

# EARLY POST-EMBRYONIC DEVELOPMENT OF MARINE CHELICERATES AND CRUSTACEANS WITH A NAUPLIUS

BY

FRANK D. FERRARI<sup>1,5</sup>), JOHN FORNSHELL<sup>1,6</sup>), ALEJANDRO A. VAGELLI<sup>2,7</sup>),  
V. N. IVANENKO<sup>3,8</sup>) and HANS-UWE DAHMS<sup>4,9</sup>)

<sup>1</sup>) IZ/MSC, MRC-534, National Museum of Natural History, Smithsonian Institution,  
4210 Silver Hill Road, Suitland, Maryland 20746, U.S.A.

<sup>2</sup>) New Jersey Academy for Aquatic Sciences, 1 Riverside Drive, Camden,  
New Jersey 08103, U.S.A.

<sup>3</sup>) Department of Invertebrate Zoology, Biological Faculty, Moscow State University,  
Moscow 119899, Russia

<sup>4</sup>) Green Life Science Department, College of Convergence, Sangmyung University,  
7 Hongij-dong, Jongno-gu, Seoul 110-743, South Korea

## ABSTRACT

Crustaceans that hatch as a nauplius-like larva, as well as xiphosuran and pantopodan chelicerates, are surveyed for five characters: presence or absence of arthrodial membranes separating somites; ventral configuration of the protopod of the second limb; number of transformed (segmented) limbs and limb buds; addition of segments to transformed limbs; fate of limb buds. An arthrodial membrane separates somites 7 and 8 of xiphosurans, a small knob articulates on the protopod of the second limb, and there are nine pairs of limbs but no limb buds. During early development, no arthrodial membranes are added, nor are segments added to limbs 1-9; limbs 10-14 are added as transformed limbs, not as limb buds, after several molts. On the post-embryonic larva of the presumed ancestral pantopodan, arthrodial membranes did not separate adjacent somites, the proximal segment of limb 2 was simple, there were three transformed limbs and no limb buds. During subsequent molts, arthrodial membranes separated somites 4-7, buds of limbs 4-7 were added in register with each molt, limb buds were reorganized in register into transformed limbs during the following molt, and two segments were added to each transformed limb in register during the next molt.

Somites of most crustacean taxa that hatch as a nauplius-like larva are not separated by arthrodial membranes on early post-embryonic stages; exceptions are posterior somites of branchiurans, mystacocaridans and cephalocaridans. Limb 2 (antenna 2) of branchiopods, copepods, thecostracans, mystacocaridans and cephalocaridans bears a naupliar arthrite on the ventral face of the coxa, on branchiurans there is an attenuation, or spine-like outgrowth, on the ventral face, and on

---

<sup>5</sup>) Corresponding author; e-mail: ferrarif@si.edu

<sup>6</sup>) e-mail: johnfornshell@hotmail.com

<sup>7</sup>) e-mail: avagelli@njaas.org

<sup>8</sup>) e-mail: ivanenko@mail.bio.msu.ru

<sup>9</sup>) Co-corresponding author; e-mail: hansdahms@smu.ac.kr

ostracodes, remipedes, euphausiaceans, mysidaceans and dendrobranchiate decapods there is no ventral structure. Most of these crustaceans hatch with three transformed limbs; exceptions include myodocopan ostracodes with six and branchiurans with seven. The number of limb buds varies from none on remipedes to three on branchiurans and cephalocaridans. Limb buds of podocopan ostracodes, branchiurans, mystacocaridans and cephalocaridans are reorganized into transformed limbs after one molt; reorganization of limb buds of branchiopods, copepods, thecostracans, remipedes, euphausiaceans, mysidaceans and dendrobranchiates are delayed several molts.

Early post-embryonic development of pantopodan chelicerates is more similar to that of podocopan ostracodes than it is to that of any other crustacean taxon, and no crustacean exhibits an early post-embryonic development similar to that of xiphosurans. Within the crustacean lineage, articulating somites, a naupliar arthrite or ventral attenuation on the coxa of antenna 2, and delayed reorganization of limb buds are apomorphies. Absence of a naupliar arthrite on ostracodes is inherited from the last stem lineage arthropod but its absence on remipedes and malacostracans is derived, and perhaps more than once.

### ZUSAMMENFASSUNG

Crustaceen die als eine dem Nauplius-ähnliche Larve schlüpfen werden im Vergleich mit den Xiphosura und Pantopoda in Hinblick auf die folgenden 5 Merkmale hin untersucht: Vorhandensein oder Fehlen von die Somite trennenden Gelenkmembranen; ventrale Ausgestaltung des Protopoditen der 2ten Extremität; Anzahl der transformierten segmentierten Extremitäten und Extremitätenanlagen; Entwicklung von Segmenten der transformierten Extremitäten; Ausgestaltung der Extremitätenanlagen. Eine Gelenkmembran trennt die Somiten 7 und 8 bei den Xiphosura, eine kleine Erhebung ist gelenkig mit dem Protopoditen der 2ten Extremität verbunden; 9 Extremitätenpaare sind hier vorhanden aber keine weiteren Extremitätenanlagen. Während der frühen Embryonalentwicklung werden keine zusätzlichen Gelenkmembranen ausgebildet, noch werden Segmente zu den Extremitäten 1-9 ergänzt; nach mehreren Häutungen werden die voll ausgebildeten transformierten Extremitäten 10-14 ergänzt. Postembryonalstadien des hypothetischen Pantopoden Vorfahren haben keine separaten Gelenkmembranen auf den anliegenden Somiten; das proximale Segment der 2ten Extremität ist einfach gebaut; es gibt 3 transformierte Extremitäten aber keine weiteren Extremitätenanlagen. Während nachfolgender Häutungen trennen Gelenkmembranen die Somiten 4-7; Anlagen der Extremitäten 4-7 werden in Serie mit jeder Häutung ergänzt.

Die Somite der meisten Crustaceentaxa, die als Nauplius-ähnliche Larve schlüpfen, weisen keine Gelenkmembranen während der frühen Postembryonalentwicklung auf; Ausnahmen sind die hinteren Somiten bei den Branchiura, Mystacocarida und Cephalocarida. Extremität 2 (= Antenna 2) der Branchiopoda, Copepoda, Thecostraca, Mystacocarida und Cephalocarida weisen einen nauplialen Coxalfortsatz ventral auf. Branchiura weisen keinen solchen ventralen Fortsatz auf. Auch bei den Ostracoda, Remipedia, Euphausiacea, Mysidacea und den Dendrobranchiata unter den Decapoda gibt es keine solche ventrale Struktur. Die meisten dieser Crustaceen schlüpfen mit 3 transformierten Extremitäten; Ausnahmen bilden myodocope Ostracoda mit 6 und Branchiura mit 7 transformierten Extremitäten. Die Anzahl der Extremitätenanlagen variiert zwischen 0 bei den Remipedia und 3 bei den Branchiura und Cephalocarida. Extremitätenanlagen podocoper Ostracoda, Branchiura, Mystacocarida und Cephalocarida werden nach einer Häutung transformiert. Diese Transformation ist für mehrere Häutungen verzögert bei den Branchiopoda, Copepoda, Thecostraca, Remipedia, Euphausiacea, Mysidacea und Dendrobranchiata.

Die frühe Postembryonalentwicklung bei den Pantopoda (Chelicerata) ist der podocoper Ostracoda ähnlicher als der anderer Crustacea. Kein Crustaceentaxon weist eine den Xiphosura ähnliche Postembryonalentwicklung auf. Apomorphien der Crustaceensektion sind: gelenkig verbundene Somite, nauplialer coxaler ventraler Fortsatz der Antenna 2 und eine verzögerte Transformation der Extremitätenanlagen. Das Fehlen des nauplialen Coxalfortsatzes wird als Merkmal von der

letzten Stammgruppe der Arthropoda übernommen; das Fehlen des nauplialen Coxalfortsatzes bei den Remipedia und Malacostraca ist abgeleitet und hat sich möglicherweise mehrmals ereignet.

## INTRODUCTION

Characters expressed during early post-embryonic development of crustaceans continue to inform crustacean phylogenetic analyses (Høeg et al., 2009; Ferrari et al., 2010). However, there remain many attributes of crustacean development that have yet to be surveyed and assessed. Among these are five characters that have been of particular interest in copepod development (Ferrari & Dahms, 2007): presence or absence of arthrodial membranes separating somites; configuration of the protopod of the second limb; number of transformed (segmented) limbs and limb buds; addition of segments to transformed limbs; and fate of limb buds. Here the states of these five characters are determined from the literature or direct observations for crustaceans that hatch as a nauplius-like larva. For analytical purposes, the states of these characters also are determined by direct observations for the pantopodan chelicerate *Ammothea glacialis* (Hodgson, 1907) and the xiphosauran chelicerate *Limulus polyphemus* Linnaeus, 1758.

## METHODS

Egg-clutches of *Limulus polyphemus* were collected at Reeds Beach, Cape May, NJ, U.S.A. (39.12°N 74.89°W) on 19 June 2007 at ~12PM. Several nests were identified by slight elevations in the sand with an approximate circular shape, and sampled nests were located at about 1.5 m (2 nests) to 3 m (1 nest) from the water line at the time of collection; eggs were buried at a depth of about 20-40 cm. Eggs were collected from three nests containing embryos that showed the least embryonic development, as judged by the grey-greenish color, and naked-eye inspection of embryos. Eggs were assumed to have been fertilized the previous night. A large egg-cluster (containing a few hundred eggs) was removed from each nest, and placed into a separated container with wet sand extracted from the nest. For daily observation of embryonic development, ten eggs from each cluster were randomly removed and placed into separated 10-cm Petri dishes containing a very thin layer of sand and seawater. Dishes were kept at room temperature (~25°C) throughout the study period. Observations on embryo development were made with an Olympus SZH (7.5-64×) dissection microscope and documented photographically using a digital Nikon Coolpix 5000 camera attached to the microscope. These fixed and preserved cultured specimens are from USNM 1146746. Specimens of *L. polyphemus* larger than cultured specimens

were examined from accessioned collections of the National Museum of Natural History, Washington, D.C.

Embryo-bearing males of *Ammothea glacialis* from the cataloged samples (USNM 123118, 122300), identified by C. Allan Child, were examined to obtain embryos and early larval stages. The embryo-carrying male used as the source of three larval stages in this study was originally collected from McMurdo Sound in the Ross Sea near the tip of Cape Armitage (77.86°N 166.67°E) at a depth of 5.5 m on 23 January 1959. Specimens initially were fixed in formaldehyde and subsequently preserved in 70% ethanol. Embryos and post-embryonic larvae were collected from limb 3 of the male. Stages were sorted by considering the configuration of the anterior three appendages and the walking legs, and three morphologically distinct stages were separated. Some of the embryos were teased open to reveal a post-embryonic stage equivalent to the least developed stage among the post-embryonic larvae.

