Abstract


As a part of a project to compare phylogenetically the larval or embryonic development of all major taxa of the Branchiopoda (Crustacea), the larval development of the Japanese spinicaudatan clam shrimp *Eulimnadia braueriana* Ishikawa, 1895, is described. Seven naupliar stages are recognized, based mainly on significant morphological differences between them, but in one case, on size alone. The seven stages range in length from 156 μm to 760 μm. Nauplius 1 is nonfeeding with incompletely developed and nonfunctional feeding structures. Nauplius 2 has apparently functional feeding structures, including a well-developed mandibular gnathobase, setulate protopodal endites of the antennae, and setules on various setae involved in swimming and food manipulation. Nauplius 3 is morphologically identical to Nauplius 2, but more than 50% larger. In nauplius 4, the coxal endite (naupliar process) of the antennae develops a bifid tip. Nauplius 5 has a lateral pair of primordial carapace lobes, and the first 4–5 pairs of trunk limb buds are weakly developed, making the anterior part of the trunk wider than the posterior. In nauplius 6, five pairs of trunk limb buds are visible externally and a small carapace has appeared, reaching approximately to trunk limbs 2; also, the pair of large buds behind the mandibles in previous stages has become divided into a large, anterior, setose bud and two smaller, posterior buds. The identities of these structures as either paragnaths or maxillules/maxillae remain uncertain. In nauplius 7, about six pairs of trunk limb buds are visible externally. The general morphology of the nauplius larvae of *E. braueriana* is much like those of the well-known *Limnadia lenticularis* (Linnaeus, 1758) and *Eulimnadia texana* Packard, 1871, including an elongate, lanceolate labrum; however, because of various heterochronies, the correspondence between the larval sequences of these species is not perfect. There is even less correspondence with the 5-stage larval development reported for *Limnadia stanleyana* King, 1855, and the spatulate labra of that species and *Jmnadia* spp. are different from those of other known limnadiid nauplii. The larvae of *E. braueriana* possess many typical (and synapomorphic) branchiopod features, such as the general morphology of the appendages involved in feeding and the mode of trunk limb development, while the small buds of the first antennae and the exact number and development of the parts of the trunk limbs are typical for the Spinicaudata.

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Introduction
Larvae of the higher taxa of ‘large’ Branchiopoda (Anostraca, Notostraca, and ‘Conchostraca’) are generally more similar than the adults. Adults of these taxa differ fundamentally in gross morphology and lifestyle (Fryer 1987; Martin 1992; Walossek 1993), for instance with respect to the limbs used for locomotion (antennae or trunk limbs), whether a carapace is present, and feeding strategy. Larvae of the Anostraca, Notostraca, and ‘Conchostraca’ are, in contrast, remarkably similar; among other things, they have a naupliar feeding apparatus consisting of virtually the same components and an identical development of the trunk limbs (Fryer 1983, 1988; Walossek 1993; Olesen in press).

Uncertainty in identifying homologous parts is a well-known cause of trouble in phylogenetic studies when morphologically very different taxa are compared (e.g. Boxshall and Huys 1992), and this has also been the case for phylogenetic studies of the Branchiopoda (see Discussions in Olesen 1998, 2000, 2002; Fryer 1999, 2001, 2002). The detailed similarity among the various branchiopod larvae suggests that a comparative study of branchiopod ontogeny could be phylogenetically useful, as it could resolve many homology problems. The present report describes the larval development of a limnadiid spinicaudatan clam shrimp (‘Conchostraca’) and is first in a series treating the development of Japanese ‘conchostracans’.

In Japan, two species of Eulimnadia have been described, E. braueriana Ishikawa, 1895, and E. packardiana Ishikawa, 1895. These have recently been considered synonyms, but with no reason given (Naganawa 2001; Naganawa and Orgiljanova 2001). At least two, and possibly three species of this genus appear to be present in the collections of the Lake Biwa Museum (Grygier unpublished data). One of these forms, with a high, triangular carapace in females and about seven growth lines, has been reported independently several times and either called E. braueriana or considered distinct from either of the two named species (Takeuchi 1982; Ida 1985). Another form has an oval carapace with up to 15 growth lines concentrated near the margins, rather like those of Eulimnadia gunturentensis Radhakrishna and Durga Prasad (1976) (q.v.). The nauplii described herein belong to the third form, the adult females of which have an oval carapace with generally three to five growth lines. Pending a comparative treatment of Eulimnadia samples from throughout Japan, specimens belonging to this form are assigned to E. braueriana.

There has been no previous treatment of the development of E. braueriana, but developmental aspects of various other species of Limnadiidae have been described. The most detailed account is that for Limnadia lenticularis (Linnaeus, 1758) given by Sars (1896), who provided more details than the earlier report on this species by Lereboullet (1866). Anderson (1967) described the development of Limnadia stanleyana King, 1855, and Streth and Sissom (1975) did so for Eulimnadia texana Packard, 1871. Eder (in press) gives an SEM-based description of the larvae of Inamadia yeyetta Hertzog, 1935. Selected developmental features of other species belonging to the Limnadiidae have been described or briefly mentioned, as for Inamadia vostetii Botnariuc & Orghidan, 1941, by Botnariuc (1947, 1948) and for Eulimnadia stoningtonensis Berry (1926) (q.v.).

