Larval and post-larval development of the branchiopod clam shrimp Cyclestheria hislopi (Baird, 1859) (Crustacea, Branchiopoda, Conchostraca, Spinicaudata)

Jørgen Olesen

Abstract

The larval and post-larval development of Cyclestheria hislopi is examined by SEM. There are at least nine stages (excluding the adult) – six larval and three post-larval stages. The first four stages are passed within the egg-membrane. The larval and the post-larval phase are separated by a profound change in morphology that takes place between stages VI and VII. The larva shifts from a dorso-ventrally flattened ‘larval’ appearance up to stage VI to a laterally flattened, more ‘adult’ appearance from stage VII. New morphological data have been revealed by this study, including (1) a large and globular larval dorsal organ; (2) the carapace starts its development from the segments of the first and second maxillae; (3) the anterior ramus of the second antenna in adult Cyclestheria hislopi is the endopod, and the posterior ramus the exopod. Direct development of the brood in Cyclestheria hislopi – unique among conchostracans – is compared with that of the Cladocera. If Cyclestheria is the sister group to the Cladocera, as favoured in this work, the classical neoteny theory of the Cladocera must be reconsidered, as there is no particular similarity between any adults of the Cladocera and any of the larval stages of Cyclestheria. It is suggested that Cyclestheria displays the type of development present in a cladoceran ancestor. A comparison between Cyclestheria and the Upper Cambrian ‘Orsten’ fossil Rehbachiella kinnekullensis reveals a remarkable similarity in the endite morphology of the trunk limbs.

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Introduction
The clam shrimp Cyclestheria hislopi (Baird 1859) (in the monotypic Cyclestheriidae) is the only conchostracan that has direct development of its offspring (larval and juvenile stages passed within the brood chamber, see below). This reproductive strategy was described by Sars (1887) and has later been mentioned or studied by various workers (e.g. Roessler and Sanchez 1986; Roessler 1995; see Olesen et al. 1997 for more references to works on Cyclestheria). A single exception has been reported from Cuba, where free-living larvae seem to hatch from resting eggs (Botnariuc and Viña Bayés 1977). In the rest of the world only populations with directly developing individuals have been reported, even from resting eggs (Roessler 1995).

It is well-known that almost all cladocerans have direct development. The only exception is Leptodora kindti, where free-living larvae are present, but solely in the sexual part of the life cycle where so-called metanauplii hatch from the shed resting eggs (Sars 1874). In the parthenoge-
netic part of the life-cycle the individuals always develop directly, both in *Leptodora* and in the remaining cladocerans. The development always takes place in a dorsally located brood chamber formed by the carapace and the dorsal side of the trunk. In the Onychopoda and Haplopoda the carapace has become further modified into a brood pouch (see Martin 1992; for review).

Because of the similarity in mode of development between *Cyclestheria hislopi* and the Cladocera (direct development in dorsal brood chamber), together with other similarities such as fused compound eyes, *Cyclestheria hislopi* has often been mentioned as a ‘link species’ between the Conchostraca and Cladocera (Tasch 1963; Schminke 1981; Martin and Cash-Clark 1995; Olesen et al. 1997; Olesen 1998). If the similarities are interpreted as synapomorphies and *Cyclestheria hislopi* therefore is the sister group to the Cladocera, then the Spinicaudata (and the Conchostraca) are paraphyletic. Against this hypothesis are some similarities between *Cyclestheria hislopi* and the remaining spinicaudate and laevicaudate conchostracans not shared by the Cladocera. The most important of these are (1) a large carapace capable of enclosing the whole animal (2) the fact that the eggs or embryos are attached by an embryonic filament to the female’s trunk limb exopods, and (3) the position of the female gonopore on the 11th trunk segment.

This paper examines in more detail the direct development of *Cyclestheria hislopi* to provide a detailed basis for a future comparison of its development with that of other branchiopods, in particular other conchostracans and the Cladocera.

**Materials and Methods**

The material for this study was collected in small permanent ponds in the Magdalena Valley, close to Bogotá, Colombia, in October 1994. The preparation for SEM followed standard procedures as described by Felgenhauer (1987). The mounted specimens were photographed with a JEOL JSM-840 at the Zoological Museum, University of Copenhagen. The material is stored at the Zoological Museum, Copenhagen (ZMUC CRU 3574).

The Cladocera are in this paper considered as a monophyletic taxon while the status of the Conchostraca is more uncertain (following Olesen 1998; see also Fryer 1987).

**Abbreviations used in figures**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>a</td>
<td>anal opening</td>
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<tr>
<td>al</td>
<td>first antenna (antennule)</td>
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<tr>
<td>a2</td>
<td>second antenna</td>
</tr>
<tr>
<td>am</td>
<td>adductor muscle (carapace)</td>
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<tr>
<td>bc</td>
<td>brood chamber</td>
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<tr>
<td>ca</td>
<td>carapace</td>
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<tr>
<td>ce</td>
<td>compound eye</td>
</tr>
<tr>
<td>do</td>
<td>dorsal organ</td>
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<tr>
<td>el-e5</td>
<td>endites 1 to 5</td>
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<tr>
<td>emf</td>
<td>embryonic filament (or frontal area to which it attaches)</td>
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<td>en</td>
<td>endpod</td>
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<tr>
<td>ep</td>
<td>epipod</td>
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<td>fc</td>
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<td>food groove</td>
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<tr>
<td>ne</td>
<td>naupliar eye</td>
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<tr>
<td>pas</td>
<td>‘post-abdominal’ setae</td>
</tr>
<tr>
<td>pgn</td>
<td>paragnath</td>
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<tr>
<td>t1-t12</td>
<td>trunk limbs 1-12</td>
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<tr>
<td>te</td>
<td>telson</td>
</tr>
<tr>
<td>tl</td>
<td>trunk limbs</td>
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**Results**

**Classification**

Branchiopoda Latreille, 1817  
Conchostraca Sars, 1867  
Spinicaudata Linder, 1945  
Cyclestheriidae Sars, 1899  
*Cyclestheria hislopi* (Baird, 1859)

**Description of larval and postlarval stages**

Nine preadult stages have been recognized, based on the present work and on Sars (1887) (Table 1). The earliest six stages (stages I-VI) are termed larval stages and the three following (stages VII-IX) are termed post-larval (= juvenile) stages. Four of these — three larval and one post-larval — are herein described using SEM.