Immature specimens of the cephalocaridan *Hutchinsoniella macracantha* Sanders, 1955 were taken from cataloged samples (USNM 112848, 279803, 279782) collected in Lydonia Canyon, Georges Bank, U.S.A. (40.54°N 67.74°W) and identified by Thomas E. Bowman. Hatchlings of *Argulus americanus* Wilson, 1902 (USNM 74307) were cultured in aquaria in Ann Arbor, Michigan, during July 1902 and identified by Charles Branch Wilson; adults (USNM 310398) were cultured in aquaria in Montreal, Quebec, Canada, in February 1970 and identified by William J. Poly. An unidentified cirripede nauplius stage VI was sorted from an 84  $\mu\text{m}$  mesh plankton sample in Link Port Dredge Channel off Indian River Lagoon, north of Ft. Pierce, FL, U.S.A. (27.54°N 80.35°W) on 5 May 1999. Early stages of the cirripede *Chelonibia testudinaria* Linnaeus, 1758 (USNM 1146761) were released from a female attached to the turtle *Caretta caretta* (Linnaeus, 1758) on 28 June 1992 in the vicinity of Minabe, Wakayama, Japan (33.77°N 139.32°W), and identified by Mark J. Grygier. Stage II of the ascothoracidan *Zibrowia* sp. (USNM 1146762) was cultured and identified by Mark J. Grygier from local specimens at the Sesoko Marine Science Center, Okinawa, Japan (26.63°N 127.87°W) on 23 June 1989. The facetotectans *Hansenocaris furcifera* Itô, 1989 and Pacific Type I of Itô (1986) were separated from a plankton sample (USNM 1146764) collected by Mark J. Grygier in the vicinity of the channel near the Seto Marine Biological Laboratory, Wakayama, Japan (33.68°N 135.35°E) on 04 September 1991. *Hansenocaris furcifera* 0.22 mm is stage II nauplius; a 0.15-mm stage I nauplius, without the bud of Mx1, also was examined; the stage of Pacific Type I is unknown.

Specimens were cleared in lactic acid and stained with chlorazol black E, and dissected and observed with a dissecting microscope or a compound microscope with camera lucida. Photomicrographs using a 1.3 megapixel digital camera and

scanning electron micrographs were also produced to facilitate descriptions of *A. glacialis*.

These terms used herein have the following definitions:

**Attenuation:** dorsal or ventral projection, often pointed, of an appendage segment.

**Limb bud:** lobe-like structure which does not articulate with its somite, and usually bears at least terminal setae of rami; earliest step of a developing limb.

**In register:** repeated during consecutive molts.

**Naupliar arthritis:** a ventrally articulating element originating on the proximal segment (coxa) of the protopod of limb 2 of a crustacean, moved by a pair of intrinsic muscles each originating on the dorsal face of the coxa and inserting proximally on the anterodorsal or posterodorsal rim of the arthritis (Ferrari & Dahms, 2007); it is absent on juvenile stages or adults, and its loss during development often identifies the termination of a naupliar phase of development (Sanders & Hessler, 1964; Hessler & Sanders, 1966; Addis et al., 2007; Ferrari & Dahms, 2007).

**Transformed limb:** reorganized limb bud with segmented protopod and rami.

The following correspondences for limbs of xiphosurans, pantopodans and crustaceans follow Brenneis et al. (2008) and Mittmann & Scholtz (2003):

**Limb 1:** chelicerae or first limb on the prosoma of xiphosurans or chelophore of pantopodans; antenna 1 of crustaceans.

**Limb 2:** pedipalp of xiphosurans; palp of pantopodans; antenna 2 of crustaceans.

**Limb 3:** walking limb 1 of xiphosurans; oviger of pantopodans; mandible of crustaceans.

**Limb 4:** walking limb 2 of xiphosurans; walking limb 1 of pantopodans; maxilla 1 of crustaceans.

**Limb 5:** walking limb 3 of xiphosurans; walking limb 2 of pantopodans; maxilla 2 of crustaceans.

**Limb 6:** walking limb 4 of xiphosurans; walking limb 3 of pantopodans; trunk limb 1 of crustaceans.

**Limb 7:** chilarium, or posterior limb of prosoma of xiphosurans; walking limb 4 of pantopodans; trunk limb 2 of crustaceans.

**Limb 8:** genital operculum, or anterior limb of opisthosoma, of xiphosurans; walking limb 5 of some pantopodans; trunk limb 3 of crustaceans.

**Limb 9:** book gill 1 of xiphosurans; walking limb 6 of some pantopodans; trunk limb 4 of crustaceans.

**Limbs 10-13:** book gills 2-5 of xiphosurans; never present on pantopodans; trunk limbs 5-8 of many crustaceans.

## RESULTS

***Limulus polyphemus* Linnaeus, 1758**

The first post-embryonic stage (figs. 1A, 3A) is released from the extra-embryonic shell (fig. 2A, B), an envelope of non-cellular material (Sekiguchi et al., 1982), as free-living larvae after the last of two embryonic molts (fig. 2C, D). The body, length 3.89-4.09 mm, is divided by an arthrodistal membrane into an anterior prosoma with 7 limbs and a posterior opisthosoma with 2 limbs.

Limb 1 (fig. 4A): 3-segmented; proximal segment simple, not extended dorsally.

Limb 2: similar to limbs 3-5 except protopod proximally with a small articulated knob and no proximal endite (fig. 4B).

Limbs 3-5: protopod with a basis of two indistinct lobes plus a well-developed endite proximal to basis; endite attached to ventral body wall and not originating on a central proximodistal shaft (fig. 4C). Endopod 5-segmented; proximal segment with 2 ventral setae, penultimate segment with proximoventral seta, penultimate and distal segments forming a chela; attenuation of penultimate segment not reaching tip of distal segment.

Limb 6 (fig. 4D): 1-segmented protopod; tooth-like attenuations proximoven- trally; endite absent. Endopod 6-segmented; proximal segment with 2 ventral setae; adjacent segment without setae but with dorsal denticles; segment 3 with 1 ven- tral seta and dorsal denticles; antepenultimate segment with four terminal setae equal in length; penultimate segment with 1 distoventral seta, shorter than distal segment; muscle pair within penultimate segment inserting proximally on atten- uate distal segment. Exopod 2-segmented; proximal segment originating between anterior and posterior protuberances of the protopod; distal segment (flabellum) elongate.

Limb 7 (fig. 5A): unsegmented with 2 large terminal and 2 small ventral attenuations.

Limb 8 (figs. 3A, 5B): protopod of proximal, triangular coxa with small dorsal setae, and distal quadrangular basis with long dorsal setae. Exopod broad, unsegmented with setae along margins. Endopod a triangular, proximal segment not articulating with the protopod and with ventral setae, and distal segment unarmed, finger-like. Contra-lateral limb pair coupled at distal edge of basis.

Limb 9 (fig. 5C, D): protopod of proximal coxa and distal basis incompletely articulated with long dorsal setae and 4 flap-like lamellae posterio proximally, decreasing in size posteriorly. Exopod broad, unsegmented with distal notch and setae along margins. Proximal segment of endopod with setae, not articulating with the protopod; middle quadrate segment with setae; distal rounded segment with setae. Contra-lateral limb pair coupled along edge of protopod and including ventral finger-like attenuation.

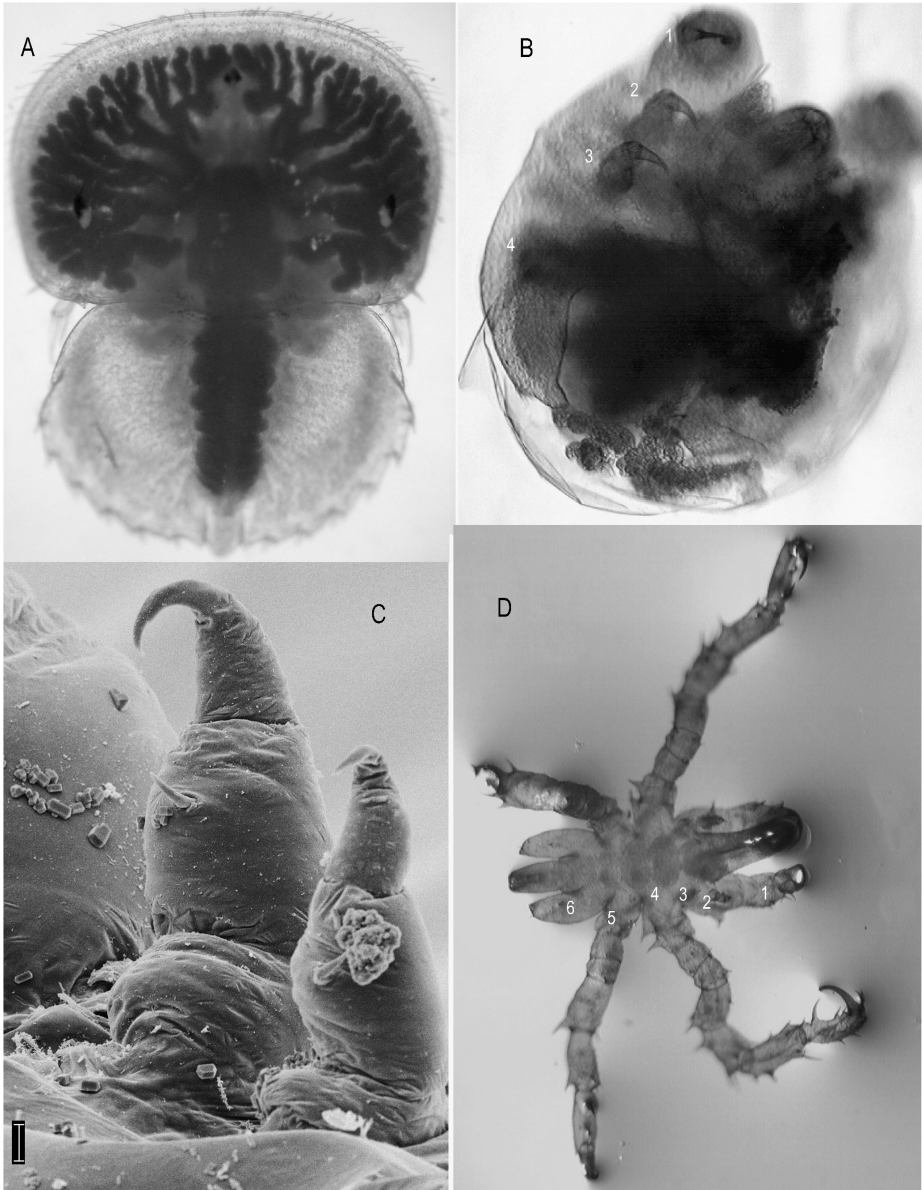


Fig. 1. *Limulus polyphemus* Linnaeus, 1758: A, first post-embryonic stage released from extra-embryonic shell, dorsal, anterior up, image 25X. *Ammothea glacialis* (Hodgson, 1909): B, first post-embryonic stage, ventral, anterior up and right, right limbs numbered, image 135X; C, limb 2, left, and limb 3; D, third post-embryonic stage, ventral, anterior right, with two transformed walking legs (limbs 4-5) and bud of limb 6, left limbs numbered, image 60X.

