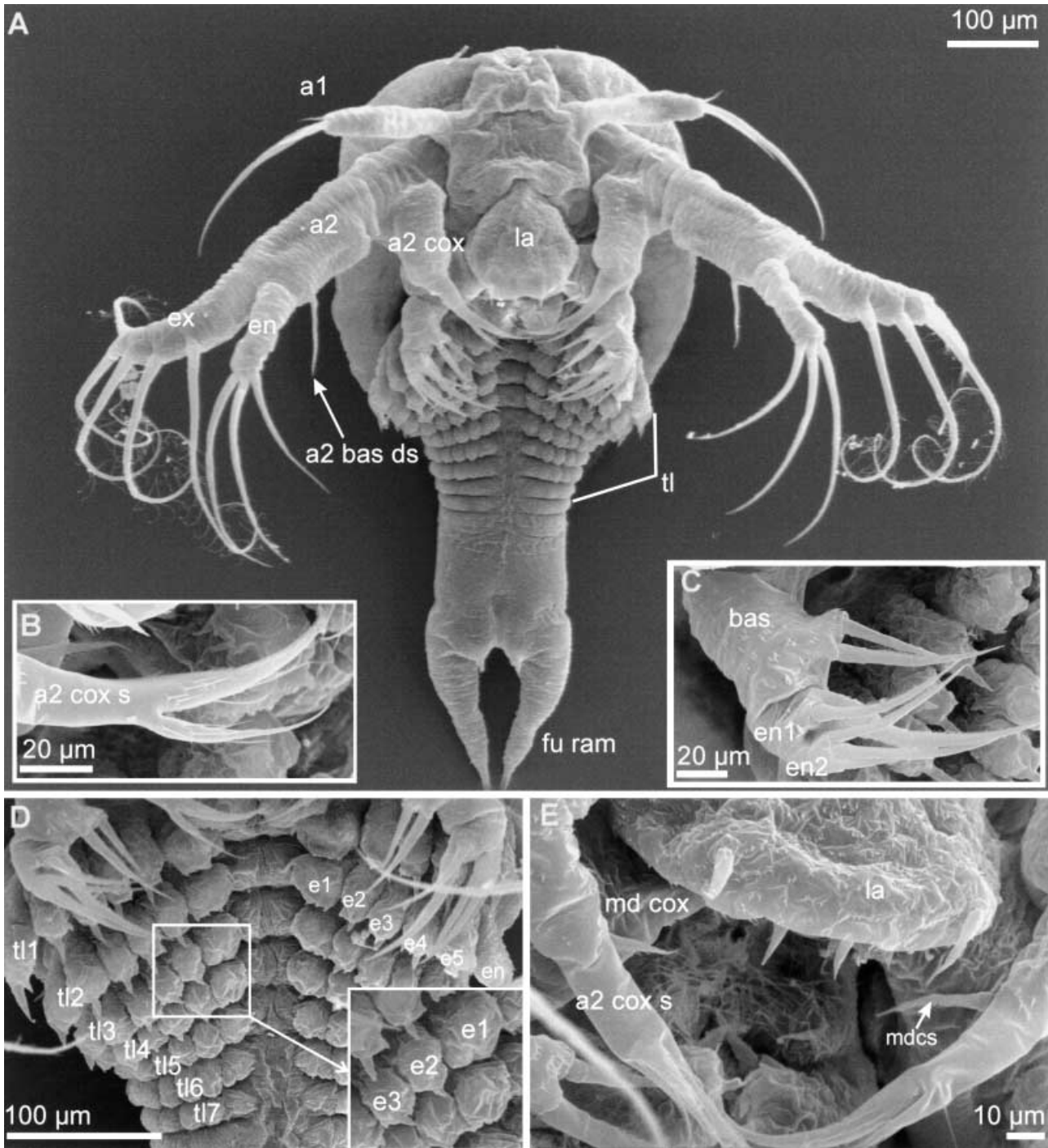


Fig. 6—*Triops cancriformis*, larval stage III. —**A**. Ventral view. —**B**. Tip of antennal coxal endite seta. —**C**. Detail of mandibular palp, ventral view. —**D**. Anterior trunk limbs, ventral view. —**E**. Detail of atrium oris underneath labrum, ventral view. a1, antennule; a2, antenna; a2 cox, antennal coxal endite;

a2 bas ds, antennal basis distal seta; a2 cox s, antennal coxal endite seta; e1–5, endites one to five; en, endopod; en1–2, endopodal segment one and two; ex, exopod; fu ram, furcal rami; la, labrum; md cox, mandibular coxa; mdcs, mandibular coxal seta; tl1–7, trunk limbs one to seven.