**Stage I (Larva 1) (Fig. 12 and Sars 1887: pl. VI, figs 3,4,5)**

Enclosed in egg-membrane (removed before photographing); length 260 μm; pear-shaped; with a dorsal organ at the back of the ‘head’ portion; a carapace not demarcated externally, but visible through the cuticle as a thickened rim behind dorsal organ (Fig. 2A); labrum, first antennae, second antennae, and mandibles have started development and constitute together a distinct ‘naupliar’ head section; first antennae and mandibles are undifferentiated limb buds; second antennae with two rami both bent dorsally;
Fig. 1—*Cyclestheria hislopi* (Baird, 1859). Lateral view of adult female with post-larvae of stage VII in dorsal brood chamber. Left half of carapace removed.

Table 1 Scheme that summarises the sources of original information on the morphology of the different stages of *Cyclestheria hislopi* (Baird, 1859)

<table>
<thead>
<tr>
<th>Stage number</th>
<th>Present study</th>
<th>Sars (1887) All re-illustrated in Fig. 12 in this work</th>
<th>Others</th>
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<tr>
<td>I</td>
<td></td>
<td>Sars: Pl VI, Figs. 3-5</td>
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<tr>
<td>II</td>
<td>• Fig. 2</td>
<td>• Sars: Pl VI, Figs. 6-8</td>
<td>Dodds (1926)</td>
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<td>III</td>
<td></td>
<td>• Sars: Pl VI, Figs. 9-11</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>• Figs 3, 4</td>
<td>• Sars: Pl VII, Figs. 1-3</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>• Sars: Pl VII, Figs. 4-6</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>• Figs 5-7</td>
<td>• Sars: Pl VII, Figs. 7</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>• Figs 8-11</td>
<td>• Sars: Pl VIII, Figs. 1-3</td>
<td>Nair (1968); Paul &amp; Nayar (1977); Egborge &amp; Ozoro (1989); Roessler (1995); Olesen et al. (1997) (see Olesen et al. for more references)</td>
</tr>
<tr>
<td>VIII</td>
<td></td>
<td>• Sars: Pl VI-V; Pl. VIII, Fig. 4</td>
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<tr>
<td>IX</td>
<td></td>
<td>• Sars: Pl VIII, Figs. 1-3</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>• Fig. 1</td>
<td>• Sars: Pl VIII, Figs. 1-3</td>
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no other limbs are recognizable externally but rudiments of first maxilla, second maxilla and 6–7 trunk limbs are visible through cuticle (Fig. 2E,F). Anal opening developed posteriorly (Fig. 2C).

Stage III (Larva 3) (Sars 1887: pl. VI, figs 6–8)

Stage IV (Larva 4) (Figs 3, 4, Fig. 12 and Sars 1887: pl. VI, figs 9–11)

Enclosed in egg-membrane (removed before photographing); length 300 μm; oval in shape; dorsal organ present on the dorsal side of the head; a short carapace originates at the segments of the first and second maxillae and reaches posteriorly to the third trunk segment; the labrum, five cephalic limbs, and 10 trunk limbs have started development; first antennae unsegmented, slightly elongated; second antennae with protopod divided into three portions, rami two-segmented; mandibles unsegmented; first and second maxillae as small, unsegmented limb buds; second maxillae slightly larger than first maxillae, each with a small pore (precursor of opening of maxillary gland); trunk limbs in varying degree of development with the anterior 5–6 limbs most developed and with the posterior 4–5 limbs as...
undifferentiated lobes, only latero-ventral edge of trunk involved in limb formation; limb tips differentiated into endopod and exopod portions of trunk limbs; same limbs with 2–4 weakly developed endites; rudiment of the epipod present dorsal to the first 5 trunk limbs; two posteriormost ‘segments’ circumscribe the trunk, the central one (telson) with an anal opening in the middle.

Stage V (Larva 5) (Fig. 12 and Sars 1887; pl. VII, figs 1–3)
Stage VI (Larva 6) (Figs 5,6,7)

Not enclosed in egg-membrane; length 440 μm; head dorsally with large inflated, globular, 35 μm wide dorsal organ at the level of the second antennae; the attachment area of the embryonic filament which connects the juvenile
to the parent’s exopod is present between the first antennae, anterior to the dorsal organ; labrum elongated, tip reaching the mandibles; the carapace is developed posteriorly, covering the dorsal side of the anterior three trunk segments, with its anterior margin indicated by a V-shaped margin dorsally (see arrows on Fig. 5A,B), apparently attached to at least the first and second maxillary segments; five pairs of cephalic limbs and 10–11 pairs of trunk limbs have started development, posteriorly 3–4 additional pairs of rudimentary trunk limbs are present; first antennae small, unsegmented, each with a central depression; second antennae with protopod divided into four portions (Fig. 6C), segments on rami only weakly indicated; mandibles divided into 2–3 portions (individual variation), which are probably artifacts; first and second maxillae are small, undifferentiated limb buds, second maxillae with a small pore (opening of the maxillary gland); anterior 8–10 trunk limbs with tips differentiated into endopod and exopod; endites present on anterior 8–10 trunk limbs, the most proximal endites being indicated on most limbs while the more distal endites are lacking on limbs more posterior than trunk limb 6; a rudimentary epipod is present until approximately trunk limb 9; hind body ends in two rounded furcal lobes; a rudimentary pair of ‘post-abdominal’ setae present.