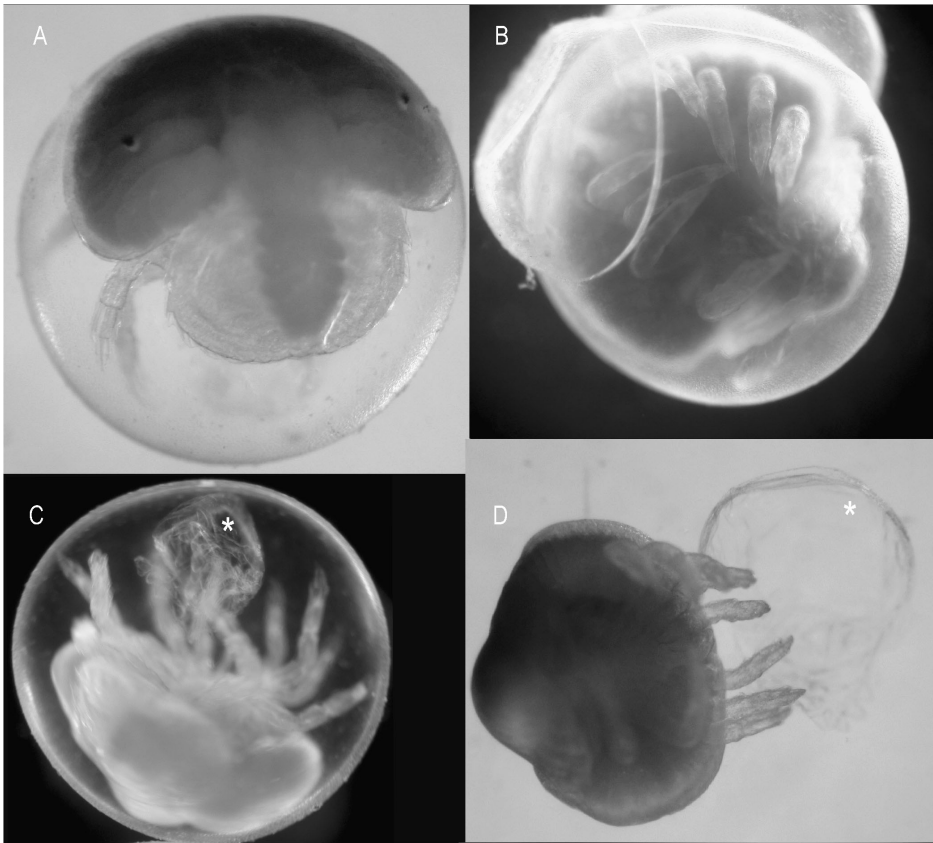


Fig. 2. *Limulus polyphemus* Linnaeus, 1758. A, first post-embryonic stage within extra-embryonic shell, anterior up, image 16X; B, large specimen within extra-embryonic shell being released from egg envelope, ventral, anterior up and left, image 22X; C, first post-embryonic stage with exuvium (asterisk) of previous stage, posterior, ventral up, image 19X; D, previous stage with exuvium (asterisk) of earlier stage, anterior, ventral right, image 80X.

The distoventral attenuation of the penultimate segment forming the chela of limbs 1-5 does not extend to the tip of distal segment of the first post-embryonic stage. On the next stage, length 4.77-6.48 mm, the proximal articulating knob on limb 2 fails to form and a caudal spine is added. On larger larvae, 9.69-57.20 mm, the distoventral attenuation on the penultimate endopodal segment on limbs 1-5 extends to the tip of distal segment and 5 book gills are present on the opisthosoma.

Remarks. — Snodgrass (1952) describes a protopod on limbs 2-6 of *L. polyphemus*, but does not mention the absence of a protopod-like segment on limb 1; limb 1 here appears as a 3-segmented ramus. Lankester (1891) illustrates a small knob articulating proximally on the basis of the protopod of limb 2, but does not note its loss later in development. Lankester (1891) states that the independently articulat-



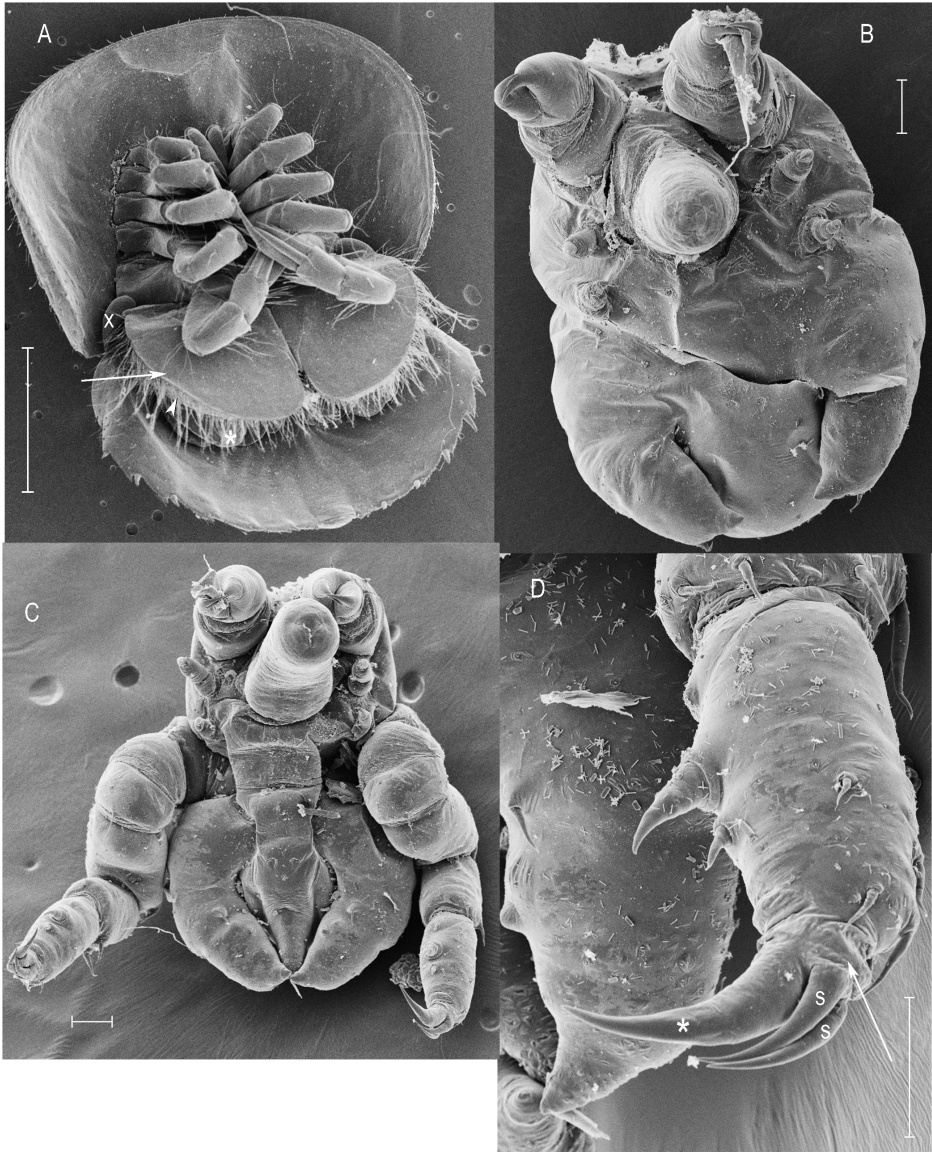


Fig. 3. *Limulus polyphemus* Linnaeus, 1758: A, first post-embryonic stage after release from extra-embryonic shell, ventral, anterior up, X on distal segment of exopod of limb 6, arrow to limb 8 (operculum), arrowhead to distal edge of limb 9 exopod, asterisk on 1 of 4 lamellae of limb 9, scale line 1.00 mm. *Ammothea glacialis* (Hodgson, 1909): B, first post-embryonic stage, ventral, anterior up, scale line 0.10 mm; C, second post-embryonic stage, ventral, anterior up, scale line 0.10 mm; D, second post-embryonic stage, distal, penultimate and antepenultimate segments of limb 4 and bud of limb 5, distal left, arrow to base of distal segment, asterisk on attenuation of distal segment, S on each of two setae on distal segment, scale line 0.10 mm.