The present paper provides a complete description of the larval sequence of Eulimnadia braueriana, member of the Limnadiidae, covering the external morphology at both the gross morphological and ultrastructural levels. This description will provide a basis for later comparison with the larval sequence of other ‘large’ branchiopods in a phylogenetic context. In this paper comparisons will largely be restricted to other limnadiids.

Materials and methods
The examined nauplii of Eulimnadia braueriana were reared under the direction of MJG between November, 2000, and February, 2001, from dried mud taken from rice paddies in the Sakamoto and Mise districts of Otsu City, Shiga Prefecture, Japan. All material used was fixed in c. 2% formaldehyde solution (i.e. c. 5% formalin). All material used for SEM was critical-point-dried from ethanol using acetone as an intermediate and sputter-coated with gold following standard procedures. A JEOL JSM-840 scanning electron microscope at the Zoological Museum, Copenhagen, was used for observations and digital photography. The dried material used for SEM is stored in the Zoological Museum, University of Copenhagen. Two to seven specimens were identified as belonging to each stage. Adults collected in May of 1999 and 2002 from the Mise paddy field and in May, 2000, from the Sakamoto paddy field are deposited as vouchers in the Lake Biwa Museum; additional nauplii reared from the same mud samples, as well as naturally hatched nauplii collected several days after flooding of the Sakamoto paddy field in late April, 2001, are also kept there.

Results
Overview of the larval development of Eulimnadia braueriana
We have identified seven naupliar stages of Eulimnadia braueriana (Fig. 1). Except in one case, the stages have been separated on distinct morphological criteria. The exception concerns nauplii 2 and 3, between which no distinct morphological differences could be found. Because nauplius 3 is more than 50% larger than nauplius 2, it has been recognized as a separate stage.

Nauplius 1 (Fig. 2)
The body length is 156 μm. This larva appears lecitrotrophic: the body and appendages are inflated with yolk,
feeding structures are not developed, segmentation of the limbs is unclear, and the setae are only weakly developed. The body is about twice as long as wide. A large dorsal organ is present, occupying the region above the ventral insertions of the three pairs of naupliar appendages (Fig. 2B,D). The labrum is approximately one third as long as the body (Fig. 2A); its sides are parallel in the proximal half and then converge distally. The antennules are represented by a pair of small, widely spaced, anterior buds, each bearing a small seta (Fig. 2A,B). The antennae are by far the largest appendages, each being approximately the same length as the entire larva (Fig. 2A,B,C,E). They are biramous with a protopod about as long as the exopod and with a slightly shorter endopod; segmentation and setation are only weakly indicated, but two
Larval development of *Eulimnadia* • Olesen and Grygier


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similarly sized portions can be recognized in the endopod. Along the median side of the protopod are two endites, each of which continues into a seta-like structure. The proximal protopodal endite, or ‘naupliar process’ (‘proximal masticatory spine’ sensu Fryer 1983, 1988), is thick and has its distal, seta-like projection directed beneath the labrum, while the distal endite (‘distal masticatory spine’ sensu Fryer 1983, 1988) is thin. The endopod is weakly two-segmented and bears three setae distally, the innermost one being less than half as long as the other two. The exopod is longer than the endopod and has three setae along the inner margin and two setae distally. The mandible is composed of four incipient segments, comprising the coxa, with a weakly developed, not yet functional gnathobase, and a weakly segmented palp with three segments (Fig. 2A,C,E,F). The proximal segment (basis) in the palp bears two undeveloped setae, the second segment bears one seta, and the distal segment bears three setae. There is a weak anal furrow terminally on the trunk (Fig. 2D).

Nauplii 2 and 3 (Figs 3, 4)

Nauplius 2 is about 195 µm long, and nauplius 3, about 315 µm. The morphology of these two stages is so similar that they are described together. Because of this detailed similarity, one could consider them to belong to the same stage; however, we consider the size difference to be too great for this and therefore recognize two stages. In addition at least one consistent difference between the stages – concerning the spination of the caudal lobes – has been identified (see below).

The larvae are more slender and elongate than Nauplius 1. They may be feeding larvae as feeding structures and setation are developed; however, Anderson (1967) reported that feeding began only in the fourth recognized naupliar stage in Limnadia stanleyana, even though apparently functional feeding armament was present on the antennae and mandibles from Nauplius 2. As in the previous stage, a large dorsal organ occupies the region overlying the insertions of the naupliar appendages (Fig. 3D,G). A carapace is not yet present. The labrum is long and narrow, tapering distally with a relatively narrow base and with the widest part one quaffer of the way distally; each side of the distal half of the labrum has approximately six irregular marginal notches approximately one third distally from the base (Fig. 3A,F). The labrum points perpendicularly away from the body. Two marginal clusters of setae are present on the surface of the labrum facing the trunk (Fig. 3C, showing cluster of setae on one side only). The antennules are represented by a pair of small buds on the anterodorsal corners of the head, each bearing one well-developed but small seta and an additional, rudimentary seta (Fig. 3A).