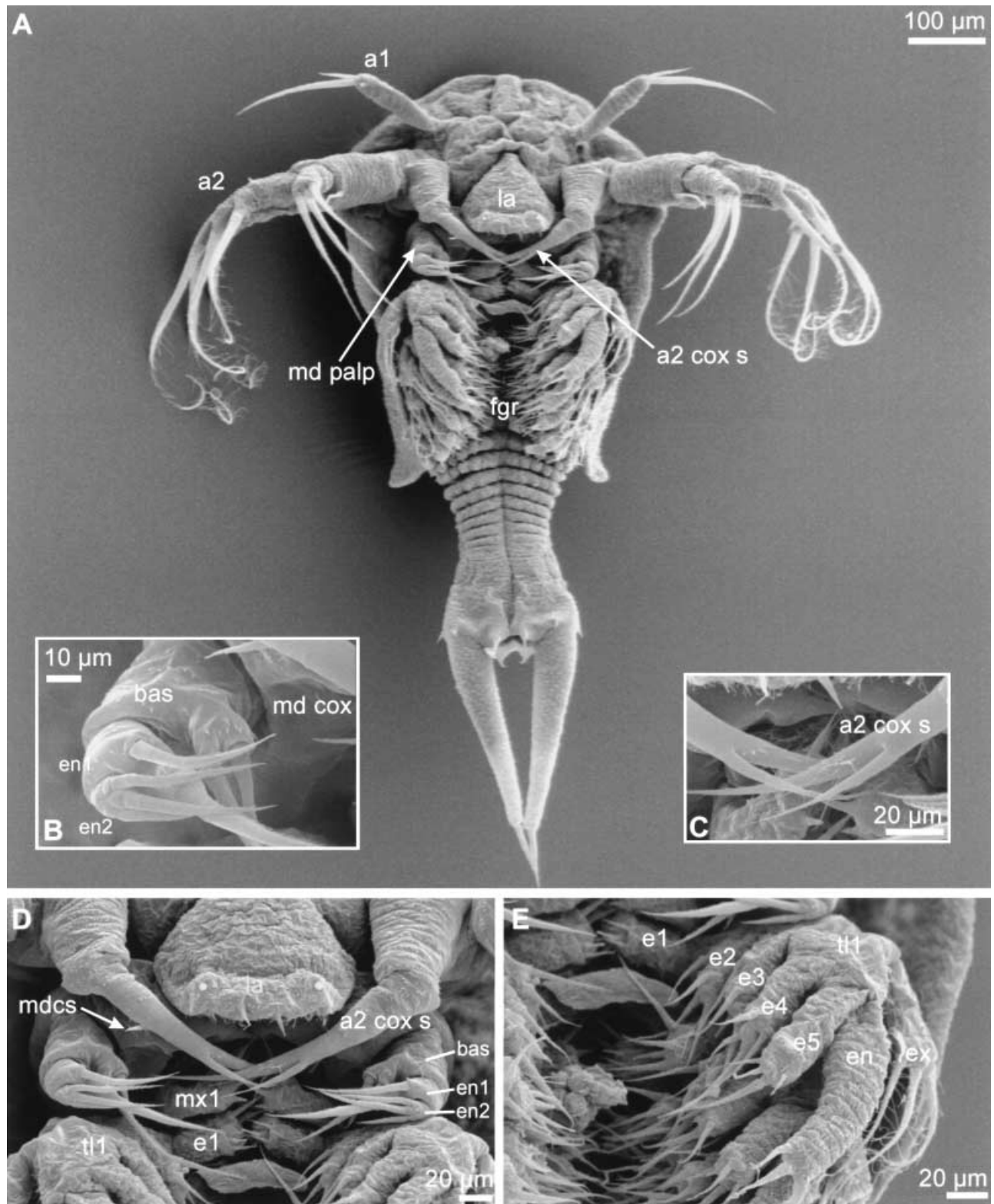


Fig. 7—*Triops cancriformis*, larval stage IV. —**A**. Ventral view. —**B**. Detail of mandibular palp. —**C**. Distal tip of antennal coxal endite seta. —**D**. Mouth region, ventral view. —**E**. Anterior trunk limbs, left side, ventral view. a1, antennule; a2, antenna; a2 cox s, antennal

coxal endite seta; bas, basis; e1–5, endites one to five; en, endopod; en1–2, endopodal segment one and two; ex, exopod; la, labrum; md cox, mandibular coxa; mdcs, mandibular coxal seta; md palp, mandibular palp; mx1, maxillules; tl1, trunk limb one.

are now present at the bases of the furcal rami and on the ventral side of the anus. The furcal rami end in well-defined setae (this is not so evident in the previous stages). The carapace now covers eight or nine trunk limbs. The anterior margin continues its extension beyond the antennules in an antero-ventral flange.

Stage V (Fig. 8)

Defining characteristics Stage V is characterized by long furcal rami, almost as long as the trunk, a reduced mandibular palp, and anterior trunk limbs with an adult upright ‘flattened’ appearance of the endites.

Description The antennules and antennae are still prominent, though their size relative to the overall length of the larva is further reduced. The antennules have moved closer to the antennae on the anteroposterior axis and retain three setae distally. The coxal endite of the antenna is still large enough to place the prominent seta just in front of the labrum. No sign of the former bifurcation of this seta remains (Fig. 8A,C).