Stage VII (post-larva 1) (Figs 8,9,10,11, Fig. 12 and Sars 1887; pl. VII, figs 4–6)

Not enclosed in egg-membrane; length 580 μm; the position of insertion of the embryonic filament is visible as an oval inflation near anterior extremity of head; dorsal organ conspicuous, globular, 40 μm wide, with a wide rim surrounding the whole organ, surface finely wrinkled;
carapace bivalved with a dorsal keel, covering approximately the anteriormost six trunk segments and part of the mandibles, the posterior margin with a large, V-shaped incision (posterolateral extension of carapace); the carapace also beginning to develop anteriorly, as indicated by the transverse rim dorsally in the position of the first and second maxillae (arrow in Fig. 9C), marking the anterior margin of the carapace in an earlier stage; labrum large and covering the distal end of the mandibles; five pairs of cephalic limbs and 13–14 pairs of trunk limbs have started development; first antennae small, unsegmented with rudimentary setae at the tips; second antennae with protopod divided into 5
portions with rows of rudimentary setae on the two distal of these, rami with 7 segments indicated by one rudimentary seta on each; mandibles relatively large compared to earlier stages; first and second maxillae are two gnathobasic lobes with basal setae pointing into the food groove, second maxilla with a large distal pore (maxillary gland); exopod and epipod well-developed in anteriormost 8–9 pairs of trunk limbs, exopods divided into a distal elongated part ventral. Arrow indicates an incipient subdivision of the mandible.

and a proximal smaller lobe posterior to the epipod; endopod not differentiated from the 5th endite in the posteriormost limbs and in the more anterior limbs resembles a ‘6th endite’; endites developed on anteriormost 11 trunk limbs, but only incipiently on the 2–3 posteriormost of these; the size of the endites decreases from proximally to distally on each limb, the proximal endite being significantly larger than the others; on some endites (1–4) is seen the

Fig. 6—Cylcestheria hislopi. Larvae of stage VI, taken from same brood chamber as Fig. 5. —A, Ventral. —B, Lateral, showing cephalon and anterior part of trunk. Arrow indicates anterior margin of carapace. —C, Cephalon and anterior part of trunk,
Fig. 7—*Cyclesteria hislopi*. Larvae of stage VI, from the same brood chamber as specimens in Figs 5 and 6. —A, Lateral view. —B, Frontal view. —C, Ventral view on left side trunk limbs showing development of these. Fifth endite becomes separated from endopod between trunk limbs 7 and 6 (white arrow). Fourth endite becomes separated between trunk limbs 10 and 9 (black arrow). —D, Latero-caudal view. —E, Caudal view. —F, Lateral view of left side trunk limbs with outer membrane removed. —G, Latero-ventral view of right side trunk limbs with outer membrane removed.
beginnings of a curved row of setae (Fig. 10E); sternitic food groove initiated between the trunk limbs; the furcal rami (furcal claws) have initiated their development, with a slit-shaped anus opening between them; ‘post abdominal’ setae present.

Stage VIII (post-larva 2) (Sars 1887; pl. VII, fig. 7)
Stage IX (post-larva 3) (Sars 1887; pl. VIII, figs 1–3)
Adult (Fig. 1 and Sars 1887; Roessler 1995; Olesen et al. 1997)
Fig. 9—*Cyclestheria hislopi*. Larvae of stage VII, from same brood chamber as specimens in Fig. 8. —**A**, Frontal view. —**B**, Lateroventral view. —**C**, Dorsal view. Arrow indicates anterior margin of attachment zone of carapace. —**D**, Caudal view. —**E**, Frontal view. —**F**, Antenna 2, left side, ‘inner’ view. —**G**, Dorsal organ. —**H**, Carapace margin with cell surfaces indicated.
Morphogenesis of appendages and other selected structures

This section is primarily based on the four stages examined using SEM in this study. Information from Sars (1887) is cited where appropriate.

General: Only the four earliest stages are passed within the egg-membrane. During the following development, the larvae are positioned in the brood chamber of the female, while still attached to this by an ‘embryonic filament’ that extends from the forehead of the larvae to the exopods of the female. The development is therefore, strictly speaking, only partly direct as not all stages are passed within the egg-membrane.

Carapace: The carapace becomes demarcated externally between stage II and stage III. In stage II it is visible latero-dorsally through the outer cuticle as a broad thickened rim immediately behind the ‘naupliar’ section, involving the first and second maxillary segments and possibly also the first trunk segment. In stage IV the carapace has grown posteriorly until trunk segment 2–3, the posterior margin is free from the trunk. It is attached latero-dorsally at the first and second maxillary segments and possibly also to a part of the first trunk segment, but if so, then only the dorsal side of the segment is involved. In the six earliest known stages the carapace is a univalved plate that follows the shape of the trunk and develops in a

**Fig. 10—Cyclestheria hislopi.** Larvae of stage VII, from same brood chamber as specimens in Figs 8 and 9. —A, Ventral view. —B, Ventral view of posteriormost trunk limbs. —C, Trunk limbs, right side, inner view. —D, Same as C. —E, Same as C. —F, Same as C.
posterior direction only. Until stage VI the carapace extends anteriorly to the ‘naupliar’ section but is distinctly separated from the mandibular region by a deep furrow in stage IV (arrow, Fig. 3B), or by a distinct margin in stage VI (arrows, Figs 5A,B and 6B). Between stage VI and stage VII the carapace becomes bivalved by the appearance of a dorsal keel and starts developing anteriorly, overgrowing the ‘naupliar’ section. In the adult the carapace is large and bivalved, with a number of growth lines reflecting the age of the animal, and it is capable of enclosing the whole body of the animal (Sars 1887; Olesen et al. 1997).