The antennae are still by far the largest appendages, and they now have well-developed setation (Fig. 3A,D,G,D). The basic components are the same as in Nauplius 1: a long protopod with two median endites, and an endopod and exopod. The protopod is slightly longer than either ramus. No clear external segmentation divides the protopod into a coxa and basis. The proximal protopodal endite, or naupliar process, has enlarged considerably compared to the previous stage; it is strongly curved and points toward the midline of the larvae (Fig. 3H, a2 pe), with an annulation at its midlength that probably indicates the setal nature of the distal part of the endite. Distally, the endite is covered with long setules arranged in an irregular bottle-brush pattern (Figs 3A,J,K, 4A, D), and each setule bears rows of small, hooked (artifact?) barbs (Fig. 3K). The distal endite on the protopod consists of a small, tubular socket that gives rise to a long seta (Fig. 3H,I, a2 de). This seta has an annulus approximately halfway to the tip; more distally it bears two rows of long, scattered setules, each provided with barb-like ornamentation. The endopod is, at least superficially, weakly subdivided into 4–5 parts by some furrows lined by spines, but only the deepest furrow will become a true articulation in later larvae (arrow on Fig. 3E). The remaining subdivisions are possibly rudiments of ancestral articulations. Distally the endopod bears four setae, three of which are fully developed, with an annulus at approximately one third length, then a dense row of setules; the fourth, innermost seta is small and undeveloped. The exopod is weakly divided into 4–5 parts. It has five long setae, three along the inner margin and two distal ones. All the setae are similar to the three fully developed endopodal setae.

The mandible is clearly composed of four segments (Fig. 4D). The most proximal of these is the enlarged coxa, followed by a three-segmented palp, wherein we interpret the most proximal segment as the basis. [Anderson (1967) interpreted the distal part of the palp as the exopod, with the single seta on the second palp segment possibly representing the endopod, but this is uncertain; in our view, the distal part of the palp may as well represent the endopod.] The coxa is large and, similarly to the protopod of the second antenna, inserts laterally on the head (Figs 3G, 4A,D). Dorso-laterally the coxa tapers, which gives it a triangular shape in lateral view, and ventro-medially it is drawn into an ornamented gnathobase endite, which is directed beneath the labrum towards the mouth. The distal margins of the pair of gnathobases lie

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**Fig. 2**—Nauplius 1. —A. Ventral view —B. Lateral view, with arrows indicating anterior and posterior margins of dorsal organ —C. Left antenna and mandible —D. Posterior view. —E. Left appendages and labrum, ventral view —F. Detail of left mandible. Abbreviations: a1, antennule; a2, antenna; ba, basis; co, coxa; de, distal endite of antenna protopod (with setae); do, dorsal organ; en, endopod; ex, exopod; la, labrum; md, mandible; pe, proximal endite of antenna protopod (naupliar process); te, telson; numerals 1–3 indicating segments of mandibular palp.

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Fig. 3—Nauplius 2 (A, C, F, J) and 3 (B, D, E, G-I, K) —A. Ventral view —B. Caudal lobes from ventral —C. Cluster of setae on inner side of labrum —D. Dorsal view —E. Endopod of left side antenna —F. Labrum —G. Lateral view —H. Left antenna —I. Detail of left antenna —J. Proximal endites (naupliar processes) of antennae, distal parts —K. Proximal protopodal endite (naupliar process) of left antenna, distal part. Abbreviations: a1, antennule; a2, antenna 2; ba, basis; co, coxa; de, distal endite of antenna protopod (with seta); do, dorsal organ; en, endopod; ex, exopod; la, labrum; md, mandible; mx, maxillulary bud (or conjoined primordia to maxillule and maxilla, perhaps also involving the paragnaths); pe, proximal endite of antenna protopod (naupliar process).
Fig. 4 caption on following page
closely adjacent, are anteriorly fringed with 3–4 irregular rows of acute teeth (7–9 teeth in each row), and bear posteriorly a large, acute tooth anterior to a row of 4–5 denticulate setae (Fig. 4B–D). In some specimens a few additional setae are present posteriorly on the gnathobase. The proximal segment (basis) of the palp is medially drawn out into two setae, which are not clearly articulated to the segment; of these, the distal seta is longer and, unlike the other, is slender and distally curled into a hook (Fig. 4D,F; possibly an artefact, but consistent for various specimens). Both setae are equipped in their distal third with long setules, which themselves bear fine barbs. The setules (c. 10) on the longer, distal seta are arranged in a collar encircling the seta, while those (c. 11) on the shorter, proximal seta are more unevenly placed. The second segment of the mandibular palp (first segment of either endopod or exopod) has a row of 4–6 short, setulose setae on its ventral face (Fig. 4D,E). The same segment bears a long seta of comparable morphology to those on the basis, with approximately nine setules (Fig. 4D,G), on its distomedial border. Different from the basis setae, this seta has an annulus and the setules appear to be slightly longer. The third and most distal segment of the palp bears three long, slender setae, each with a row of dense setules on the inner side facing the body of the larva and with scattered setules along the outer, opposite side (Fig. 4D,H).

A pair of elongate knobs or swellings fringed with small, medially directed setae, some of which bear setules (Figs 3J,K, 4D), is situated on the latero-ventral corners of the body immediately behind the mandibles. It is uncertain whether they represent the conjoined primordia of the maxillules and maxillae or only the maxillules. They may also represent the paragnaths, at least in part. The posterior part of the body is tube-shaped with parallel sides and terminates in a pair of caudal lobes with an anal opening in between (Fig. 3B,D,G). One clear-cut and consistent morphological difference between stages 2 and 3 (apart from the difference in size) concerns the spination and degree of development of these lobes. In nauplius 2 the caudal lobes are only weakly developed and only very few irregularly placed spines are present (Fig. 3A), whereas in nauplius 3 the caudal lobes have protruded more and are equipped with many more spines of which some are arranged in short rows (Fig. 3B).