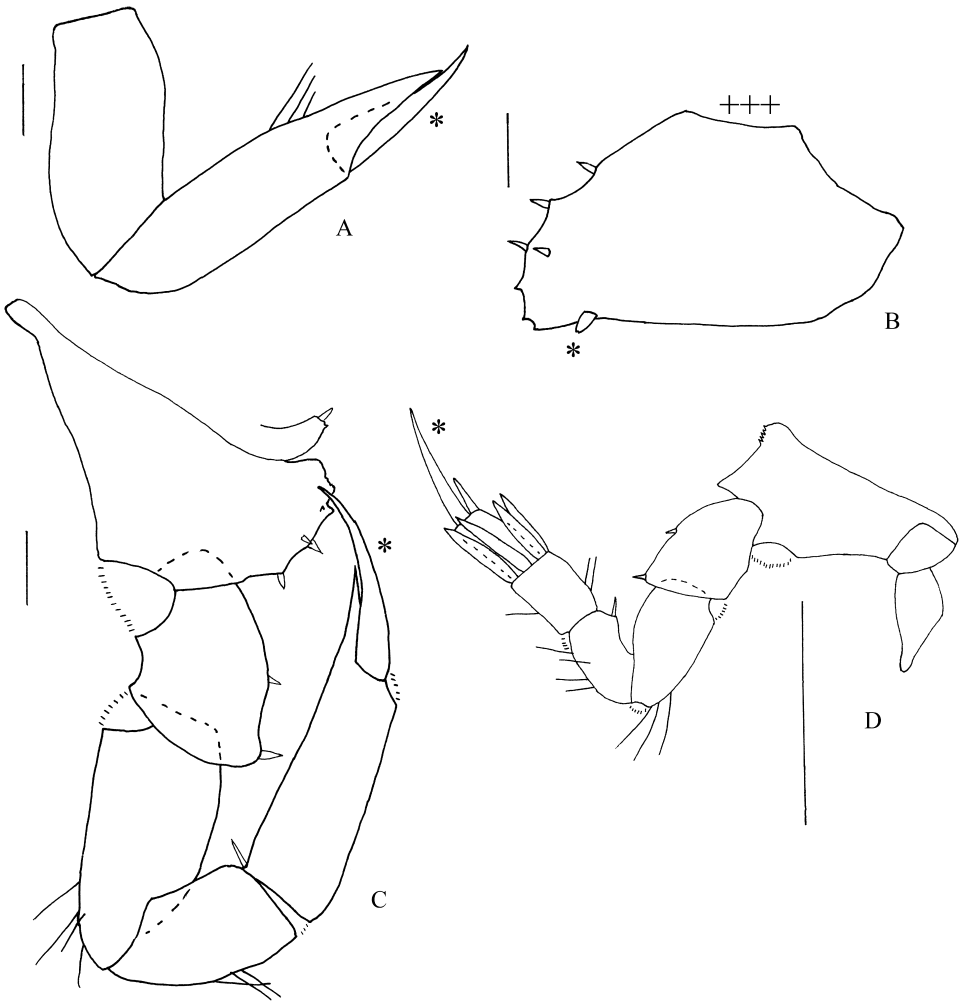


Fig. 4. *Limulus polyphemus* Linnaeus, 1758, first post-embryonic stage: A, limb 1, posterior, proximal up, asterisk adjacent to distal segment that extends beyond tip of attenuation of penultimate segment, scale line 0.10 mm; B, protopod of limb 2, posterior, ventral left, proximal down, asterisk adjacent to proximal articulated knob, three "plus" signs adjacent to point of attachment of endopod, scale line 0.10 mm; C, limb 3, posterior, proximal up, asterisk near tip of distal segment, scale line 1.00 mm; D, limb 6, posterior, proximal up and to right, asterisk near distal segment; scale line 1.00 mm. On C, D, well-developed arthrodistal membranes indicated as broken lines.

ing endite proximal to the basis on limbs 3-5 is an epicoxite. Walossek & Müller (1990) propose that a similar structure, referred to as a "proximal endite", originated on limbs of stem-lineage crustaceans, although this structure is not restricted to that lineage. The small knob on limb 2 does not appear to be a serial homolog of the proximal endite on limbs 3-5. Owen (1873), Lankester (1891), and Snodgrass (1952) figure the tip of the distal attenuation of the penultimate segment reaching

to the tip of the distal segment although it does not reach the tip in the earliest post-embryonic stages here. Snodgrass (1952) figures limb 6 with a chela. Here the ventral element originating on the penultimate segment of limb 6 articulates and no muscles insert on its base; this ventral element is a seta and so limb 6 has no chela. The dorsal ramus of limb 6 is described here as a 2-segmented exopod; the proximal segment articulates with the dorsal extension of the protopod and the distal segment is the flabellum. The configuration of the operculum and book gills is interpreted here as a biramal limb with segmented protopod and endopod, and unsegmented exopod.

The first post-embryonic stage of *L. polyphemus* has a single arthrodistal membrane separating somites 7-8, nine transformed limbs and no limb buds; a single protopodal segment of limb 2 bears a proximal knob which is lost during the following molt. During early post-embryonic molts no arthrodistal membranes are added, no segments are added to the transformed limbs, and limbs 10-13 are added as transformed limbs, not as buds, apparently during a single molt.

#### ***Ammonothea glacialis* (Hodgson, 1907)**

The first post-embryonic stage of (figs. 1C, 3B) is ellipsoidal, length 0.60-0.82 mm, without arthrodistal membranes separating somites.

Limb 1: originating slightly anterior to proboscis; 3-segmented, middle and distal segments curved, forming a chela (fig. 3B); proximal segment with mid-ventral seta, middle segment with seta proximal to origin of distal segment.

Limb 2: originating posterior to proboscis; 3-segmented; distal segment tapering to a point and forming a subchela with the middle segment; proximal and distal segments with one mid-ventral seta (fig. 1C); middle segment with a proximoventral seta.

Limb 3: 3-segmented subchela; distal segment tapering to a point and forming a subchela with the middle segment; proximal segment unarmed; middle segment with mid-ventral seta.

Limb 4: elongate bud, longer than the anterior limbs, oriented posteroventrally, pointed distally with dorsal seta towards distal tip (fig. 3B).

Second post-embryonic stage posteriorly elongate, length 0.71-0.83 mm; without arthrodistal membranes between somites (fig. 3C).

Limbs 1-3: unchanged.

Limb 4: 8-segmented (fig. 7A); proximal arthrodistal membrane of segment 1 weakly developed; segments 1-3 unarmed; segment 4 with 1 ventral seta; segment 5 with 1 dorsal and 2 adjacent terminal setae; segment 6 with 1 ventral, 1 dorsal

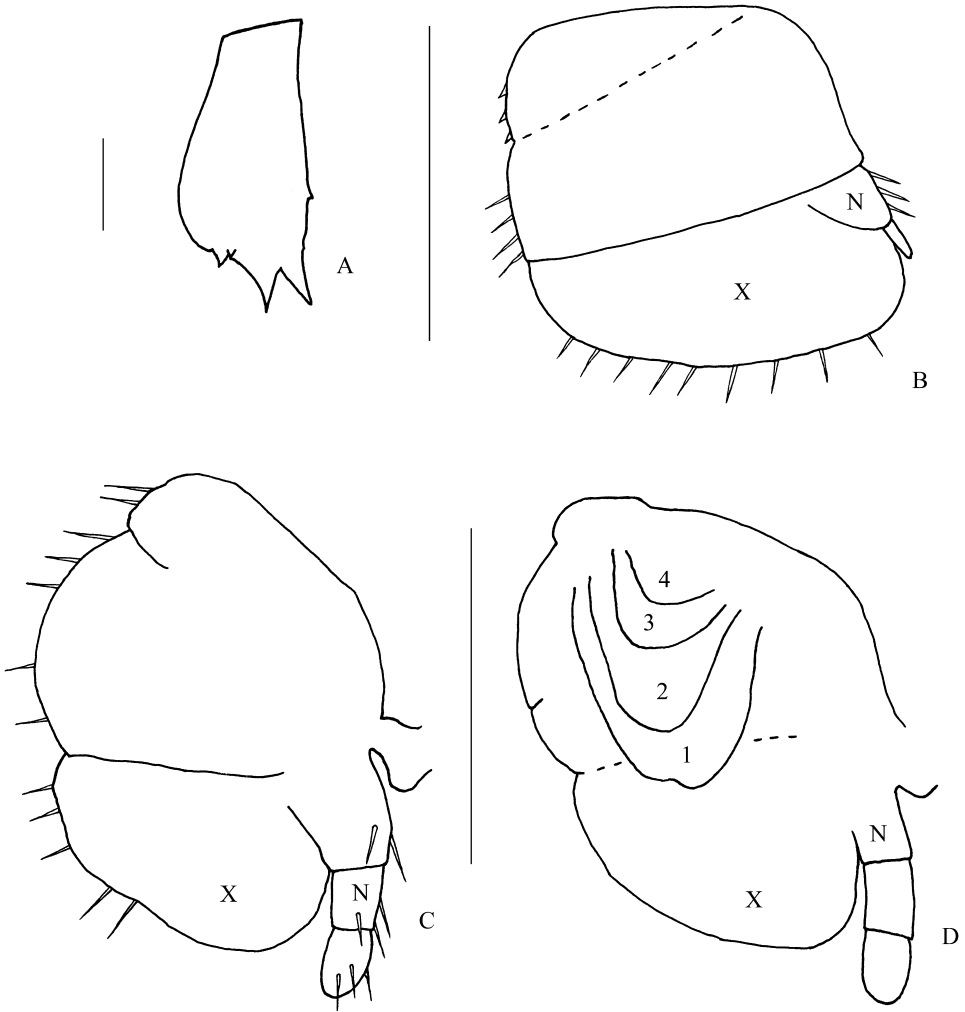


Fig. 5. *Limulus polyphemus* Linnaeus, 1758, first post-embryonic stage: A, limb 7, posterior, proximal up, scale line 0.10 mm; B, limb 8, operculum, anterior, proximal up, not all setae indicated; X on exopod, N on endopod; broken line indicates line of poor sclerotization posteriorly; arrow to point of attachment with contra-lateral limb, scale line 1.00 mm; C, limb 9, book gill, anterior, proximal up, X on exopod, N on endopod, scale line 1.00 mm; D, limb 9, book gill, posterior, proximal up, X on exopod, N on endopod, lamellae numbered, scale line 1.00 mm.

and 2 terminal setae; segment 7 with 2 dorsal, 2 ventral and 2 adjacent, terminal setae; terminal segment curved, tapering to a point, with 2 dorsal setae.

Limb 5: elongate bud, oriented posteriorly, pointed distally with dorsal seta towards distal tip (fig. 3C).

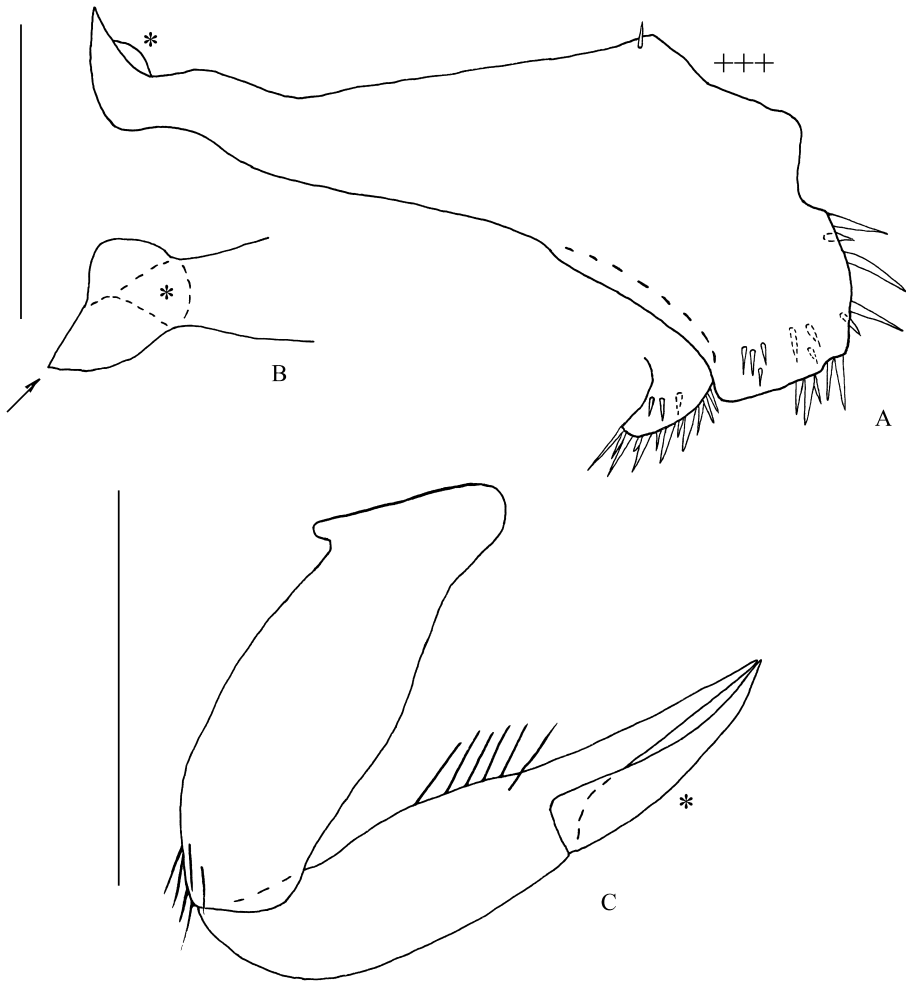


Fig. 6. *Limulus polyphemus* Linnaeus, 1758, 9.00-mm specimen: A, limb 3, protopod, posterior, proximal down, ventral right, not all setae drawn on ventral face, three "plus" signs adjacent to point of attachment of endopod, asterisk adjacent to point of attachment of proximal exopodal segment on limb 6, scale line 1.00 mm; B, limb 3 dorsal part of protopod, broken lines indicate depressions in cuticle, arrow to posterior dorsal tip, asterisk on point of attachment of proximal exopodal segment of limb 6, scale line 1.00 mm; C, limb 1, posterior, asterisk near tip of distal segment that does not extend beyond tip of attenuation of penultimate segment, scale line 1.00 mm.