Dorsal organ: A dorsal organ is present in all known stages. In the two earliest stages it is a flattened, rounded, and wrinkled structure near the back of the ‘naupliar’ head section (termed ‘the occipital process’ by Sars 1887). In stages III and IV an enlargement of the ‘head’ (the head ‘bends’ forward) brings the dorsal organ into a relatively more anterior position, and it now lies approximately at the level of the second antennae. In stage V it becomes a large, inflated, globular structure. In stage VI it displays a number of large pits, which are probably, at least partially, critical point drying artifacts (Fig. 5D). In stage VII the organ is even larger, with an evenly wrinkled surface, and it is encircled at the base by a wide rim (Fig. 9G). In stage VIII it looses its globular shape but still occupies a relatively large part of the dorsal side of the head (Fig. 12 and Sars 1887; pl. VII, Fig. 7). The dorsal organ of the adult is a small, rectangular, flattened structure encircled by a rim and with a large pore in the centre (Olesen 1996; Olesen et al. 1997).

Labrum: The labrum is present in all stages. It starts as a large, broadly rounded structure and gradually becomes longer and acquires a more pointed tip. Between stages VI and VII it becomes very large and covers the distal ends of the mandibles.

First antennae: The first antennae do not appear externally until stage II. They are always unsegmented and uniramous. They originate as small, flattened lobes lateral to the proximal part of the labrum (stage II). Between stages II and VII they only elongate slightly, and it is not before stage VIII that they have elongated significantly...
(Fig. 12 and Sars 1887; pl. VII, Fig. 7). The adult first antenna is a long tubular appendage, that of the female with distal sensillae only but that of the male often with lateral sensillae also (Olesen et al. 1997).

Second antennae: The second antennae are biramous in all stages and are, throughout development, the largest appendages of all. In the two earliest stages (e.g. stage II, Fig. 2) both the protopod and the rami are unsegmented. During later development a number of articulations appear. In stage IV (Figs 3, 4) the protopod has become divided into 3 portions. Eventual homologies to a coxa and basis are uncertain. The most distal portion of the protopod carries the rami. At stage VI the protopod has become divided into 4 portions (Fig. 6C) and in stage VII 5 portions are recognizable (Fig. 9F). In the adult the protopod is divided into 5 portions, some carrying characteristic rows of stout setae (Olesen et al. 1997). The development of some of these setae is initiated in stage VII. The two rami gradually change from being unsegmented in the earliest stages (e.g. stage II, Fig. 2). At stage IV the rami are divided into 2–3 portions, at stage VI into 5 portions, and in stage VII into 7 portions. At the earliest stages (stages I–IV) the exopod is slightly longer than the endopod and has a u-shaped bend with the tip pointing dorsally. The endopod is bent in the same direction, but less so than the exopod. Traces of the curved appearance of the exopod are retained in later stages. The whole appendage makes during development (between stage VI and stage VII) a 90° turn that brings the inner ramus – the endopod – to an anterior position. The outer ramus – the exopod – is correspondingly brought from a lateral to a posterior position. This observation makes it possible to homologize the endopod with the ‘anterior ramus’ and the exopod with the ‘posterior ramus’, which were the terms used to describe an adult male in Olesen et al. (1997). That the appendage has turned this way – and not the opposite way, which would have brought the exopod to an anterior position – can be seen by using the position of the setae on the rami as indicators when comparing stages VI and VII, as these setae are only present on one side of the respective rami (compare position of exopodal setae Fig. 6C with exopodal setae on Fig. 9A).

Mandibles: Mandibles arize in the earliest stages as small limb buds posterior to the second antennae at about the level of the tip of the labrum. In some specimens are seen a division of the mandible into a number of portions (e.g. Fig. 6B). This is variable between specimens and is an artifact. Another division into a rounded distal part and elongate proximal part (Fig. 6C, arrow) is more significant and presumably corresponds to the division of the adult mandible into a proximal, large, leaf-shaped section and a distal molariform section (Sars 1887; pl. 4, Fig. 3.). In stage VII the mandibles have become very large and are covered distally by the labrum – as they are in the adults. A palp is lacking in all stages of development.

Maxillules: The maxillules are present as small, paired elevations posterior to the mandibles in stage II (Fig. 2E). During later development a number of articulations appear. In stage IV (Figs 3, 4) the protopod has become divided into 3 portions. Eventual homologies to a coxa and basis are uncertain. The most distal portion of the protopod carries the rami. At stage VI the protopod has become divided into 4 portions (Fig. 6C) and in stage VII 5 portions are recognizable (Fig. 9F). In the adult the protopod is divided into 5 portions, some carrying characteristic rows of stout setae (Olesen et al. 1997). The development of some of these setae is initiated in stage VII. The two rami gradually change from being unsegmented in the earliest stages (e.g. stage II, Fig. 2). At stage IV the rami are divided into 2–3 portions, at stage VI into 5 portions, and in stage VII into 7 portions. At the earliest stages (stages I–IV) the exopod is slightly longer than the endopod and has a u-shaped bend with the tip pointing dorsally. The endopod is bent in the same direction, but less so than the exopod. Traces of the curved appearance of the exopod are retained in later stages. The whole appendage makes during development (between stage VI and stage VII) a 90° turn that brings the inner ramus – the endopod – to an anterior position. The outer ramus – the exopod – is correspondingly brought from a lateral to a posterior position. This observation makes it possible to homologize the endopod with the ‘anterior ramus’ and the exopod with the ‘posterior ramus’, which were the terms used to describe an adult male in Olesen et al. (1997). That the appendage has turned this way – and not the opposite way, which would have brought the exopod to an anterior position – can be seen by using the position of the setae on the rami as indicators when comparing stages VI and VII, as these setae are only present on one side of the respective rami (compare position of exopodal setae Fig. 6C with exopodal setae on Fig. 9A).