Nauplius 4 (Figs 5 and 6)
The body length is 360–392 μm. This stage is in many ways quite similar to the previous two stages, so only the aspects by which it differs are mentioned here. The setation of the small buds of the antennules has increased to one large seta and three very small, undeveloped setae (Fig. 5E). The most striking new feature is the bifid tip of the naupliar process of the antennae (Figs 5A,F–J, 6B). Both branches of this long endite have long, ornamented setules, the 8–12 setules on the anterior branch being arranged as a comb on the anterior side only, while the setules of the posterior branch are arranged unevenly, like the hairs on a bottle-brush and similarly to those on the uniramous endite in Nauplii 2 and 3 (Fig. 5G,H). The posterior branch has an annulus close to its base (Fig. 5J). Close to the base of the entire endite is another constriction or an annulus, a vestige of the more distinct change in thickness seen there in Nauplius 1 (Fig. 5G, arrow). The fourth seta on the antennal endopod, which was small and undeveloped in Nauplius 2 and 3, is now fully developed (Fig. 5F). The mandible is still composed of four segments, but the proximal segment (the coxa) has become much larger (Fig. 6A,B,D). The gnathobase has become more setose and now bears, in addition to a row of four stout setae, four to five additional, smaller setae on its posterior face (Fig. 6C). The two setae of the first segment of the mandibular palp (i.e. the basis) have the same morphology as in the previous stages and bear approximately the same number of setules (Fig. 6B,D,H). The setules on the more distal of these two setae are arranged in a ring encircling the seta, as in the previous stage, while the setules on the proximal seta are arranged somewhat more irregularly. The whole larva has a more elongate appearance. Compared to the previous stage, the relatively slender mid-section is followed by an inflated hind body with more pointed caudal lobes, on which the spination is arranged in 4–5 regular rows (Fig. 5A,B).
Nauplius 5 (Figs 7, 8)

The body length is 407–568 \( \mu m \). This stage is somewhat similar to the previous one, but a number of features set it apart. The most obvious of these is that the Anlagen of the carapace have appeared as a pair of dorso-lateral triangular swellings between the mandibles and the first trunk limbs. Due to the lack of separate maxillules and maxillae, the precise segmental origin of the carapace cannot be determined. The carapace Anlagen make the anterior part of the trunk characteristically broad (Fig. 7A). In addition, the labrum has become more pointed distally (compare Figs 5C and 7C). The setation of the small buds of the antennules has increased to one large seta and four (previously three) very small, undeveloped setae (Fig. 7D). The tip of the mandibular gnathobase has basically the same morphology as in Nauplius 2 (that of Nauplius 4 was not examined in detail): approximately three rows of small spines on the anterior half and a large spine followed by some setae posteriorly (Fig. 7G, I). Different from Nauplius 2, however, are the smaller gap between the anterior spine rows and the posterior large spine, a more gradual change in spine size from anterior to posterior, and a great increase in the number of posterior setae. Furthermore, the number of setae on the median side of the second segment of the mandibular palp has increased from 5–6 in one row to 13–15 in two rows: an anterior row with 9–11 large setae and a smaller posterior row with four setae (Fig. 8A, B). The two pairs of elongate knobs or swellings fringed with small, medially directed setae situated immediately behind the mandibles are more clearly expressed than before (representing either the conjoined primordia of the maxillules and maxillae, or only the maxillules, and, perhaps, also including the paragnaths; we have no basis for judging their precise homologies) (Fig. 7G). Anlagen of the 5–6 anterior trunk limbs can also be recognized (Fig. 7B). In general, various setae have more setules than before. The terminal part of the body appears more inflated than in the previous stage and the caudal lobes are relatively longer and more pointed with the spination arranged in 6–7 regular rows.

Nauplius 6 (Fig. 9)

The body length is about 625 \( \mu m \). This stage is clearly separated from the previous one by the presence of elongate, subdivided trunk limb buds and a small, free carapace reaching approximately the level of the second pair of trunk limbs. The setation of the small buds of the antennules has increased to one large seta and six (previously four) very small, undeveloped setae (not shown). The basal portion of the labrum has become wider relative to the width of the body and the labrum is in general significantly larger than in the previous stages. The number and position of the major setae of the antennae and mandibles are the same as in the previous stage. Behind the mandibles there are now three buds on each side (Fig. 9C, D); a relatively large bud, bearing setae and situated immediately behind the mandibular gnathobase, probably represents the maxillule. Two smaller buds, placed next to each other between the larger bud and the first pair of trunk limb buds, probably represent different parts of the maxilla (perhaps the vestigial maxilla itself and the duct of the maxillary gland?). If so, this would be similar to the vestigial, two-divided maxilla in adults of the Notostraca (see Cannon & Leak 1933). Four pairs of trunk limbs are developed to the extent that all limb parts – five endites, endopod, exopod, and epipod – can be recognized as small, separate limb portions (Fig. 9A–C). A fifth pair of limb buds can be clearly recognized together with the Anlagen of two more. The anterior trunk limbs are the best developed but are still oriented horizontally, their distal ends positioned laterally. Anlagen of setae are clearly present on the endites, endopod, and exopod of the two anterior pairs of limb buds. Each endite has a posterior row of setae with some additional setae in the centre of the endites (not shown in detail for this stage, but see Figs 9 and 10 for a similar arrangement in the next stage). A pair of small, dorsal, telsonal setae has appeared (Fig. 9C). The caudal region – or telson – is more clearly set off from the more anterior part of the body than in the previous stage (Fig. 9A). The caudal spines have become more elongate and pointed and relatively more slender than before (Fig. 9A, C).