Length of third post-embryonic stage 0.83-0.88 mm; somite 4 articulates with somite 5.

Limb 1 (fig. 7B): 3-segmented; proximal segment with proximodorsal attenuation and 4 distodorsal setae; seta of middle segment larger and thicker; distal segment unchanged.

Limb 2 (fig. 7D): 2-segmented, small; proximal segment unarmed; distal segment tapering more abruptly with 1 ventral and 2 dorsal setae.

Limb 3 (fig. 7C): unsegmented, small, tapering to a point, with 1 ventral seta.

Limb 4 (fig. 7E): 10-segmented; segment 1 fused to somite; segments 1 and 2 with paired dorsal attenuations but without setae; segment 3 with 2 ventral setae; segment 4 with 1 ventral seta; segment 5 with 1 ventral, 1 dorsal and 2 terminal setae; segment 6 with 1 ventral, 4 dorsal (2 pairs) and 1 terminal seta; segment 7 with 2 ventral, 5 dorsal (2 pairs + 1), and 3 terminal setae; segment 8 with 1 ventral seta; segment 9 with 3 dorsal, 2 ventral and 2 terminal setae; terminal segment curved, tapering to a point, with 2 dorsal setae. A pair of tendons from muscles within segment 9 attach to the proximal rim of terminal segment.

Limb 5 (fig. 7F): 8-segmented; proximal arthroal membrane of segment 1 poorly developed; segments 1 and 2 with dorsal attenuation; segments 1-3 without setae, segment 4 with 1 ventral seta; segment 5 with 1 dorsal and 3 terminal setae; segment 6 with 1 ventral, 2 dorsal, 1 anterior and 3 terminal setae; segment 7 with 2 dorsal, 2 ventral and 2 terminal setae; terminal segment curved, tapering to a point, with 2 dorsal setae. A pair of tendons from muscles within segment 7 attach to proximal rim of terminal segment.

Limb 6: elongate bud, oriented posteriorly, pointed distally with dorsal seta towards distal tip (fig. 1D).

Remarks. — The three stages of *A. glacialis* described here are attributed to stages II-IV by Cano & López-González (2009); stage I, a protonymph, with three anterior transformed appendages but without the bud of limb 4, was assumed by them to have molted immediately before or after hatching from the egg. However, an exuvium representing this stage was not recovered from specimens here, and so the first post-embryonic stage bears the bud of limb 4. More setae are found here on the three transformed limbs of all stages than are reported by Cano & López-González (2009), a proximal segment with a poorly developed proximal arthroal membrane is present on all reorganized walking limbs, the tapering terminal element of the walking legs with muscles attaching proximally is a segment, the bud of limbs 5-6 is the posterior limb on second and third stages, and no posterior incipient limb bud is present on those stages.

Based on morphology, it is not possible to identify a protopod on any pantopodan limb; these limbs may be simple rami, like limb 1 of *L. polyphemus*. Several authors have explained the addition of segments to the pantopodan limb from two or more sources along the limb (Morgan, 1891; Nakamura, 1981; Vilpoux & Waloszek, 2003; Gillespie & Bain, 2006). An alternate approach here is to assume only one segment is the source for all new segments (Ferrari & Dahms, 2007),

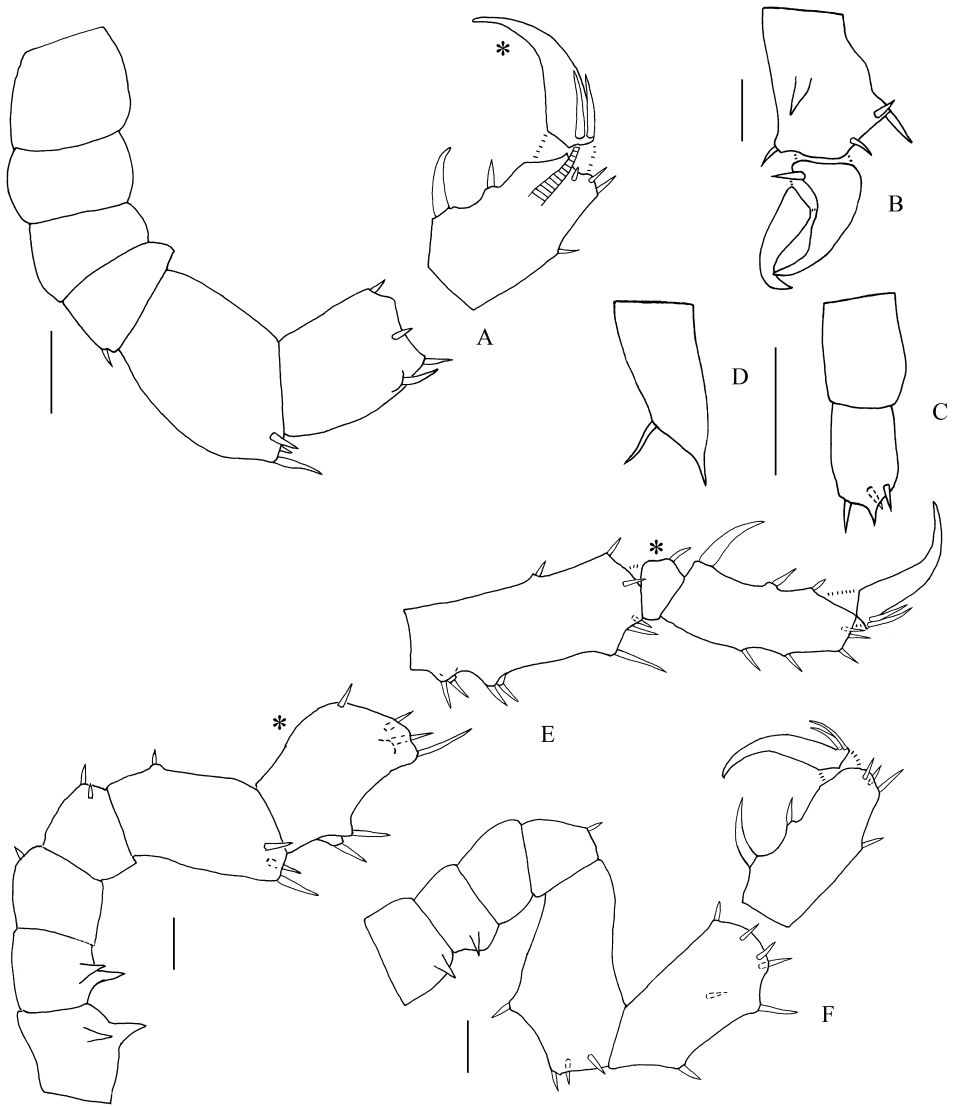


Fig. 7. *Ammothea glacialis* (Hodgson, 1909): A, second post-embryonic stage, limb 4, posterior, proximal left, muscle inserting on distal segment as enclosed hatched lines, asterisk adjacent to distal segment, scale line 0.01 mm; B, third post-embryonic stage, limb 1, dorsal, proximal up, scale line 0.10 mm; C, limb 3, posterior, proximal up, scale line 0.05 mm; D, limb 2, posterior, proximal up, scale line 0.05 mm; E, limb 4, posterior, proximal left, "S" on source segment, asterisks adjacent to segments added from stage II, scale line 0.10 mm; F, limb 5, posterior, proximal left, scale line 0.10 mm. On A, B, E, F, well-developed arthrodial membranes indicated as broken lines.

and that new segments have fewer setae and attenuations. Segment 6 on the 8-segmented walking leg 1 of the second stage of *A. glacialis* is the source of a new segment distal to itself that becomes segment 8 on the 10-segmented leg of the third stage; in addition, segment 6 on the 8-segmented walking leg 1 adds a new segment proximal to itself that becomes segment 6 on the 10-segmented leg of the third stage. Segment 6 on the 8-segmented leg is segment 7 on the 10-segmented leg.

The addition of limb 4 is variable among pantopodans. It may be present at hatching [see Cano & López-González (2009) and present study for *A. glacialis*, Ammonotheidae], added during the first molt [see Meinert (1899) for *Nymphon grossipes* (Fabricius, 1780), Nymphonidae; Okuda, 1940 for *A. alaskensis* Cole, 1904, Ammonotheidae], added during the second molt [see Gillespie & Bain, 2006 for *Tanystylum* sp., Ammonotheidae, and Vilpoux & Waloszek, 2003 for *Pycnogonum litorale* (Ström, 1762), Pycnogonidae], or added during the third molt [see Morgan, 1891 for *Tanystylum orbiculare* (Wilson, 1878), Ammonotheidae]. During subsequent molts, in all four cases, buds of four walking limbs are added posterior to the transformed limb and in register with molts. Each limb bud is reorganized in register one molt later, and segments are added to the limbs, also in register. During a fifth developmental pattern of pantopodans, there is no change during the first molt, but during the second molt buds of limbs 4-7 are added simultaneously; during subsequent molts limbs 4-7 are reorganized and add segments simultaneously [see Ohshima, 1937 for *Nymphonella tapetis* Ohshima, 1927 and Salazar-Vallejo & Stock, 1987 for *Ammonothea spinifera* (Cole, 1904), both Ammonotheidae]. Based on recent morphological analyses (Dunlop & Arango, 2004; Bamber, 2007), Nymphonidae is the sister family to the remaining pantopodan families, and here nymphonid development is assumed to be the ancestral condition of pantopodans.