Maxillae: The maxillae are visible as small, paired elevations lateral/posterior to where the maxillules are indicated in stage II (Fig. 2E). In stages IV and VI they are small buds with pores at the tip, presumably the future openings of the maxillary glands. In stage VII the maxillae are still small lobes but now with large median pores, and incipiently developed setae pointing into the food groove (Fig. 8B–E). In the adult they are represented by the food-forwarding gnathobases (Sars 1887; pl. 4, Fig. 6). The maxillules and maxillae in certain stages (Figs 4A, 6A, 8D) very similar in morphology, size and position to the proximal endite portion of the trunk limbs, and these structures are therefore probably serially homologous.

Trunk limbs: The development of a single trunk limb and the order in which the various parts appear can conveniently be divided into two phases, a first phase during which the different limbs parts appear and become differentiated (the trunk limb differentiation phase), and a second phase during which certain parts begin extensive growth (the trunk limb growth phase). It is important to note that this distinction applies to the development of a single limb and not to the whole animal as such. The description of the development of a single limb is based on the examination of three stages (stages IV, VI and VII), which, because of the serial displacement in the degree of development in each individual, display all degrees of limb development.

The trunk limb differentiation phase occurs as follows (based primarily on stages IV and VI, Figs 3, 4, 5, 6, 7): the ventro-lateral edge of the trunk becomes sliced into rectangular lobes; the dorsal ends of the lobes become separated from the dorso-lateral side of the trunk; a rudimentary epipod becomes visible (Figs 3A, 4E, 4B); the limb tip becomes divided into a small endopodal and a large exopodal part (arrows, Fig. 3A) and some of the endites become indicated, the separation between the 3
most proximal endites appearing first (arrows, Fig. 4A); the endopodal and exopodal parts become equal in size and the 4 most proximal endites are recognizable, the largest proximally, the smallest distally (trunk limb 1, Figs 4A, 7C); a 5th endite becomes separated from the endopod (Fig. 7C). Not surprisingly, the endites are ‘budded off’ distally from the inner branch of the limb.

The trunk limb growth phase, when the endopod, the epipod, and especially the exopod, start an extensive growth, occurs as follows (all based on stage VII, Figs 8,9,10,11): the exopod grows in the distal as well as proximal direction and becomes distally a large rectangular plate with a small, proximal lobe; the epipod becomes club-shaped, situated anteriorly to the small proximal exopodal lobe; the gaps between the endites become deeper; setation on the endopod, endites, and exopod starts appearing more clearly. A noteworthy observation is that the endopod becomes separated late from the 5th endite and in the latest known juvenile stage basically looks like a ‘6th endite’ (e.g. Fig. 10C).

Immediately after the enlargement phase has started, the two limb rows, from a flattened lateral orientation, start bending towards each other and take on a dorso-ventral orientation. The actual flexing zone of the limb is between the proximal (first) endite and the second endite (Fig. 10A-D, arrows). The proximal endite maintains its position except that it is orientated more ‘upright’ in the later stages of limb development.

The limb orientation and morphology of the most developed (anterior) limbs in stage VII approach those of the adults. The following adult features are different from stage VII: the limbs are much more flattened; the exopods are much larger relative to the rest of the limb, distally as well as proximally, where the small lobe from stage VII has grown into a large, quadrate plate; the endopod is no longer of the same size as the endites but significantly larger; the proximal endite has a characteristic gnathobasic morphology that is different from that of the other endites; setation is present along all margins except on the epipod (information on the adult from Sars 1887 and Olesen 1997).

Telson, anal opening, furcal rami, and the ‘postabdominal’ setae: Neither furcal rami nor ‘post-abdominal’ setae are present in stages (stage I-IV). In stage IV the hind body is divided in two segments circumscribing the trunk. The central of these, the telson, have an anal opening in the middle. These structures are difficult to follow in later stages. In stage VI the posterior-most hind body ends in two caudal lobes with an anal opening between. The ‘post-abdominal’ setae are not visible until stage VI and are in this stage attached to a section that may correspond to the telson (Fig. 9D). In stage VII a pair of furcal claws have started developing from the caudal lobes (Fig. 9D). The adult has two articulated furcal claws terminally on the body.

Summary of the development of Cyclestheria hislopi with indication of key events (Fig. 13A).

- Stages I-IV are passed within the egg-membrane.
- Stage II: The antennular buds appear.
- Stage III: The carapace begins its external development; the trunk limbs become demarcated; the first and second maxillae appear.
- Stage IV: Incipient division of anterior trunk limbs into endopod, exopod, endites, and epipod; protopod and rami of second antenna become segmented.
- Stage V: The dorsal organ attains its globular structure.
- Stage VII: The body has become laterally instead of dorso-ventrally flattened; body curved with concave side ventral (opposite of earlier); the carapace develops its bivalved structure and starts developing in the anterior direction; the second antennae have made a 90 turn; the two rows of trunk limbs start, from the anterior end, to bend towards each other and develop a ventral orientation; incipient development of furcal claws.
- Stage VIII: All appendages developed, functional, and movable; the dorsal organ has lost its globular shape and become more or less flattened.