Nauplius 7 (Figs 10 and 11)

The body length is 713–760 \( \mu m \). This stage differs from the previous one by its larger carapace, which reaches approximately the level of trunk limbs 3, and in having five (rather than four) pairs of trunk limb buds with developed limb portions. Besides a general enlargement of the body and increase in the number of setules of various setae, a number of other

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**Fig. 6**—Nauplius 4. — A. Lateral view. — B. Basal parts of right antenna and mandible. — C. Gnathobases of mandibles partly hidden by labrum, and region from which buds of supposed maxillules and maxillae will differentiate. — D. Left mandible, arrow pointing to annulation of proximal protopodal endite (naupliar process) of antenna. — E. Setae of mandibular palp segments 2 and 3. — F. Detail of characteristic cluster of setae of segment 2 of mandibular palp. — G. Seta of segment 2 of mandibular palp. — H. Setae of segment 1 (basis) of mandibular palp. — I. Detail of seta of third, distal segment of mandibular palp. Abbreviations: a2, antenna; ba, basis; co, coxa; de, distal endite of antenna protopod; do, dorsal organ; la, labrum; md, mandible; mx, maxillulary bud (or conjoined primordia to maxillule and maxilla, perhaps also involving the paragnaths); pe, proximal endite of antenna protopod (naupliar process); numerals 1–3 indicate segments of mandibular palp.
changes compared to the previous stage can be mentioned. The antennules are now a pair of distinct globular buds, each with one fully developed seta and six small, undeveloped setae (setation as in previous stage) (Fig. 10G). The endopod of the antennae is now clearly two-segmented but is still superficially divided into some smaller portions distally (Fig. 11A). The coxa of the mandible is now very large compared to the palp (Fig. 11B). The tip of the gnathobase is much like that of Nauplius 5, but the anterior spination is denser and the spines tend to appear in groups of three (see inset in Fig. 11E). The five anterior pairs of limb buds have well-differentiated limb portions, and approximately three more pairs of limb buds can be recognized (Fig. 10A, B, D, F, H, J). The anterior limbs are the most fully developed. The proximal endites of the first pair are relatively large with incipient setation along the medio-posterior margin. These endites are somewhat offset from the other limb buds and point inward, perpendicular to the main axis of the body. The more distal four endites are quite similar to, but smaller than, the proximal endite, each with a cluster of setal Anlagen vaguely arranged in two rows. The endopod, located distally on the limb bud, is small, less than half the size of each endite, and bears about three distal setal Anlagen. The exopod is considerably larger than in Nauplius 6 and projects distally with incipient setation along the distal and lateral margins. The epipod, which is attached proximal to the exopod, has also attained a considerable size, about double the size of an endite. Posteriorly along the body, the limb buds become more and more undeveloped until the small buds of the sixth pair of limbs are only slightly elevated and subdivided. The caudal spines are elongate and slender (as in previous stage) with rows of spines along the proximal half but largely lack spines along the distal half (Fig. 10I).

Discussion

Comparison with the larval development of other limnadiids

Seven naupliar stages are described here for Eulimnadia braueriana. A close comparison of its naupliar development with those of congeneric species is appropriate. Sars (1896) provided the most detailed description of the development of any species of the Limnadiidae until now. He depicted seven different naupliar stages of Limnadia lenticularis, but he did not state unambiguously that this was the full number of stages. The general morphology of the respective larval stages is very similar to that of E. braueriana. Some of these similarities, such as the long, pointed labrum (relatively longer in L. lenticularis) and the fact that the trunk limbs do not appear externally until a number of stages has passed, are apparently typical for at least some limnadiids, while other similarities are shared with other spinicaudatans or even with other branchiopods. Three other purportedly complete limnadiid naupliar series have been reported: Eulimnadia texana supposedly has six naupliar stages, including two orthonauplii and four metanauplii (Strenth and Sissom 1975), and Limnadia stanleyana supposedly has five, including two orthonauplii and three metanauplii (Anderson 1967), while Eder's (in press) describes five stages for Imnadia yeyetta. Strenth and Sissom (1975) did not state precisely how staging was determined in their study. Anderson's 1967 work was based in part on individual rearing to the early fourth stage, with escape from the inner egg membrane occurring at either the first or second stage, and in part on mass-cultured and field-collected larvae. Eder's (in press) study was based on mass-cultured larvae.