The coxae of the mandibles are large. The seta on the posterior face of the coxa is reduced in size and is further away from the mouth region due to the continued growth of the coxa. The mandibular palps are very reduced and further apart, and appear nonfunctional (Fig. 8D). The paragnaths are easily recognizable, and the maxillules are almost identical to the proximal endites of the trunk limbs (Fig. 8A,D).

Seventeen to eighteen pairs of trunk limbs are visible, and the anterior seven or eight have well-developed endites and setae. As in the previous stage the exopods of the trunk limbs in this region are large and well-developed. The fourth and fifth endites of the first and second trunk limbs are still ‘juvenile-like’ and have not yet elongated into the adult shape. Thus the endopod is the largest of the endites at this stage (Fig. 8E). The endites of the remaining five to six free trunk limbs are still mostly tubular and thus subadult in appearance. Posterior to the eighth or ninth trunk segment, the trunk limbs are still typically rows of limb primordia showing progressively less differentiation into endites.

From the eleventh and twelfth trunk segments and continuing backwards, the trunk limbs make up an increasingly small part of the ventral surface of the larva. This moves the delimitation of the most laterally placed limb primordia (i.e. the epipod precursor) from a point high on the dorsolateral side (in the first to seventh trunk limb) to a point on the ventral side in the 17th to 18th trunk limb [not shown on this stage; see Fig. 10(D) for the subadult condition]. This is also the case in the previous stages but the pattern only becomes clear in this stage as the development has progressed sufficiently.

While the telson looks almost identical to that of the previous stage, the furcal rami have increased their length up to one-third of the whole length of the larva. The carapace

extends posteriorly to the ninth or tenth trunk limb. Laterally the carapace almost reaches the distal seta on the basis of the antennae, and the development of the antero-ventral flange is greater.

Discussion

This study describes the first five larval stages of *Triops cancriformis*. Claus (1873) described similar stages using light microscopy, but this study is the first to investigate the development using SEM. We discuss the ontogeny of head appendages, the ontogeny and morphology of the feeding mechanism, and finally some issues of limb homologization patterns.

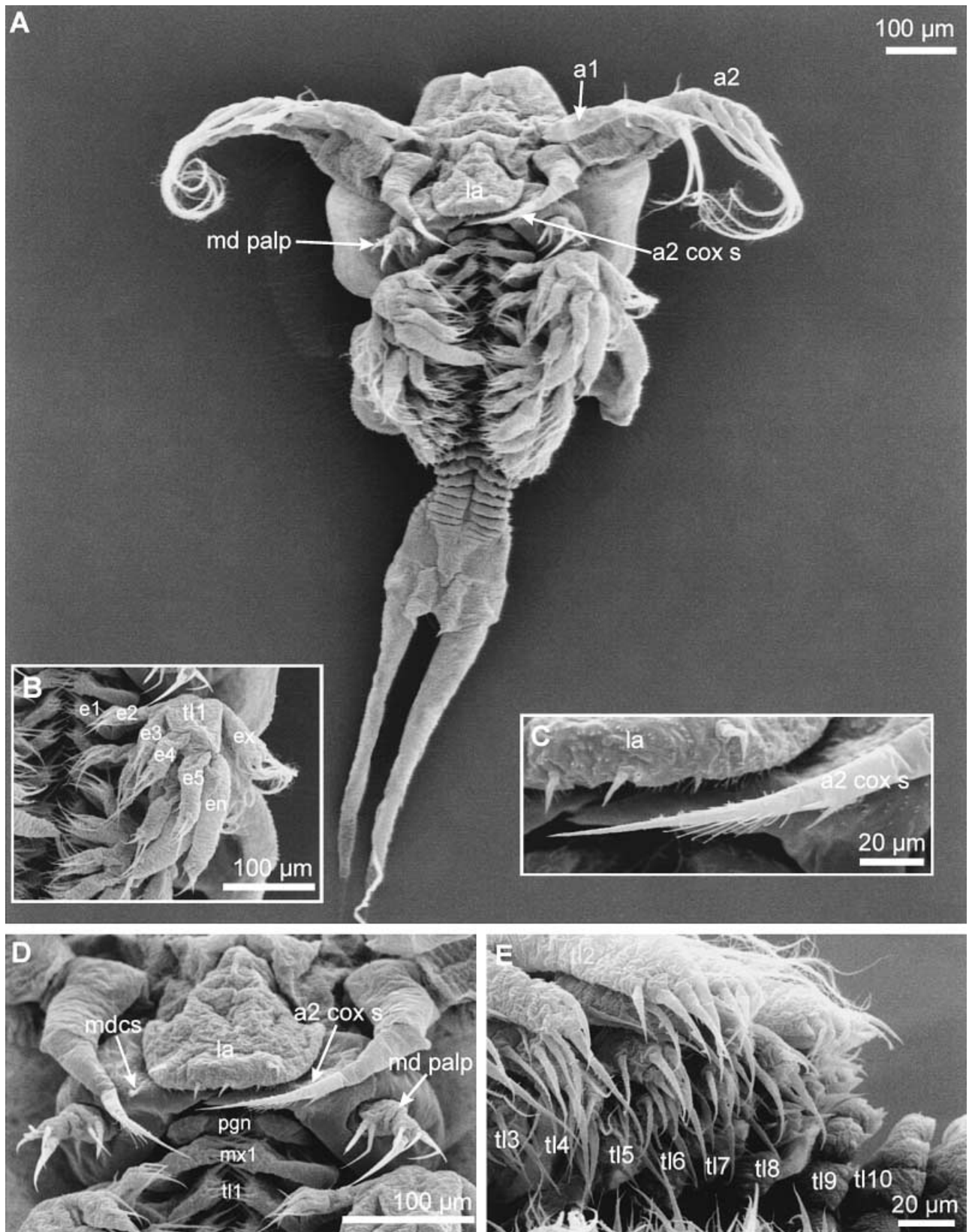
Head appendages and carapace: ontogeny and homologization pattern