Discussion

The development of Cyclestheria hislopi as compared to other conchostracans

Four stages of Cyclestheria are described here. Sars (1887) described seven stages including a male embryo at a late developmental stage. Two of the stages described in this work are different from any described by Sars and the number of stages passed in the brood chamber is therefore at least 9, possibly higher. Four of these are passed within the egg-membrane. In several ways the most significant changes in morphology take place between stages VI and VII. These are so profound that they can be used as a convenient marker to divide the development into two distinct phases, the ‘larval’ and the ‘post-larval’ or ‘juvenile’ phases. The ‘larvae’ – stages I-VI – are characterized by a slightly concave dorsal side, a univalved carapace, and trunk limbs laterally directed and widely separated and exhibit a general dorso-ventrally flattened. In the first post-larval stage (stage VII) the morphology changes. The carapace is now bivalved and the two rows of trunk limbs have begun to bend towards each other, which gives the body a bilaterally compressed appearance. Additionally, the posterior part of the trunk is bent in a ventral direction and the ventral side is slightly concave.
Fig. 13—Schematic representation of the development of three spinicaudate conchostracans. —A, Development of *Cyclestheria hislopi* (Baird, 1859) based on the present study and on Sars (1887). —B, Development of *Cyzicus (Estheria) packardi* (Brady) based on Sars (1896). —C, Development of *Limnadia stanleyana* (King) based on Anderson (1967).
Other important changes occur later when the limbs become functional, between the 2nd and 3rd post-larval stages (stages VII and VIII), and also earlier in development, between larvae 2 and 3 (stages II and III), where 8 trunk limb buds become demarcated in one step.

As parthenogenetically reproducing Cyclestheria hislopi have no free-living larvae, this development is different from that of all other conchostracans, all of which have free-living larvae. The morphology of the various stages are most similar to those of other spinicaudates, whereas the larvae of the levaicicudies are very modified. A comparison between Cyclestheria and two other spinicau-
dates, Cyzicus (Estheria) packardi (Brady) and Limnadia stanleyana King – larval development described by Sars (1896) and Anderson (1967), respectively, – show a close similarity both in number of ‘larval’ stages before the ‘post-larval’ phase is initiated, and in morphological similarity between some of the stages. In all three species the first six stages have a dorso-ventrally flattened appearance, a univalved carapace and two rows of trunk limbs widely separated from each other. Stage VII of C. hislopi and C. packardi significant changes have taken place, including the carapace becoming bivalved and the rows of trunk limbs bending towards each other. The precise time that this profound change in morphology occurs in L. stanleyana is unknown as Anderson (1967) examined the first six stages only. The distinction between the two different phases in conchostracan development applies both to species with free-living larvae and to the single known species with direct development (C. hislopi).

Anderson (1967) used another developmental marker to distinguish between the larval and the juvenile (post-
larval) phase for C. packardi, namely the time that the trunk limbs become functional. This happens in L. stanleyana as early as stage VI, in C. packardi in stage VII, and in C. hislopi as late as stage VIII. It is to be expected that the functionality of the limbs in Cyclestheria has been secondarily delayed, but as noted, some variation is also seen in conchostracans with free-living larvae. In Cyzicus the functionality of the trunk limbs is correlated with significant changes in morphology between stage VI and stage VII (larval and post-larval phases), while the trunk limbs in L. stanleyana become functional one stage earlier and before the two rows of limbs start bending towards each other. It can be concluded that the advent of func-
tionality of limbs is a labile feature, the timing of which seems to differ significantly between taxa. A complication is the problems of defining precisely when limbs are functional and not just movable. The usefulness of such a character as a developmental marker to homologize stages among taxa is therefore limited. Instead, I propose the use of morphological changes as those taking place between stage VI and VII in both Cyzicus and Cyclestheria (see earlier and Fig. 13). These are probably equivalent (homologous) between these spinicaudates and can therefore be used to distinguish between phases in their development.

Comparison of morphological details between Cyclestheria and other conchostracans

Many of the differences in the morphology between Cyclestheria hislopi and other spinicaudates such as Cyzicus packardi and Limnadia stanleyana are related to the direct development of the offspring in the former where the appendages are not functional until late in development. The second antennae and the mandibles, which are used for locomotion and feeding already in the very earliest stages of other spinicaudates, are highly modified and reduced in early stages of Cyclestheria, where they do not become functional until stage VIII. There is, for example, no trace of the food-manipulating ‘naupliar process’ on the second antennae of early stages of parthenogenetically reproducing Cyclestheria hislopi as seen in other spinicaudates. Additionally, the mandible in the early stages of Cy-

A comparison of the direct development in Cyclestheria and in the Cladocera

With the exception of Leptodora kindti, all cladocerans have direct development of their offspring. Leptodora kindti is not even a true exception, as free-living larve hatch from resting eggs. Parthenogenetic development is direct. Direct development in Cyclestheria and the Cladocera is a possible synapomorphy for these taxa, as suggested by Olesen et al. (1997) and Olesen (1998). However, direct development has certainly appeared independently a number of times within the Crustacea and may, for that reason, appear less convincing as support for Cyclestheria and the Cladocera as sistergroups. A significant difference between Cyclestheria and the Cladocera is the presence of an embryonic filament in Cyclestheria extending between some of the trunk limb exopods of the adult and the frontal part of the
heads of the embryos. No such structure is found in any cladoceran taxa. An embryonic filament is shared with all other conchostracans and therefore, depending on the accepted phylogenetic position of Cyclestheria, is either a synapomorphy of the conchostracan taxa or plesiomorphic for these, and later lost in the cladocerans (see Olesen et al. 1997). If the direct development in the Cladocera is homologous to that of Cyclestheria, and Cyclestheria therefore is the sistergroup to the Cladocera, as is favoured in this study, then the developmental modes in the Cladocera should be considered as further developments of that seen in Cyclestheria.