The earliest described stages of L. lenticularis and E. braueriana are very similar. They are both clearly nonfeeding, with a yolk-inflated hind-body and poorly developed setae on the feeding appendages. The second-stage nauplii in the two species are also very similar; the body is more slender than in Nauplius 1 and the various setae and spines have developed setulation and are now prepared to function in feeding. The larval sequence from Nauplius 3 to 7 is not precisely the same in these two species, although the proximal naupliar process of the antenna becomes bifid between Nauplius 3 and 4 in both species. Nauplius 4 of E. braueriana has no clearly equivalent stage in the sequence described for L. lenticularis. The fourth stage of the latter looks more like Nauplius 5 of E. braueriana in terms of the development of the trunk limbs and carapace; Anlagen of both are present, and this makes the anterior part of the trunk appear broad. On the other hand, Sars (1896) described three stages for L. lenticularis in which the trunk limb buds are visible externally, namely stages 5 to 7, whereas this is only the case in Nauplius 6 and 7 of E. braueriana. Nauplius 6 of E. braueriana apparently corresponds closest to stage 5 of L. lenticularis (based on carapace size), while Nauplius 7 of the former corresponds closest to stage 6 or 7 of the latter.

Nauplii of E. texana and E. braueriana are rather similar. Despite some heterochronic differences, correspondences between naupliar stages are easier to draw than between E. braueriana and L. lenticularis. Only one stage was described of E. texana corresponding to Nauplius 2 and 3 of

Fig. 7—Nauplius 5.A. Ventral view—B. Trunk limbs of right side—C. Labrum —D. Left antennule —E. Caudal region—F. Lateral view—G. Gnathobases of mandibles and region of maxillules and maxillae (not yet differentiated into buds) —H. Lateral view showing coxa of mandible, early carapace fold, and early rudiments of anterior trunk limbs—I. Close-up of mandibular gnathobases. Abbreviations: a2, antenna; ca, carapace; co, coxa; de, distal endite of antenna protopodium; do, dorsal organ; en, endopod; ex, exopod; md, mandible; mx, maxillulary bud (or conjoined primordia to maxillule and maxilla, perhaps also involving the paragnaths); pe, proximal endite of antenna protopod (naupliar process); tl1, trunk limb 1.
Larval development of *Eulimadia* • Olesen and Grygier


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**Fig. 8**—Nauplius 5 —A. Left mandibular palp —B. Left mandible and proximal protopodal endite of antenna —C. Proximal protopodal endite of left antenna —D. Right antenna and mandible —E. Setae of antennal endopod and exopod —F. Distal protopodal endite of antenna with seta —G. Detail of same. Abbreviations: a2, antenna; ba, basis; co, coxa; de, distal endite of antenna protopod; en, endopod; ex, exopod; md, mandible; pe, proximal endite of antenna protopod (naupliar process); numerals 1–3 indicate segments of mandibular palp.
*E. braueriana*. Otherwise, the stages match except for the following points. The first three stages of *E. texana* are much bigger than the corresponding stages of the present species, e.g. Nauplius 1 is 230 μm long vs. 156 μm, and Metanauplius 1 is 400–470 μm long vs. 360–392 μm for the present Nauplius 4. There are no setae on the antennular rudiments at any stage in *E. texana*. The caudal spines are considerably shorter in *E. texana*, and in some final-instar individuals they are articulated as ‘cercopods’, unlike those of *E. braueriana*. Natatory setae in *E. texana* are described as simple in Nauplius 2, whereas they bear setules in Nauplius 2 and 3 in *E. braueriana*. The naupliar process of the antenna becomes bifid in the third-stage larva of *E. texana* and its equivalent Nauplius 4 of the present species, but, as noted above for *L. lenticularis*, the beginnings of carapace development and traces of about three trunk segments are apparent as well at this stage (not until the following stage in *E. braueriana*). While *E. braueriana* has exposed limb buds with *Anlagen* of setae in both of its final naupliar instars, *E. texana* has them only in the last stage. There is some evidence, though, that Streth and Sissom (1975) have confounded certain stages: (1) in the third-stage larva (Metanauplius 1) the number of delimited trunk segments behind the region giving rise to the carapace varied between zero and three; (2) only in some individuals of Metanauplius 3 (the stage-5 larva) was a newly formed minute outer spine seen on the exopod of the antenna (no equivalent in *E. braueriana*); and (3) only in some last-stage individuals were the caudal spines articulated.

Nauplii of *Limnadia stanleyana* are similar to nauplii of *Imnadia yeyetta* in having a rather short, spatulate labrum instead of a lanceolate one as in *E. braueriana* and *L. lenticularis*. The nauplii of *Limnadia stanleyana* are also much bigger than those of *E. braueriana*, ranging from 400 μm long in Nauplius 1 to 800 μm in the final Nauplius 5. There is a single seta on the antennal rudiment, at least through Nauplius 4, whereas additional elements develop in *E. braueriana*. Many aspects of timing of development differ between these two species, and it is not possible to match Nauplii 2–4 of *L. stanleyana* precisely with stages of *E. braueriana*. Nauplius 1 is similar in form in both species, but in the former the natatory setae are already articulated at the base and there are four (not three) distal setae on the antennal endopod. The innermost of these setae is still short in Nauplius 2 of *L. stanleyana*, and the body shape at that stage is reminiscent of Nauplius 2 and 3 of *E. braueriana*. Nauplius 3 of *L. stanleyana* agrees with Nauplius 4 of *E. braueriana* in having a bifid naupliar process of the antenna, but, like *L. lenticularis* and *E. texana*, and differing from *E. braueriana*, this is already accompanied by the development of paired carapace rudiments and the beginning of (in this case, six) segmental subdivisions of the trunk. On the other hand, the development of posterior telsonic spines is delayed in *L. stanleyana*; they don’t appear until Nauplius 4 and are much smaller than those of *E. braueriana*. As in *E. texana*, trunk limb buds are not exposed until the final naupliar instar in *L. stanleyana*; also, the separate rudiments of the maxillules and maxillae are only exposed at this stage (in the last two stages in *E. braueriana*).