Notostracans have a very accelerated development compared to anostracans and the ‘Orsten’ fossil *Rehbachella kinnekullensis* (Walossek 1993). As shown in this study, *Triops cancriformis* employs only five stages to develop seven to eight well-developed trunk limbs while anostracans and *R. kinnekullensis* employ more than twice as many stages.

Adult Notostraca have strongly reduced antennules and antennae, but it is clear from the ontogeny, that these appendages start out as ‘normal’ naupliar appendages in *T. cancriformis*. This is the pattern for both the Notostraca and the Anostraca, while the antennules of ‘conchostracan’ nauplii differ somewhat in being reduced to small buds (Spinicaudata and Cyclotherida) or to unique, laterally placed, unjointed branches (Laevicaudata) (Olesen in press). In *T. cancriformis*, both the antennules and antennae are reduced during the late larval development, and their functions are gradually transferred to the trunk limbs. For a period of time, swimming is accomplished with the mixed propulsion of the still-not-fully developed trunk limbs and the still-not-reduced antennae. As this occurs, the animal also shifts from being an active swimmer to being more sedentary, with frequent rests on the bottom (Fryer 1988 and our unpublished observations).

The antennules differ from the posterior naupliar appendages in being unbranched and annulated rather than truly segmented. The antennae are large, well-developed and biramous, while the mandibles are uniramous and much smaller. Both pairs have a specialized coxa, i.e. the coxa of the antenna carries an enlarged enditic process with a large spine/seta, and the mandibular coxa is very large and develops a medial cutting/grinding edge in the later stages. The endopod of the antenna is not clearly segmented, while the mandibular palp comprises three segments: basis and two endopodal segments.

Based on investigations of the spinicaudate *Limmadia stanleyana* King and the anostracan *Artemia salina* L., Anderson (1967) interpreted the mandibular palp as consisting of



the distal protopod and the remnants of both an exo- and endopod. This interpretation was used again by Schrehardt (1987) for *Artemia salina*, but several facts militate against this view. Anderson's suggestion that the mandibular endopod is represented by one or two setae is not explained morphologically. Olesen and Grygier (2003), left the homologies unresolved, but based on Olesen (in press), we suggest the following homology pattern: The most proximal and enlarged segment is the coxa, which carries the macerating surfaces medially. Next comes the basis, which bears two medially orientated setae. The two distal segments we interpret as endopodal segments carrying two and three setae, respectively. No remnant of the exopod is found (Figs 3A,D, 5A, 6C and 7B,D). The mandibular palp is most prominent in stages II and III, and signs of its reduction can be seen in stage IV.

The maxillules appear in stage II. Based on the stages documented here, it is evident that they are represented only by the most proximal part of the appendage, the proximal endite, as suggested by Cannon (1933). The maxillae appear after stage V (not shown). The reduction of maxillae is apparently species-specific to *T. cancriformis* and not a general trait for the Notostraca (see Longhurst 1955). A set of paragnaths is present in stage IV, and by stage V they are almost as large as the proximal endites of the first trunk limbs. The large setae on the coxal endites of the antennae are most likely still able to take part in the food handling in stage IV and V. The mandibular palp is present and most probably active in stage IV. It is evident that its reduction has started in stage V, and the growth of the mandibular coxae move the palps away from the active food handling area.

The early development of the posterior part of the carapace – as a pair of 'humps' immediately behind the dorsal organ – is similar to that of *Cyclotheria hislopi* Baird, and various cladocerans (see Olesen 1998, 1999; Olesen *et al.* 2003). This indicates that at least the posterior part of the carapace in the Notostraca is homologous to the different types of more or less bivalved carapaces seen in the Diplostrostraca (the latter termed 'secondary shield' by Walossek 1993). This similarity furthermore suggests that the carapace in the Notostraca has been evolutionarily derived from a carapace more distinctly bivalved than the one found in recent notostracans. The ultimate (adult) shape of the carapace is illustrated by the subadult specimen in Fig. 9(D).