The first post-embryonic stage of the ancestral condition of pantopodans had no arthroal membranes separating somites and there were three transformed limbs; the proximal segment of limb 2 was a simple cylinder. During early post-embryonic molts, arthroal membranes were added posterior to somite 4, buds of limbs 4-7 were added in register with each molt, and two segments are added to each transformed limb in register with each molt.

#### Early development of crustaceans

The first post-embryonic stage of crustaceans that hatch as a nauplius-like larva (nauplius, metanauplius or nauplioid larva) and changes during the immediate several molts of these larvae are compared and contrasted with xiphosurans and pantopodans in table I. Attributes include presence of an arthroal membrane



TABLE I

Attributes of early post-embryonic stages of xiphosurans, pantopodans and crustaceans hatching as a nauplius-like larva: A, number of arthrodistal membranes separating somites; B, ventral configuration of protopod of limb 2: simple cylinder (c), attenuation of proximal segment (at), arthrite on proximal segment (ar); C, number of transformed limbs + limb buds. Changes during immediate molts; D, number of arthrodistal membranes added; E, ventral configuration of protopod of limb 2: simple cylinder (c); proximal knob (k); attenuation of proximal segment (at); arthrite on proximal segment (ar); F, maximum number of limbs as buds posterior to mandible; G, number of molts before one or more new limb buds appear; H, maximum number of new limb buds added per molt: one (1); two or more (+); I, addition of segments to some transformed limbs (+). Number in parentheses indicates variation in taxon

	A	B	C	D	E	F	G	H	I
Xiphosurans	1	na	9 + 0	0	—	n	u	4	—
Pantopodans	0	na	3 + 0(1)	0	na	1	1-2	1	+
Branchiopods	0	ar	3 + 1	0	ar	6	3	+	+
Branchiurans	4	at	7 + 3	4	at	0	na	0	—
Cephalocaridans	4	ar	3 + 3	4	ar	1	1	1	—
Copepods	0	ar	3 + 1(2)	0	ar	4(5)	1-2	1	+
Dendrobranchiates	0	c	3 + 1	0	c	4(5)	4	+	—
Euphausiaceans	0	c	3 + 0	0	c	3	2	+	—
Mysidaceans	0	c	3 + 1	0	c	0	0	+	—
Mystacocaridans	3	ar	3 + 2	4	ar	1	1	1	+
Ostracodes	0	c	3(6) + 1(2)	0	c	1	1	1	+
Remipedes	0	c	3 + 0	0	c	8	1	+	+
Thecostracans	0	ar	3 + 1	0	ar	1	5	1	+

between adjacent somites, configuration of the protopod (specifically the proximal segment or coxa) of limb 2 (antenna 2), number of transformed limbs and limb buds, fate of limb buds and addition of segments to transformed limbs. The following notes clarify some of the states of these attributes as published or as observed here (for explanation of letter coding A-I, see table I).

**Xiphosurans:** A. segment corresponding to a coxa is absent on limb 2; G. stage during which four posterior book gills are added is unknown. [Source: present study.]

**Pantopodans:** A. segment corresponding to a coxa on limb 2 cannot be determined; C. bud of limb 4 is present on some species; D. arthrodistal membranes separating posterior somites are added later in development. [Source: Bain, 2003; present study.]

**Branchiopods:** D. furrows separating limb buds not considered arthrodistal membranes; F. number refers to limb buds bearing setae; H. buds of maxilla 1, maxilla 2 and trunk limbs 1-3 present after third molt. [Sources: Anderson, 1967; Ferrari & Grygier, 2003; Olesen & Grygier, 2004.]

- Branchiurans:** C. caudal ramus is a transformed limb because it articulates with its somite; D. some arthrodial membranes fail to form during later stages but telson articulates; F. trunk limbs 2-4 are transformed during first molt and no more limbs are added. [Source: Shimura, 1981; Rushton-Mellor & Boxshall, 1994; present study.]
- Cephalocaridans:** A. and D. arthrodial membranes separate limbless trunk somites; B. naupliar arthritis present (fig. 8A); G. addition of new trunk limbs delayed for 2 or 3 molts. [Sources: Sanders, 1963; Sanders & Hessler, 1964; Addis et al., 2007; present study.]
- Copepods:** B. some parasitic copepods lack a naupliar arthritis (e.g., Ivanenko et al., 2001; Dojiri et al., 2008); some free-living copepods lack a naupliar arthritis on the first, non-feeding stage although the arthritis is added on later feeding stages (Ferrari & Dahms, 2007); C. bud of caudal ramus present on all copepods, bud of maxilla 1 present on thaumatopsylloids; F. gymnopleans with five, podopleans with four; G. new limb buds appear after the first molt of podopleans, and after the second molt of gymnopleans. [Sources: Ferrari & Dahms, 2007; Dojiri et al., 2008.]
- Dendrobranchiates:** C. ventral setae on rami of antenna 1, antenna 2 and mandible suggest that each ramus is a segment complex and so these are transformed limbs; F. buds of maxilla 1-2 and maxilliped 1-2 are present early in development, and later in development those limbs are reorganized and buds of five pereopods are added, finally five pereopods are reorganized and buds of five pleopods are added. [Sources: Cook & Murphy, 1971; Chong & Sasekumar, 1994.]
- Euphausiaceans:** C. ventral setae on rami of antenna 1, antenna 2 and mandible suggest that these limbs are transformed; caudal ramus may be absent or present on first post-embryonic stage of different species; F. not including mandible which has been reorganized as a secondary bud. [Sources: Gurney, 1942; Boden, 1951; Gómez-Gutiérrez, 2003.]
- Mysidaceans:** C. antenna 1, antenna 2 and mandible elongate, latter two are biramus and not considered buds here. [Source: Wittmann, 1981.]
- Mystacocaridans:** C. buds of caudal ramus and maxilla 1 are present; F. trunk limb buds 2-5 are excluded because they are never reorganized into transformed limbs; H. thoracic limb 5 appears as a bud earlier than buds of thoracic limb 2-4 and 6. [Sources: Hessler & Sanders, 1966; Olesen, 2001; Ferrari et al., 2010.]
- Ostracodes:** C. first post-embryonic stage of some podocopans with three transformed limbs and bud of the caudal ramus, other podocopans bear the bud of maxilla 1; mydocopans have six transformed limbs, five anterior limbs plus caudal ramus. [Sources: Hiruta, 1980; Smith & Kamiya, 2002, 2008.]

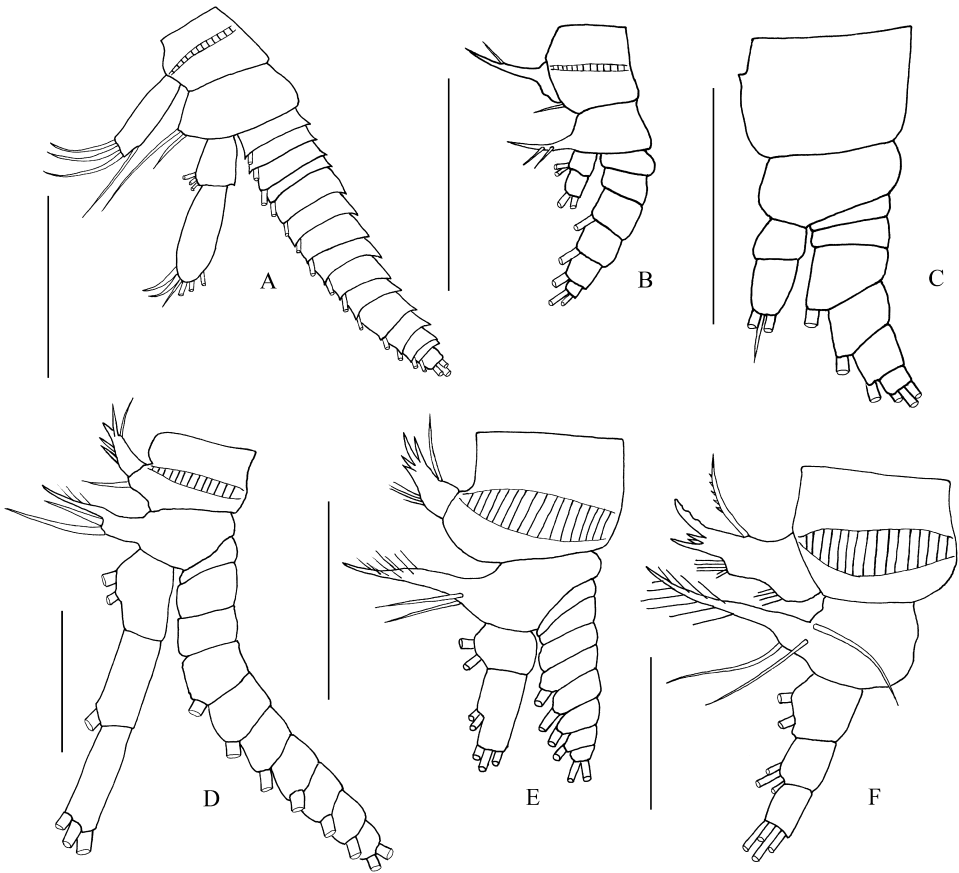


Fig. 8. Antenna 2, proximal up, with long setae on rami cut off near base; muscle inserting on dorsoproximal border of naupliar arthrite enclosed hatched lines. A, *Hutchinioniella macracantha* Sanders, 1955, stage III, scale line 0.10 mm; B, *Hansenocaris furcifera* Itô, 1989, stage II, scale line 0.05 mm; C, facetotectan Pacific Type I, stage undetermined, scale line 0.05 mm; D, *Zibrowia* sp., stage II, scale line 0.10 mm; E, *Chelonibia testudinaria* Linnaeus, 1758, early stage, scale line 0.10 mm; F, unidentified cirripede from Fort Pierce, FL, stage VI, exopod behind endopod, not shown, is 10-segmented with only proximal segment unarmed, scale line 0.10 mm.

**Remipedes:** C. setae on rami of antenna 1, antenna 2 and mandible indicate that limbs are transformed; F; asetose bud of caudal ramus initially appears during development later than buds of maxilla 1-2 and maxilliped. [Sources: Koenemann et al., 2007, 2009.]

**Thecostracans:** B. naupliar arthrite of cirripedes, ascothoracidans and facetotectans (fig. 8B, D-F); some species of ascothoracidans and facetotectans do not have a naupliar arthrite (fig. 8C); C. bud of maxilla 1 absent on 0.15 mm specimen of facetotectan *Hansenocaris furcifera* Itô, 1989 (pers. obs., FDF); G. bud of maxilla 1 of facetotectans and ascothoracidans added after one

molt; after five molts seven or eight transformed limbs appear on cirripedes. [Sources: Barnes & Costlow, 1961; Itô, 1990; Itô & Grygier, 1990; present study.]