The only other SEM-based description of limnadiid nauplii is of *Imnadia yeyetta* by Eder (in press). The larval sequence differs from that of *E. braueriana* in many respects, including a different labrum morphology (spatulate versus lanceolate), different number of stages, and different timing in the appearance of various structures. The size range of the larvae in the two species is approximately the same. A striking difference is that in contrast to the seven nauplii recognized for *E. braueriana*, only five were recognized by Eder (in press). Nauplius 1 is very similar in both species with their lecithotrophic appearance. Nauplii 2 and 3 are also similar, at least in general proportions of the body. It is important to note, however, that the proximal process of the antenna has become bifid already in Nauplius 3 of *I. yeyetta*, while this does not happen until Nauplius 4 of *E. braueriana*. In both species, externally visible limb buds are present in the two last stages, but in *E. braueriana*, five stages have passed before this situation appears, whereas only three stages have passed in *I. yeyetta*. Furthermore, it is difficult to find a complete match between these two last stages. The very last stages of both species are similar with respect to the number of developed limb buds clearly subdivided into enditic portions (5–6 limbs), but the carapace is larger in *E. braueriana* than in *I. yeyetta*. Of the penultimate stages in the two species, that of *E. braueriana* is the most developed as it has partly free limb buds and a larger carapace. Hence, the major differences between the two species are in the middle phase of the development, where two more stages have been identified for *E. braueriana* compared to *I. yeyetta*.

A few larval stages have been described for *Imnadia voitestii* by Botmuriuc (1948) and for *Eulimnadia stoningtonensis* by Berry (1926), but as no complete treatment of the whole larval sequence has been attempted in these two cases, no comparison will be provided here.

Precise correspondence among the naupliar stages of the species compared above is difficult to establish. Some of the differences in detail in the descriptions may be attributable to the use of different methods (SEM vs. light microscopy), but others are probably real. Three possibilities exist to explain the divergence in early larval sequences between *E. braueriana* and the three mentioned species: (1) the described sequences reflect true differences in their larval development; (2) earlier authors failed to recognize two morphologically similar early stages for their species; and (3) we have recognized too many larval stages in the early sequence of *E. braueriana* herein. Indirect support for our staging of *E. braueriana* is provided by the timing of the appearance of a bifid naupliar process on the antenna in Nauplius 4 of both this species and *L. lenticularis* (if Nauplius 2 and 3 of *E. braueriana* are indeed distinct). However, the bifid tip appears in third-stage larvae of both *E. texana* and *L. stanleyana*.
All three of these earlier described species differ from *E. braueriana* in developing the bifid tip of the naupliar process, rudimentary carapace lobes, and the beginnings of trunk segmentation simultaneously. The latter two features are delayed one further molt in *E. braueriana*. This difference is very clear-cut, but its phylogenetic significance cannot yet be ascertained. A related point is that we are not able to exclude completely the possibility that three stages (instead of only two) with externally demarcated limb buds in the late part of the larval sequence are present in *E. braueriana* (which would then be similar to *Eulimnadia lenticularis* as described by Sars (1896); see comparison between these two species above). One specimen treated as Nauplius 7 actually had better developed trunk limb setation than the other stage-7 nauplii examined and perhaps we should have interpreted it as a later stage, Nauplius 8. Two other apparently real differences, which will have to be taken into account in any future phylogenetic analysis, can be mentioned. Limnadiids apparently differ in the number of late naupliar stages in which buds of the trunk limbs are visible externally: one in *L. stanleyana* and *E. texana*, two in *E. braueriana*, three in *L. lenticularis*, and one in *I. yeyetta*. They also appear to differ in the presence and number of antennular setae in the nauplii: no setae in *E. texana*, one seta in *L. stanleyana*, and five setae in *E. braueriana*, but this may be due in part to different investigation techniques. Another interesting difference among species of the Limnadidae concerns the shape of the larval labrum. In *I. yeyetta*, *I. coxestii*, and *Limnadia stanleyana* the labra are more or less short and spatulate, whereas the labra are elongate, pointed, and lanceolate in *E. texana*, *L. lenticularis*, and *E. braueriana*. The latter morphology should be treated as a possible synapomorphy for certain taxa of the Limnadidae.

**Comparison with other branchiopods**

A closer comparison with other branchiopods, and in particular with other ‘conchostracans’, will be delayed until later, but some general comments can be provided here. In terms of general morphology, the larvae of *Eulimnadia braueriana* possess the characteristics recognized as synapomorphies of the Branchiopoda by Olesen (in press). One of the most important of these is a naupliar feeding apparatus that includes (1) very large second antennae, each with two characteristic protopodal endites with manipulatory spines or setae, the more proximal of the two becoming bifid at approximately the same molt in all taxa, and (2) a uniramous, three-segmented palp of the mandible. Another important synapomorphy that *E. braueriana* shares with other Recent branchiopods is the form of trunk limb development, beginning as elongate, laterally placed limb buds, which become subdivided into a number of endites before shifting to an ‘upright’, vertical orientation (Olesen in press).