Of the four cladoceran orders, the embryos of the Ctenopoda (e.g. Sida and Diaphanosoma) are most similar to those of Cyclestheria (Olesen, unpublished data). They appear, with respect to the trunk limbs, like reduced forms of the Cyclestheria embryos, but with fewer trunk limbs (six pairs) and with fewer endites indicated along the limbs' inner margins. As in the Cyclestheria embryos the ctenopod embryos have serially similar limbs with the tip of each limb divided into an endpod and exopod, and with a distinct proximal endite lateral to a deep food groove. Also, the carapace originates from the first and second maxillae region in a very similar way in Cyclestheria and in the ctenopods (Olesen, unpublished data). In other respects the ctenopods have their own modifications. Further comparison with the various cladoceran orders is postponed until more information about these becomes available.

If Cyclestheria is accepted as the sister group of the Cladocera, then the classical theory of a neotenic origin of the Cladocera with a free-living conchostracan larva as starting point should be seriously reconsidered; these two theories conflict. Having Cyclestheria as the sister group of the Cladocera implies that their common ancestor probably had direct-developing larvae (see below for another possibility), which means that a completely different scenario of the origin of the Cladocera would have to be established. Classical neoteny would involve an origin from free-living conchostracan larvae such as larva VI of Cyzicus packardi (see Fig. 14) (theory first suggested by Claus 1876; summarized by Schminke 1981). At this point in its development Cyzicus packardi has only six pairs of trunk limbs developed; the carapace is short, not yet covering the head, and the sensillae of the first antenna largely restricted to the tip. This larval stage is in these respects similar to certain cladocerans, especially to the ctenopods which have retained six serially similar pairs of trunk limbs, and the neotenic theory has therefore always been appealing to many workers (e.g. Claus 1876; Margalef 1949). However, if Cyclestheria eventually turns out to be the sister group of the Cladocera, and direct development therefore a part of the groundplan for the two taxa, then the classical neoteny scenario must be rejected. The Cladocera cannot be derived from any of the larval stages of Cyclestheria described herein and by Sars (1887) as none of them show any specific resemblance to any adult cladocerans, so the cladoceran origin – given that the...
mentioned position of Cyclestheria and the homologous status of the direct development—cannot be neotenic in the classical sense. In this situation it would be easier to assume a simple reduction in the number of body segments in the branch leading to the Cladocera, not markedly related to neotenic processes.

However, it cannot be excluded that development of direct development is a much more labile feature in crustacean evolution than assumed so far in this paper, and developed independently in Cyclestheria and in the Cladocera—perhaps even several times within the Cladocera. This could be indicated by the fact that free-living larvae hatch from resting eggs in the cladoceran Leptodora kindti and supposedly also in a single population of Cyclestheria hislopi (in Cuba, Botnariuc and Viña Bayés 1977). If this is a retained primitive condition, it means that free-living larvae have been lost independently within Cyclestheria and the Cladocera. This does not preclude Cyclestheria as the sistergroup to the Cladocera but it does mean that an important argument for this is lost. In light of the pivotal role of Cyclestheria in discussions of the origin of the Cladocera, a re-examination of the reported free-living larvae of Cyclestheria is strongly needed.

The carapace of Cyclestheria hislopi

The larvae of C. hislopi provide good data on the ontogeny of the carapace. This is first visible as a dorsal, thickened transverse rim posterior to the dorsal organ, more exactly at the level of the first and second maxilla in a posterior position at the cephalon (Stage II, Fig. 2A). It grows posteriorly in the first stages of development, then anteriorly and finally covers the head as well as the trunk. In some intermediate stages, the carapace is clearly not morphologically continuous with the ‘naupliar’ region anterior to it (Figs 3A, 6B). These two structures are separated from each other externally by a dorsal furrow immediate behind the mandibular level (Figs 3B, 6B). The first part of the development is very similar to that of ctenopod cladocerans (Olesen, unpublished data on Diaphanosoma brachyurum). In Diaphanosoma the carapace also originates dorsally from the posterior part of the cephalon, from where it continues its growth posteriorly. In contrast to Cyclestheria there is no later anterior growth in Diaphanosoma, and in the adult the carapace covers the trunk section only and not the head. Because of the general similarity in position and early development of the carapace in Cyclestheria and Diaphanosoma there is good reason to believe that the carapace is homologous in these taxa and therefore possibly also in other spinicaudate conchostracans and other cladocerans.

Walossek (1993, 1995) suggested that the carapace in the Conchostraca and Cladocera is formed as a new structure behind the naupliar shield, nonhomologous with the carapace in other crustaceans, and thereby constituting a synapomorphy for these two taxa. This type of carapace was termed a ‘secondary shield’ and this interpretation was followed by Olesen et al. (1997) and by Olesen (1998) in advocating the monophyly of the Diplostraca (Conchostraca and Cladocera), together with other characters such as the presence of male claspers. The presence of a ‘secondary shield’ was later rejected by Fryer (1996), who stated that the carapace in all the orders of the Conchostraca and Cladocera develops via a cephalic fold from the back of the cephalon in the classical manner of other crustacean carapaces (as viewed by Calman, 1909). The carapace development of Cyclestheria appears to agree with the carapace view of Fryer (1996) and Calman (1909), as it develops from the posterior part of the cephalon. However, the carapace has a unique way of development in Cyclestheria and in other conchostracans (at least spinicaudates). As mentioned above, it overgrows the head section in later stages of Cyclestheria (Fig. 9C), becoming large and bivalved, capable of enclosing the whole body (Fig. 1). Conchostracans (at least spinicaudates) differ in this respect from all other branchiopods. This morphology of the carapace is a possible synapomorphy for either the Conchostraca (at least the Spinicaudata) or for the Conchostraca plus the Cladocera, but then it must be viewed as lost in the Cladocera. This is not to say that the spinicaudate carapace necessarily is non-homologous to that of the Notostraca or, e.g. the Upper Cambrian branchiopod fossil, Rehbachiella. It could with equal right be viewed as a further development of a carapace type as found in these taxa where the carapace is continuous with the ‘naupliar’ region and develops as a simple posterior prolongation of the cephalic shield.