#### DISCUSSION

Branchiurans, cephalocaridans and mystacocaridans are the only crustaceans with one or more somites separated by arthrodial membranes on a nauplius-like, first post-embryonic stage. Branchiurans do not add somites during their post-embryonic phase, and only the telson is separate. Early in the development of mystacocaridans and cephalocaridans, posterior somites that will not bear limbs are separated by arthrodial membranes while anterior somites that bear limbs are not separated, including the anterior five that are always fused on crustaceans.

Presence of a naupliar arthritis on the coxa of antenna 2 during early post-embryonic development is shared by branchiopods, copepods, thecostracans, mystacocaridans and cephalocaridans. The naupliar arthritis originates directly on the coxa of copepods (Ferrari & Dahms, 2007), thecostracans and cephalocaridans (fig. 8A-E), or on a quadrate-like projection of the coxa of branchiopods and mystacocaridans (Ferrari & Dahms, 2007). Arthritis morphology varies as a simple setal-like or modified setal-like element on many copepods, an enditic-like element on cephalocaridans, or more complex morphologies on mystacocaridans, branchiopods and thecostracans. The morphology also may vary during the development of branchiopods from a simple setal-like element to a forked element (Olesen & Grygier, 2004). The nature of the elongate process on the coxa of the Upper Cambrian orstenocarid crustacean *Bredocaris admirabilis* Müller, 1983 (cf. Müller & Walossek, 1988) and the Upper Cambrian branchiopod *Rehbachiella kinnekulensis* Müller, 1983 (cf. Walossek, 1993) cannot be determined. Branchiurans bear an attenuation on the ventral face of the coxa, but it is retained throughout development (Shimura, 1981; Rushton-Mellor & Boxshall, 1994). The coxa of antenna 2 on early post-embryonic stages of ostracodes, remipedes, euphausiaceans, mysidaceans and dendrobranchiates has no ventral structure. The ventral “proximal endite” of limb 2 of stem lineage crustaceans (Walossek & Müller, 1990) is not a naupliar arthritis but appears to correspond to the well-developed endite proximal to basis on limbs 3-5 of xiphosurans.

During early post-embryonic development, most post-mandibular limbs initially are added as a limb bud. The bud of the caudal ramus is an exception; it usually is present as a seta on the first post-embryonic stage of most crustaceans considered here. However, the bud of the caudal ramus is absent on euphausiaceans and remipedes; it appears as a seta after the first molt of euphausiaceans (Gurney, 1942; Boden, 1951) or as a pair of unarmed knobs after the second molt of remipedes (Koenemann et al., 2007). The bud of maxilla 1 initially is present on the first

post-embryonic stage of some podocopan ostracodes, thaumatopsylloid copepods, mystacocaridans, and cephalocaridans. However, the bud of maxilla 1 is added one molt later on podocopan ostracodes and podoplean copepods, and on gymnoplean copepods it is added two molts after hatching. The bud of maxilla 2 also is present on the first post-embryonic stage of cephalocaridans. On branchiurans, the last three posterior thoracic limbs are the only limbs present as buds.

New limb buds of podocopan ostracodes, branchiurans, cephalocaridans and mystacocaridans usually are reorganized into a transformed limb during the molt immediately following the initial appearance of the bud. Segments may be added to transformed limbs during later molts. In contrast, limb buds are retained for more than one molt on branchiopods, copepods, thecostracans, dendrobranchiates, euphausiaceans and remipedes. On mysids, limb buds elongate, become segmented but remain within the naupliar cuticle (Wittmann, 1981). Some post-mandibular limbs of the orstenocarid *Bredocaris admirabilis* appear to be retained as buds over two molts (Müller & Walossek, 1988), and some post-mandibular limbs of the branchiopod *Rehbachella kinnekullensis* appear to be retained as buds during at least one molt (Walossek, 1993). Some post-mandibular limbs of podoplean copepods (e.g., maxilla 2 and maxilliped) initially appear as transformed limbs, not as limb buds (Ferrari & Dahms, 2007).

Similarities and differences. — Early post-embryonic development of pantopodans appears more similar to that of podocopan ostracodes than to that of any other crustacean taxon, and no crustacean develops like the xiphosuran (table I). Similarities between pantopodans and podocopan ostracodes include: absence of arthrodial membranes between somites during early development; three transformed limbs; bud of limb 4 may be absent or present on different species of both taxa; each limb bud is reorganized to a transformed limb after one molt while a limb bud is added posterior to the transformed limb; transformed limbs may add segments during subsequent molts. Significant morphological changes within the crustacean lineage include: arthrodial membranes separate one or more limbless posterior somites (branchiurans, cephalocaridans and mystacocaridans); coxa of antenna 2 with a ventral, naupliar arthrite moved by muscles (cephalocaridans, mystacocaridans, branchiopods, copepods and thecostracans) or a ventral attenuation (branchiurans); reorganization of limb buds delayed so that limbs are retained as buds (branchiopods, copepods, thecostracans, dendrobranchiates, euphausiaceans and remipedes). Absence of a naupliar arthrite on antenna 2 of ostracodes can be explained as inherited from the last stem lineage arthropod. On branchiurans, the ventral attenuation of the coxa of antenna 2 may not be a simple convergence with a naupliar arthrite because similar structures are present proximally on antenna 1, maxilla 1 and maxilla 2 throughout development. Absence of

a naupliar arthritis on antenna 2 of remipedes, euphausiaceans and dendrobranchi-ates, taxa that also delay reorganization of some limb buds, may be a secondary loss for remipedes and malacostracans. This absence appears to be derived within the crustacean lineage that includes copepods, thecostracans and branchiopods that have a naupliar arthritis, but that delay reorganization of limb buds. The absence of a naupliar arthritis in this lineage may be a convergence among remipedes and malacostracans.

These nested sets of character states may be derived through descent with modification from a common ancestor, or through an origin by larval hybridization (Williamson, 2006). The latter hypothesis proposes that Upper Cambrian adult naupliar-like crustaceans, the Naupliomorpha Williamson, 2001, hybridized with other crustaceans that expressed direct development. Support for the hypothesis is found in the apparent absence of metanauplii in the Upper Cambrian, although crustaceans like *Bredocaris admirabilis* and *Rehbachella kinnekullensis* have metanaupliar-like stages, one pair of pre-oral appendages on nauplii vs two pairs on non-naupliar stages, and the discordant presence of naupliar development among crustacean taxa. As proposed at present, this hybridization resulted in crustaceans with three kinds of development: gradual metamorphosis, e.g., branchiopods (Wilson, 2006) and presumably ostracodes, cephalocaridans and mystacocaridans; metamorphosis by substitution for the remaining crustaceans expressing naupliar development, i.e., thecostracans, copepods, remipedes, euphausiaceans and dendrobranchiates; and the ancestral direct development, presumably retained by branchiurans.

#### ACKNOWLEDGEMENTS

The Smithsonian Institution's Office of Fellowships supported visits of H.-U.D. and V.N.I.; V.N.I. also was supported by The Ministry of Education and Science of the Russian Federation. We extend special thanks to Lana Ong (National Museum of Natural History, Smithsonian Institution) for specimen and figure preparations and to Scott Whittaker (National Museum of Natural History, Smithsonian Institution) for SEM preparations. Mark J. Grygier (Lake Biwa Museum) provided specimens of thecostracans and Rob Smith (Lake Biwa Museum) answered questions about ostracodes.

#### REFERENCES

- ADDIS, A., F. BIAGI, A. FLORIS, E. PUDDU & M. CARCUPINO, 2007. Larval development of *Ligitiella magdalenina* (Crustacea, Cephalocarida). *Marine Biology*, Berlin, **152**: 733-744.
- ANDERSON, D. T., 1967. Larval development and segment formation in the branchiopod crustaceans *Limnadia stanleyana* King (Conchostraca) and *Artemia salina* (L.) (Anostraca). *Australian Journal of Zoology*, **15**: 47-91.

- BAIN, B. A., 2003. Larval types and a summary of postembryonic development within the pycnogonids. *Invertebrate Reproduction and Development*, **43**: 193-222.
- BAMBER, R. N., 2007. A holistic re-interpretation of the phylogeny of the Pycnogonida Latreille, 1810 (Arthropoda). *Zootaxa*, **1668**: 295-312.
- BARNES, H. & J. D. COSTLOW, JR., 1961. The larval stages of *Balanus balanus* (L.) Da Costa. *Journal of the Marine Biological Association of the United Kingdom*, **41**: 59-68.
- BODEN, B. P., 1951. The egg and larval stages of *Nyctiphanes simplex*, a euphausiid crustacean from California. *Proceedings of the Zoological Society of London*, **121**: 515-527.
- BRENNEIS, G., P. UNGERER & G. SCHOLTZ, 2008. The chelifores of sea spiders (Arthropoda, Pycnogonida) are the appendages of the deutocerebral segment. *Evolution & Development*, **10**: 717-724.
- CANO, E. & P. J. LÓPEZ-GONZÁLEZ, 2009. Novel mode of postembryonic development in *Ammonothea* genus (Pycnogonida: Ammonotheidae) from Antarctic waters. *Scientia Marina*, **73**: 541-550.
- CHONG, V. C. & A. SASEKUMAR, 1994. Larval development of the fiddler shrimp, *Metapenaeopsis stridulans* (Alcock, 1905) (Decapoda: Penaeidae) reared in the laboratory. *Journal of Natural History, London*, **28**: 1265-1285.
- COLE, L. J., 1904. Pycnogonids of the west coast of North America. *Harriman Alaska Expedition*, **10**: 249-298, pls. 4-26.
- COOK, H. L. & M. A. MURPHY, 1971. Early developmental stages of the brown shrimp, *Penaeus aztecus* Ives, reared in the laboratory. *Fishery Bulletin, U.S.*, **69**: 223-239.
- DOJIRI, M., G. HENDLER & I.-H. KIM, 2008. Larval development of *Caribeopsyllus amphiodiae* (Thaumatosyllidae: Copepoda), and enterozoic parasite of the brittle star *Amphiodia urtica*. *Journal of Crustacean Biology*, **28**: 281-305.
- DUNLOP, J. A. & C. P. ARANGO, 2004. Pycnogonid affinities: a review. *Journal of Zoological Systematics and Evolutionary Research*, **43**: 8-21.
- FABRICIUS, O., 1780. *Fauna Groenlandica, systematicae Animalia Groenlandica occidentalis, hactenus indagata: 1-452.* (Ioannis Gottlob Rothe, Hafniae et Lipsiae).
- FERRARI, F. D. & H.-U. DAHMS, 2007. Post-embryonic development of the Copepoda. *Crustaceana Monographs*, **8**: i-vi, 1-226.
- FERRARI, F. D. & M. J. GRYGIER, 2003. Comparative morphology among trunk limbs of *Caenestheriella gifuensis* and of *Leptestheria kawachiensis* (Crustacea: Branchiopoda: Spinicaudata). *Zoological Journal of the Linnaean Society of London*, **139**: 547-564.
- FERRARI, F. D., V. N. IVANENKO & H.-U. DAHMS, 2010. Body architecture and relationships among basal copepods. *Journal of Crustacean Biology*, **30**: 465-477.
- GILLESPIE, J. M. & B. A. BAIN, 2006. Postembryonic development of *Tanystylum bealensis* (Pycnogonida, Ammonotheidae) from Barkley Sound, British Columbia, Canada. *Journal of Morphology*, **267**: 308-317.
- GÓMEZ-GUTIÉRREZ, J., 2003. Hatching mechanism and accelerated hatching of eggs of a spawning euphausiid *Nematoscelis difficilis*. *Journal of Plankton Research*, **25**: 1397-1411.
- GURNEY, R., 1942. Larvae of decapod crustaceans. *The Ray Society Monograph*, **125**: 1-306.
- HESSLER, R. R. & H. L. SANDERS, 1966. *Derocheilocaris typicus* revisited. *Crustaceana*, **11**: 141-155.
- HIRUTA, S., 1980. Morphology of the larval stages of *Vargula hilgendorffii* (G. W. Müller) and *Euphilomedes nipponica* Hiruta from Japan (Ostracoda: Myodocopina). *Journal of Hokkaido University of Education, (II) (B)* **30**: 145-167.
- HODGSON, T. V., 1907. Pycnogonida. National Antarctic Expedition 1901-1904. Reports of the National Antarctic expedition of (1904-1907), (Natural History) **3**: 1-172.
- HØEG, J. T., M. PÉREZ-LOSADA, H. GLENNER, G. A. KOLBASOV & K. A. CRANDALL, 2009. Evolution of morphology, ontogeny and life cycles within the crustacean Thecostraca. *Arthropod Systematics and Phylogeny*, **67**: 199-217.