The extent to which the present study’s findings can contribute to a better understanding of the phylogeny of the taxa previously known as the Conchostraca is uncertain, because only *Cyclestheria hislopi* Baird 1859, is known in similar detail (see Olesen 1999). It is clear, however, that the development of the trunk limbs in *E. braueriana* is practically identical to that of *C. hislopi*: both have laterally placed limb buds that become gradually divided into a number of subparts. The buds have exactly the same components with the same relative sizes and develop in the same order in the two taxa. One difference is that trunk limb buds are present along the whole length of the body in *C. hislopi* in quite early stages, whereas the appearance of externally delimited trunk limbs in *E. braueriana* is delayed until Nauplius 6. A number of other features are quite similar for at least the late stages of *C. hislopi* and *E. braueriana*, including (1) similarly small limb buds of the antennules, (2) similar formation of the lateral, paired carapace primordia in a region between the mandibles and the first pair of trunk limbs, corresponding to the region of the future maxillules and maxillae. Many differences are also present, most notably that the developmental stages of *C. hislopi* lack all modifications for feeding activity since they are all passed through in the embryo.

*Eulimnadia braueriana* and *C. hislopi* appear closely related based on the many similarities that have been mentioned; probably, however, the similarities are synapomorphies if the sister-group relationship suggested for *C. hislopi* and the Cladocera is correct (e.g. Martin and Cash-Clark 1995; Olesen et al. 1997; Olesen 1998; Negrea et al. 1999; Spears and Abele 2000; Braband et al. in press; see also Martin and Davis 2001 for a review). On the evolutionary level, and based on the mentioned similarities between the two species, it is not difficult to infer how the embryonized direct mode of development seen in *C. hislopi* could have evolved from the mode of development with free-swimming larvae seen in *E. braueriana*. In this context it is striking to note that in the only report of free-living larvae of *C. hislopi* the illustrated larva has a pointed labrum (Botnariuc and Viña Bayés 1977), exactly like the ones in larvae of *E. braueriana* and a number of other limnadiid nauplii described.

More precise considerations on this subject will be provided when the larval sequences have been described for species of the remaining three families of the ‘Conchostraca’.

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**Fig. 9**—Nauplius 6 — **A**. Ventral view — **B**. Trunk limbs of right side, close-up of A — **C**. Lateral view — **D**. Mandibular region showing proximal endites of antennae, proximal parts of mandibles, and buds of maxillules and maxillae — **E**. Right maxillule and maxilla, latter consisting of two separate buds — **F**. Left antenna and mandible — **G**. Seta of distal protodotal endite of antenna, endopodal setae, and one exopodal seta of antenna. Abbreviations: a1, antennule; a2, antenna; ca, carapace; de, distal endite of antenna protopod; la, labrum; md, mandible; mx1, maxillule; mx2, maxilla; pe, proximal endite of antenna protopod (naupliar process); t1, trunk limb 1; t4, trunk limb 4; numerals 1–3 indicate segments of mandibular palp.
the Cyzicidae, Leptestheriidae, and Lyncidae. As the first step towards meaningful evolutionary speculation is to establish a robust phylogeny, it will be necessary to include a larger taxonomic sampling of the ‘Conchostraca’ in the analysis.

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References


Fig. 10—Nauplius 7 — A. Ventral view — B. Lateral view — C. Cluster of setae on inner side of labrum — D. Left trunk limbs 1–6, ventral view — E. Caudal region, dorso-lateral view — F. Lateral view showing posterior margin of carapace and anterior trunk limbs of right side — G. Right antennule — H. Lateral view showing six right trunk limbs at differing degree of development

—I. Caudal region, ventral view. Abbreviations: a1, antennule; a2, antenna; ca, carapace; do, dorsal organ; e1–e5, endites 1–5; en, endopod; ep, epipod; ex, exopod; la, labrum; md, mandible; tes, telsonal setae; tI1, trunk limb 1; tI5, trunk limb 5; tI6, trunk limb 6; numerals 1–3 indicate segments of mandibular palp.
Fig. 11—Nauplius 7 —A. Antenna of left side —B. Mandible and proximal protopodal endite (naupliar process) of right antenna, with arrow pointing to annulation of endite —C. Proximal endite of protopod of left antenna, with arrow pointing to annulation of posterior prong —D. Detail of same —E. Gnathobases of mandibles; insertion is a close-up of spination of mandibular coxa —F. Two setae of segment 1 (basis) of mandibular palp —G. Detail of characteristic cluster of setae of segment 2 of mandibular palp —H. Detail of distal part of seta on segment 2 of mandibular palp —I. Setae on distal segment of mandibular palp. Abbreviations: a2, antenna; ba, basis; ca, carapace; co, coxa; en, endopod; ex, exopod; la, labrum; md, mandible; pe, proximal endite of antenna protopod (naupliar process); te, telson; tl1, trunk limb 1; numerals 1–3 indicate segments of mandibular palp.


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