Larval feeding – general

Development starting with a classical orthonauplius is considered the most plesiomorphic in Crustacea (= Eucrustacea *sensu* Walossek 1999) (Gruner 1993; Walossek 1993; Dahms

2000). In all taxa the antennules in this larval form differ in morphology from the more posterior appendages in being uniramous. Within the Copepoda and the Cirripedia the naupliar antennae and mandibles have almost identical morphologies (see Walossek 1993). The situation in *Triops*, where the mandibles and antennae differ from each other already in first stage (ortho)nauplii, is therefore unusual, and indicates a functional specialization between the postantennular limbs very early in development.

Only limited research has been carried out on the feeding mechanism of nauplii. Gauld (1959) investigated these larvae in several taxa (Anostraca, Cirripedia, Copepoda and others) while Rainbow and Walker (1976) and Moyses (1984) concentrated on cirripede nauplii. The feeding mechanism of anostracan nauplii is relatively well-studied because of the works of Barlow and Sleight (1980) and Fryer (1983). According to Fryer (1988), many principles of the feeding mechanism in anostracan larvae can be transferred to notostracan larvae.

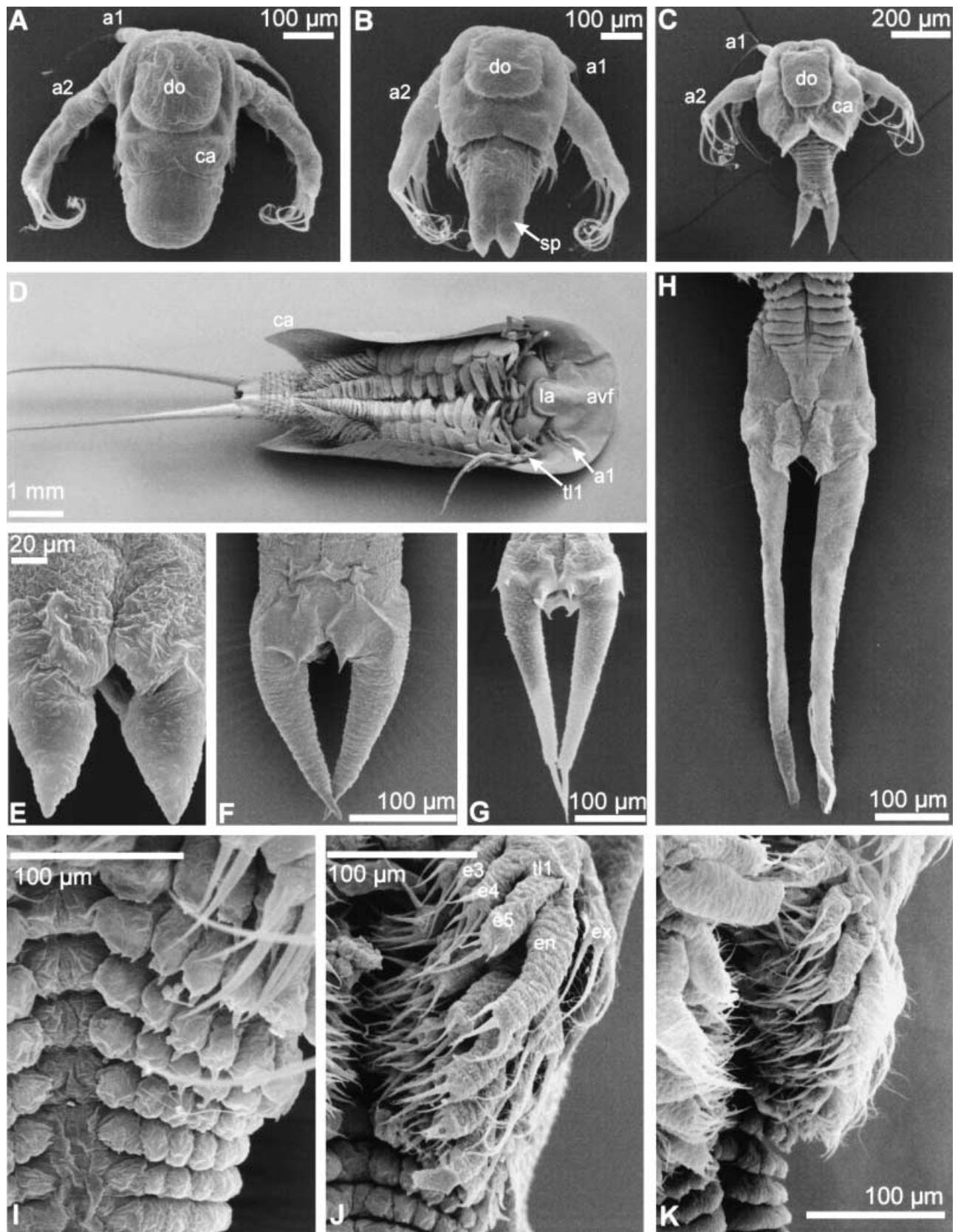
Walossek (1993) suggested that the larvae of Upper Cambrian Crustacea, like *Rehbachella kinnekullensis* and *Bredocaris admirabilis* were feeding in a manner similar to that of lepadomorph barnacles (see Moyses 1984, 1987). In extant crustacean larva it is very common that the earliest nauplius stage is nonfeeding. This is also the case in extant Branchiopoda, although not in *Rehbachella kinnekullensis* (see Walossek 1993). The naupliar phase of cirripedes and copepods often has several instars ending with a (more or less) complete metamorphosis. Dahms (1992) described the complete metamorphosis from nauplius to copepodid stages in some harpacticoid copepods, while several authors have described the nauplius-to-cypris metamorphosis in the Cirripedia (Nott and Foster 1969; Walley 1969). In these examples, naupliar feeding comes to an abrupt end. In the Copepoda the naupliar feeding is replaced by the copepodid feeding mechanism, which is very different and resembles the adult mode. In the Cirripedia the naupliar feeding mechanism is replaced by the adult filter-feeding mechanism with an intermediate nonfeeding cypris stage.