- ITÔ, T., 1986. Three types of “nauplius y” (Maxillopoda: Facetotecta) from the North Pacific. *Publications of the Seto Marine Biological Laboratory*, **31**: 63-73.
- —, 1989. A new species of *Hansenocaris* (Crustacea: Facetotecta) from Tanabe Bay, Japan. *Publications of the Seto Marine Biological Laboratory*, **34**: 55-72.
- —, 1990. Naupliar development of *Hansenocaris furcifera* Itô (Crustacea: Maxillopoda: Facetotecta) from Tanabe Bay, Japan. *Publications of the Seto Marine Biological Laboratory*, **34**: 201-224.
- ITÔ, T. & M. J. GRYGIER, 1990. Description and complete larval development of a new species of *Baccalaureus* (Crustacea: Ascothoracida) parasitic in a zooanthid from Tanabe Bay, Honshu, Japan. *Zoological Science*, **7**: 485-515.
- IVANENKO, V. N., F. D. FERRARI & A. V. SMUROV, 2001. Nauplii and copepodids of *Scottomyzon gibberum* (Copepoda: Siphonostomatoida: Scottomyzontidae, new family), a symbiont of *Asterias rubens* (Asteroidea). *Proceedings of the Biological Society of Washington*, **114**: 237-261.
- KOENEMANN, S., J. OLESEN, F. ALWES, T. M. ILIFFE, M. HOENEMANN, P. UNGERER, C. WOLFF & G. SCHOLTZ, 2009. The post-embryonic development of Remipedia (Crustacea) — additional results and new insights. *Development Genes and Evolution*, **219**: 131-145.
- KOENEMANN, S., F. R. SCHRAM, A. BLOECHL, T. M. ILIFFE, M. HOENEMANN & C. HELD, 2007. Post-embryonic development of remipede crustaceans. *Evolution & Development*, **9**: 117-121.
- LANKESTER, E. R., 1891. *Limulus* an arachnid. *Quarterly Journal of Microscopical Science*, **24**: 504-548.
- LINNAEUS, C., 1758. *Systema Naturae, per Regna tria Naturae, secundum Classes, Ordines, Genera, Species, cum characteribus, differentiis, synonymis, locis.* (Ed. decima, reformata.) **I**, Regnum animale: 1-823. (Laurentius Salvius, Holmiae).
- MEINERT, F., 1899. Pycnogonidae. The Danish Ingolf-Expedition, **3**(1): 1-71.
- MITTMANN, B. & G. SCHOLTZ, 2003. Development of the nervous system in the ‘head’ of *Limulus polyphemus* (Chelicerata: Xiphosura): morphological evidence for a correspondence between the segments of the chelicerae and of the (first) antennae of Mandibulata. *Development Genes Evolution*, **213**: 9-17.
- MORGAN, T. H., 1891. A contribution to the embryology and phylogeny of the pycnogonids. *Studies from the Biological Laboratory of Johns Hopkins University, Baltimore*, **5**(1): 1-76.
- MÜLLER, K. J., 1983. Crustacea with preserved soft parts from the Upper Cambrian of Sweden. *Lethaia*, **16**: 93-109.
- MÜLLER, K. J. & D. WALOSSEK, 1988. External morphology and larval development of the Upper Cambrian maxillopod *Bredocaris admirabilis*. *Fossils and Strata*, **23**: 1-70.
- NAKAMURA, K., 1981. Post embryonic development of a pycnogonid, *Propallene longiceps*. *Journal of Natural History, London*, **15**: 49-61.
- OHSHIMA, H., 1927. Píknogono parazite vivanta en Bivalvo. *Bulteno Scienca Fakultato Terkultura Kjusu Imperia Universitato*, **2**(5): 366-377.
- —, 1937. The life-history of “*Nymphonella tapetis*” Ohshima (“Pantopoda, Eurycyridae”). *Comptes Rendus du XIIème Congrès International de Zoologie, Lisbonne*, **1935**: 1616-1626.
- OKUDA, S., 1940. Metamorphosis of a pycnogonid parasitic in a hydromedusa. *Journal of the Faculty of Science, Hokkaido Imperial University, (Zoology, 6)* **7**(2): 73-86.
- OLESEN, J., 2001. External morphology and larval development of *Derocheilocaris remanei* Delamare-Deboutteville & Chappuis, 1951 (Crustacea, Mystacocarida), with a comparison of crustacean segmentation and tagmosis patterns. *Biologiske Skrifter*, **53**: 1-59.
- OLESEN, J. & M. J. GRYGIER, 2004. Larval development of Japanese ‘conchostracans’: part 2, larval development of *Caenestheriella gifuensis* (Crustacea, Branchiopoda, Spinicaudata, Cyzicidae), with notes on homologies and evolution of certain naupliar appendages within the Branchiopoda. *Arthropod Structure & Development*, **33**: 453-469.



- OWEN, R., 1873. Anatomy of the king crab (*Limulus polyphemus*, Latr.): 1-50. (Taylor and Francis, London).
- RUSHTON-MELLOR, S. K. & G. A. BOXSHALL, 1994. The developmental sequence of *Argulus foliaceus* (Crustacea: Branchiura). *Journal of Natural History*, London, **28**: 763-785.
- SALAZAR-VALLEJO, S. & J. H. STOCK, 1987. Apparent parasitism of *Sabella melanostigma* (Polychaeta) by *Ammothella spinifera* (Pycnogonida) from the Gulf of California. *Revista de Biología Tropical*, **35**(2): 269-275.
- SANDERS, H. L., 1955. The Cephalocarida, a new subclass of Crustacea from Long Island Sound. *Proceedings of the National Academy of Sciences, U.S.A.*, **41**: 61-66.
- —, 1963. The Cephalocarida, functional morphology, larval development, and comparative external anatomy. *Memoirs of the Connecticut Academy of Arts & Sciences*, **15**: 1-80.
- SANDERS, H. L. & R. R. HESSLER, 1964. The larval development of *Lightiella incisa* Gooding (Cephalocarida). *Crustaceana*, **7**: 81-97.
- SEKIGUCHI, K., Y. YAMAMICHI & J. D. COSTLOW, 1982. Horseshoe crab developmental studies I. Normal embryonic development of *Limulus polyphemus* compared with *Tachypleus tridentatus*. In: J. BONAVENTURA, *Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals*: 53-73. (Alan R. Liss, Inc., New York).
- SHIMURA, S., 1981. The larval development of *Argulus coregoni* Thorell (Crustacea: Branchiura). *Journal of Natural History*, London, **15**: 331-348.
- SMITH, R. J. & T. KAMIYA, 2002. The ontogeny of *Neonesidea oligodentata* (Bairdioidea, Ostracoda, Crustacea). *Hydrobiologia*, **489**: 245-275.
- — & — —, 2008. The ontogeny of two species of Darwinuloidea (Ostracoda, Crustacea). *Zoologischer Anzeiger*, **247**: 275-302.
- SNODGRASS, R. E., 1952. A textbook of arthropod anatomy: 1-363. (Comstock Publishing Associates, Ithaca, New York).
- STRÖM, H., 1762. Physik og Oeconomisk Beskrivelse over Fogderiet Søndmør, beliggende i Bergens. *Stift i Norge*, **1**: 208-209.
- VILPOUX, K. & D. WALOSZEK, 2003. Arthropod structure & development: Larval development and morphogenesis of the sea spider *Pycnogonum litorale* (Stroem, 1762) and the tagmosis of the body of Pantopoda. *Arthropod Structure and Development*, **32**: 349-383.
- WALOSSEK, D., 1993. The Upper Cambrian *Rehbachella* and the phylogeny of Branchiopoda and Crustacea. *Fossils & Strata*, **32**: 1-202.
- WALOSSEK, D. & K. J. MÜLLER, 1990. Upper Cambrian stem-lineage crustaceans and their bearing upon the monophyletic origin of Crustacea and the position of *Agnostus*. *Lethaia*, **23**(4): 409-427.
- WILLIAMSON, D. I., 2001. Larval transfer and the origins of larvae. *Zoological Journal of the Linnean Society*, London, **131**: 111-122.
- —, 2006. Hybridization in the evolution of animal form and life-cycle. *Zoological Journal of the Linnean Society*, London, **148**: 585-602.
- WILSON, C. B., 1902. North American parasitic copepods of the family Argulidae, with a bibliography of the group and a systematic review of all known species. *Proceedings of the United States National Museum*, **25**(1302): 635-742.
- WILSON, E. B., 1878. Synopsis of the Pycnogonida of New England. *Transactions of the Connecticut Academy of Arts & Sciences*, **5**: 1-26.
- WITTMANN, K., 1981. Comparative biology and morphology of marsupial development in *Leptomysis* and other Mediterranean Mysidacea (Crustacea). *Journal of Experimental Marine Biology and Ecology*, **52**: 243-270.

First received 18 April 2011.

Final version accepted 16 May 2011.