Comparison with Rehbachiella kinnekuellensis Müller 1983, and the phylogenetic position of Rehbachiella

There are many general, and some very specific, similarities between certain late juvenile stages of Cyclestheria and the largest known stage of the Upper Cambrian ‘Orsten’ fossil, Rehbachiella kinkekuellensis Müller 1983, described in much detail by Walossek (compare Fig. 8A and Walossek, 1993; Fig. 15d). Adults of Cyclestheria are much less similar to Rehbachiella (compare Fig. 1 and Rehbachiella). This can possibly be interpreted as Cyclestheria going through a ‘Rehbachiella-stage’ during its development. Most striking are the similarities in the enditic arrangement on the inner side of the trunk limbs (compare Fig. 10 and Walossek, 1993; Fig. 13 and pl. 17 : 2). In both taxa, the endites are formed as globular lobes, 5 in Cyclestheria and 7–9(? in Rehbachiella, and the proximal endites, closest to the food groove, are larger than the more distal ones in both taxa. In Cyclestheria the clefts between the endites are generally not as deep as in Rehbachiella but this is probably due to a membrane covering of the still nonfunctional trunk limbs of Cyclestheria. Even the
setation pattern of the endites of *Rehbachiella* is remarkably similar to the rudimentary setation of certain endites of *Cyclestheria*. In both taxa a row of setae encircles the tip of each endite (at least some endites in *Cyclestheria*). These similarities have to be interpreted as the result of common ancestry, and, unless interpreted as plesiomorphies, there can be little doubt about the branchiopod affinity of *Rehbachiella*, as suggested by Walossek (1993, 1995).

Other parts of *Cyclestheria* trunk limb morphology are quite different from those of *Rehbachiella*. In *Cyclestheria* each trunk limb has an external epipod, which appear to be absent in *Rehbachiella*, fewer lobate endites, and an unsegmented endopod, in contrast to the 4-segmented endopod of *Rehbachiella*. Another difference is seen in the early development of the limbs. Those of *Cyclestheria* are formed as elongated, laterally, pair-wise separated limb buds (see above), with the endite bearing side facing ventrally, and the future lateral (outer) limb margin not yet visible. This is similar to early limb development of *Artemia* (see Schrehardt 1987) and *Branchipus schaefferi* (see Schlögl, 1996), and possibly to other extant branchiopods and hence is a possible synapomorphy for these (see below). In *Rehbachiella* the early limb development is different as the rudimentary, biramous trunk limbs are in a vertical position already from the onset of trunk limb development (Walossek, 1993; Fig. 7, Fig. 12, pl. 19 : 7). Also the head appendages, the carapace, the furcal rami and the dorsal organ in *Cyclestheria* are different from those of *Rehbachiella*.

*Rehbachiella* was convincingly placed among the branchiopods by Walossek (1993, 1995), based on the shared presence of a similar postnaupliar filter-feeding apparatus (Walossek, 1993; p. 71, character 3). This feeding apparatus was identified as a autapomorphy for the Branchiopoda by Walossek (1993), and includes a deep food groove, posteriorly concave limb corms, many posteriorly directed lobate endites (maximum of 5 in extant branchiopods), and with the setation of the proximal endites pointing into the food groove. The similarity between *Cyclestheria* and *Rehbachiella* in these features brings further support to the validity of this character. The anostracan affinities of *Rehbachiella*, tentatively suggested by Walossek (1993, 1995), appear less well-supported, and *Rehbachiella* may be placed better further down the stem, possibly as sister group to the rest of the branchiopods. This is based on the following 4 limb characters, shared by all or most other branchiopods. (1) Reduction of both pairs of maxillae to small food-forwarding gnathobases. The Devonian fossil *Lepidocaris rhyniensis* Scourfield 1926, with supposedly anostracan affinities (see Walossek, 1993, 1995), may be an exception to this as it apparently has the first maxillae modified as claspers. *Rehbachiella* has a large, unsegmented first and second maxillae. (2) An enlarged protopodal part of the second antennae found in the larvae or the adult (the character was first identified by Sanders 1963). This is seen in larvae of the Anostraca, Notostraca, Conchostraca (Spinicaudata) and in various adults representing all 4 cladoceran orders. However, a number of cladocerans are quite modified and deviate from this pattern. *Also Lepidocaris rhyniensis* has elongated second antennae protopods (Scourfield 1926). In Kazacharthra, another extinct branchiopod group, with notostracan affinities, the second antennae are reduced in size (McKenzie et al. 1991). The laevidaudates have not modified the second antennae the described way, not even as larvae (see Gurney 1926), which must be interpreted as secondary if the suggested character is valid. (3) A similar way of trunk limb development is seen in most recent branchiopods. In *Artemia* (see Schrehardt 1987; Fig. 24) and *Branchipus schaefferi* (see Schlögl 1996; Fig. 9), representing the anostracan branchiopod line, in *Triops longicaudatus* (see Williams and Müller 1996), representing the notostracan branchiopod line, in *Cyclestheria* (this work) and in certain cladocerans (Olesen, unpublished), the trunk limbs are formed as two rows of elongated, lateral limb buds, with the lobate, endite-bearing side facing ventrally, and with the future lateral (outer) limb margin not yet developed. The two limb rows are separated by the incipiently developing food groove. (4) An unsegmented endopod is seen in the trunk limbs of most recent branchiopods. Since *Rehbachiella* has none of these four appendage characters it is best placed outside the Branchiopoda, possibly as its sistergroup.

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