Feeding mechanism: ontogeny and morphological transition

In the Notostraca and Anostraca, we find a gradual transition from a feeding mechanism based on naupliar appendages to an adult-like mechanism with highly specialized components including mouth parts and trunk limbs. Our SEM studies document many of the observations on ontogeny made by Fryer (1988) [e.g. compare Figs 105 and 106 in Fryer (1988) with our Figs 5 and 6]. The stage I larva (in *T. cancriformis*) is most likely nonfeeding. The small

Fig. 8—*Triops cancriformis*, larval stage V. —A. Ventral view. —B. Anterior trunk limbs, left side, ventral view. —C. Detail of antennal coxal endite seta. —D. Mouth region, ventral view. —E. Developed to nondeveloped trunk limb transition zone, left side. a1, antennule;

a2, antenna; a2 cox s, antennal coxal endite seta; e1–5, endites one to five; en, endopod; ex, exopod; la, labrum; mdcs, mandibular coxal seta; md palp, mandibular palp; mx1, maxillules; pgn, paragnaths; t11–10, trunk limbs one to 10.



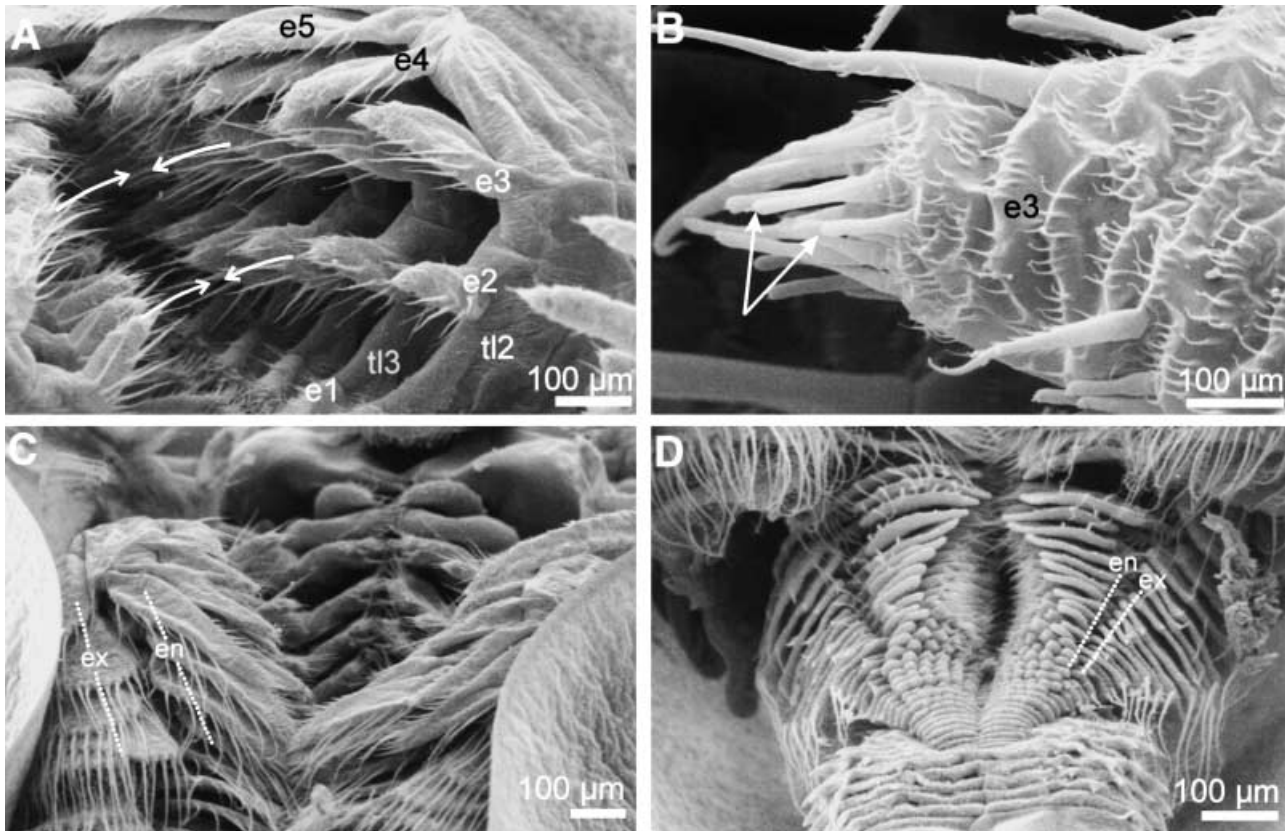


Fig. 10—*Triops cancriformis*, subadult specimen. —**A**. Distal endites of anterior trunk limbs in food handling space, oblique view in posterior direction. Arrows indicate possible path of movement. —**B**. Medially orientated tip of endite 3, trunk limb 2. Arrows

indicate possible chemosensory setae. —**C**. Distal endites of trunk limbs, ventral face. —**D**. Posterior trunk limbs, caudal view. en, endopod; e1–5, endites one to five; ex, exopod; tl2–3, trunk limbs two and three.

setae on the posterior face of the mandible point posteriorly, indicating that they are not yet active. When functional, these setae point medially towards the mouth. The stage II larva shows precursors of maxillules and has a small seta on the posterior face of the mandible; the stage III larvae have a bifid spine/seta on the enlarged coxal endite of the antenna. In addition, we show that the functional group of trunk limbs consists of five to seven limbs in stage IV and eight to 10 limbs in stage V.

When comparing the trunk limb morphology of the (late) larval stages with the subadult, some clear patterns emerge. The food handling space between the medially orientated endites of the trunk limbs arise during ontogeny as a direct consequence of the ventral growth of the outer margin of all

trunk limb bases (see, e.g. Figs 9J,K and 10). The first pair of trunk limbs does not follow this pattern.

The rapid growth brings the distal tip (i.e. the endopod) of the limbs from a lateral position (as seen in stage III) to a fully ventral position (as seen in stage V). Thus the proximal/distal axis of the limbs starts in the plane of the anteroposterior axis (long axis) of the larva and ends up being almost perpendicular to it (in plane with the dorsoventral or short axis). The basis enlarges and forms what is sometimes referred to as the ‘limb corm.’ Most significantly, there is an enlargement of the outer margin of the limbs, which effectively causes the endites to become directed toward the ventral mid-line of the animal forming the upper part of the characteristic median food handling space (probably equivalent to what

Fig. 9—*Triops cancriformis*, ontogeny of selected features.

—**A**. Larval stage I, dorsal view. —**B**. Larval stage II, dorsal view. —**C**. Larval stage III, dorsal view. —**D**. Sub-adult specimen. —**E**. Furcal rami, larval stage II, ventral view. —**F**. Furcal rami, larval stage III, ventral view. —**G**. Furcal rami, larval stage IV, ventral view. —**H**. Furcal rami, larval stage V, ventral view.

—**I**. Anterior trunk limbs, left side, larval stage III. —**J**. Anterior trunk limbs, left side, larval stage IV. —**K**. Anterior trunk limbs, left side, larval stage V. a1, antennule; a2, antenna; avf, anteroventral flange; ca, carapace; c, pro, do, dorsal organ; e1–5, endites one to five; en, endopod; ex, exopod; la, labrum; sp, setal precursor; tl1, trunk limb one.

was termed ‘food cage’ by Fryer 1988) (Figs 6, 7, 8 and 9J,K). This morphology is first seen in stage IV larva and in stage V the food handling space is clearly enclosed by the trunk limbs. In stage IV this space is formed by a functional group made up of the first five to seven trunk limbs, all having well-developed endites and exites (Fig. 9J). In stage V, eight to 10 trunk limbs are involved (Fig. 9K). This functional group gives the typical ‘layered’ appearance of the adult trunk limbs where the larger anterior limbs overlap their smaller posterior neighbours.

The food handling space is clearly subdivided into different compartments: (1) a proximal portion that is laterally enclosed by the proximal endites (gnathobases) of the trunk limbs and dorsally in the mid-line by the ridged cuticle of the sternum, and (2) an upper or distal portion which is laterally enclosed by endites two to five and ventrally (or distally) by the prominent endopods. In the subadult, the food handling space becomes subdivided into several ‘channels’ in different planes, when the median sides of the limbs oppose each other in the midline (Fig. 10A). The distal space is widest anteriorly and narrows posteriorly with the progressively smaller trunk limbs (Fig. 10A,C,D). The proximal food handling space, i.e. between the proximal endites (gnathobases), remains more constant along the trunk. The endites of the anterior trunk limbs are clearly large enough to have mechanical functions, and their tips are equipped with a group of setae likely to have chemosensory capabilities, as suggested by Rieder (1978) (Fig. 10B). The independently controllable proximal endite (gnathobase) is required to attain the functional separation between the proximal and distal food handling space. This separation is very clear in the highly specialized first pair of trunk limbs. The distal endites (endites four and five) are long, filiform and used as long-range sensors emulating the function of the antennae. The proximal endite remains active in the food manipulation process in the proximal food handling space. These two mechanisms can work either separately or together according to the situation, emphasizing the versatility of the phyllopod limb type. The meta-chronal limb movement along the trunk makes it difficult to understand the fine details of the situation in the live animal. This emphasizes that SEM micrographs alone cannot provide an exhaustive account of such complex patterns of movement.

Cannon (1933) offered the first detailed study of feeding in the (adult) Notostraca. According to him, smaller food particles are passed forward to the mouth by the proximal endites (gnathobases) of the trunk limbs. Larger pieces of food are held by the distal endites and brought to the mouth parts for processing, but it is not quite clear from his account what role (if any) the proximal endites play in final ingestion of such food items (Cannon 1933). Cannon’s interpretation is supported by Fryer (1988) who supplied SEM documentation of the adult feeding structures in *Lepidurus apus* and a description of the ontogeny of feeding.

Trunk limb ontogeny and homology

The notostracan trunk limb ontogeny as exemplified by *Triops cancriformis* is similar to many of the other ‘large branchiopods’ (Olesen 1999; Olesen *et al.* 2001). The typical pattern of elongated rows of ventrally facing limb primordia was suggested as a synapomorphy for the Branchiopoda by Olesen (1999), but it has recently been noted that the Devonian *Lepidocaris rhyniensis* Scourfield is a significant exception to this pattern (Schram and Koenemann 2001; Olesen in press).

The notostracan trunk limb condition with five endites plus an endo-, exo-, and epipod was (structurally) recognized in adults of *T. cancriformis* by Schäffer (1756) and in the developing larvae of the same species by Claus (1873). Williams and Müller (1996) showed, using phalloidin-staining and light microscopy, that the eight subdivisions of the limb are already evident in the first larval stage of *T. longicaudatus*. Williams (1998) investigated *Distalless*-gene expression in a *Triops* sp. and found *dLL*-expression in the endo- and exopod precursors in the earliest larvae. Investigating *Cyclestheria hislopi* (Cyclestherida) using similar methods, Olesen *et al.* (2001) found a similar pattern. The earliest expression of *dLL* is in the distal tip of the trunk limbs and only later in the proximal part. In *T. cancriformis* it is also evident that the first externally visible subdivision of the trunk limb is the endopod/exopod division taking place at the distal end of the basis.

Borradaile (1926) divided the notostracan limb into six endites, an exopod, and epipod, thus arriving at eight ‘lobes’ in all. From his drawings, it is clear that what is considered the ‘apical lobe’ (or the sixth endite) by Borradaile (1926; Figs 17 and 18) is now interpreted as the endopod. This was proposed by Linder (1952; Fig. 26) and is accepted in many recent works (e.g. Walossek 1993). What Borradaile (1926), Linder (1952), and Longhurst (1955) did not explain was the limb structure from which the endites, exopod and epipods actually originate. As a result of a somewhat vague explanation of the ‘protopod’ concept, one way of interpreting Borradaile (1926) is that this structure is the proximal part of the endites themselves. But this explanation is not sufficient to explain the limb ontogeny documented here.

Fryer (1988) described the ‘protopod’ structure of the first trunk limb in a notostracan as follows: the distal part is the ‘limb corm’ and the proximal part is ‘a basal endite’ with a gnathobase. Only endites, endo-, exo- and epipods are identified, while no structural homologizations were given for the ‘protopod’. Detailed ideas about the homologies of the various parts of the trunk limbs of the Notostraca to those of other Crustacea were advocated by Hansen (1925). He suggested that the median part of the notostracan (*Lepidurus*) trunk limb – the part we refer to as a basis with five endites and an unsegmented endopod – is composed of a *precoxa*, a *coxa*, a *basis* (together forming a ‘sympod’), and three endopodal segments. In contrast to this idea, our interpretation of

the extant notostracan appendage is this (following Walossek 1993). The trunk limb consists of a proximal endite, an enlarged basis lobed into endites and carrying the endo- and exopod distally and the epipod more proximo-laterally. Thus the ‘basal endite’ or gnathobase of Fryer (1988) is the proximal endite, while the ‘limb corm’ is the enlarged basis. The first pair of trunk limbs in an adult notostracan has a highly specialized morphology and this does not simplify the identification of homologous structures.

According to Walossek (1993) and Walossek and Müller (1997) the ‘proximal endite’ of the trunk limbs of the Branchiopoda (including the Upper Cambrian *Rehbachella*) is in its plesiomorphic condition while it has ‘spread across’ the limb and forms a separate and articulating segment (the coxa) in, for example, the Malacostraca. Based on comprehensive work on Cambrian crustacean-like fossils, Walossek and Müller (1990) and Walossek (1993) concluded that the presence of a distinct ‘proximal endite’ goes far back in the evolution of Crustacea (present in stemgroup Crustacea), and that its transformation into a coxa, in at least the antennae and mandibles, was a novelty (synapomorphy) for the Crustacea (= Eucrustacea *sensu* Walossek 1999). Hence, the distinct proximal endites of the trunk limbs in *Triops cancriformis*, and other branchiopods, are serially homologous to the coxa of the antennae and mandibles of branchiopods.

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