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## Laboratory Rearing of Ascothoracidan Nauplii (Crustacea: Maxillopoda) from Plankton at Okinawa, Japan

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*With Text-figures 1-7 and Table 1*

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**Abstract** Seven ascothoracidan nauplii of instars II-IV and one ascothoracid-larva were captured in inshore plankton at Sesoko Island, Okinawa, Japan, and the nauplii reared in the laboratory until, in two cases, they metamorphosed into ascothoracid-larvae. Naupliar instars II-VI and the ascothoracid-larvae are described and compared to larvae of *Baccalaureus falsiramus* Itô & Grygier from Tanabe Bay, Honshu, which they closely resemble. Pigmented structures in the ascothoracid-larvae possibly represent vestigial compound eyes. Competing hypotheses about the claw guard and proximal sensory process of ascothoracidan antennules are discussed in light of new ontogenetic data.

Crustacean larvae are a major component of marine meroplankton, and in order to identify the adults and determine the stage of development of a given larva, laboratory rearing is usually necessary. The planktonic larvae of the maxillopodan superorder Ascothoracida, which as adults are parasites of anthozoans and echinoderms, have rarely been reported but are striking and easily recognizable (e.g., Grygier, 1987, 1988, 1990; Boxshall & Böttger-Schnack, 1988). They include nauplii and bivalved ascothoracid-larvae. Itô & Grygier (1990) recently described the complete larval series of a new species of laurid ascothoracidan, *Baccalaureus falsiramus*, from Tanabe Bay, Honshu, Japan, based on larvae initially removed from a brooding female, but did not determine when they are normally released into the plankton. The present paper concerns several larvae similar to those of *B. falsiramus* that were captured in plankton at Sesoko Island, Okinawa, and subsequently reared in the laboratory.

A single ascothoracid-larva was found in a preserved sample of light-attracted plankton taken Aug. 16, 1988, off the pier of the Sesoko Marine Science Center, on the southeastern shore of Sesoko Island, Okinawa, Japan. In August and September, 1989, plankton samples taken several times a week at different times of day and evening with a small plankton net tossed from the same pier were examined immediately in order to cull living larvae of Ascothoracida. Four ascothoracidan nauplii (D1-D4) were taken after dark on Aug. 31 and three more (D5-D7) similarly on Sept. 20. Each nauplius was placed in a 10 ml vial filled with sea water (52  $\mu$ m mesh filter), kept at air-conditioned room temperature (22-28°C), and transferred by pipette to a fresh vial each morning or every other day; any exuviae, or

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Table 1. Timing of molts of ascothoracidan nauplii in culture. D4 was fixed Sept. 19 before the molt to the ascothoracid-larva; D5 was fixed upon capture. Last previous date of examination given in parentheses if not previous day.

Specimen and Data of Capture	Naupliar Instars and Dates of Finding Their Cast Exuviae				
	II	III	IV	V	VI
D1, Aug. 31		Sept. 5			
D2, Aug. 31			Sept. 7	Sept. 9	Sept. 18
D3, Aug. 31		Sept. 5			
D4, Aug. 31			Sept. 6 <sup>1)</sup>	Sept. 11 (9)	
D6, Sept. 20	Sept. 25	Sept. 26	missed	Oct. 1	Oct. 13 (2)
D7, Sept. 20	Sept. 20	Sept. 23	Sept. 25		

1) It is unconfirmed that D4 was at instar IV upon capture.

nauplii which failed to properly shed an exuvia, were preserved in 8% buffered seawater formalin. During a 10-day absence, D6 was left untended in a 125 ml jar. The dates on which naupliar exuviae were discovered are listed in Table 1. The two ascothoracid-larvae that were eventually obtained (D2, D6) were not fixed until 14 and 27 (or more) days, respectively, after their last molt in order to be sure there were no additional planktonic instars. The nauplii were lecithotrophic, so no attempt was made to feed them.

Fixed exuviae were transferred to glycerine and thence into glycerine jelly mounts for microscopical examination with phase contrast optics. Fixed nauplii were examined microscopically in aqueous mounts; D4 (ready to molt to the ascothoracid-larva) was dissected and mounted in glycerine jelly, and D1 and D3, both at instar IV, were prepared for scanning electron microscopy according to the procedure outlined in Itô & Grygier (1990) but omitting postfixation in osmic acid. Developmental stages were determined on the basis of antennular setal armament (Itô & Grygier, 1990). Instar II was described from whole D5 and exuviae of D6 and D7; instar III from exuviae of D3, D6, and D7; instar IV from whole D7, D1, and D3 (including SEM of latter two) and the exuvia of D2; instar V from exuviae of D2, D4, and D6; instar VI from whole and dissected D4 and exuviae of D2 and D6. Fixed ascothoracid-larvae were partly dissected and examined microscopically in glycerine jelly mounts. Drawings were done with the aid of a drawing tube; while accurately representing the best displayed preparations, Figs. 3 and 4 are not meant to be idealizations, and the text should be consulted for full details.

### Nauplii

(Figs 1-5)

#### *Common features of instars II-VI*

Instars II-VI actually metanauplii with rudimentary maxillules. Dorsal outline rounded, kite-like, 0.61-0.65 mm long, 0.49-0.57 mm wide (Figs 1A, B, 5A).

Equatorial and dorsal pores, 2-cupped nauplius eye with red pigment, and pair of frontal filaments present, though last not often visible in exuviae. Labrum a short, raised, triangular lobe (Fig. 5C). Appendages arising from within bowl-shaped ventral concavity, natatory setae about 0.32–0.39 mm long, not reaching rear margin of body. Caudal armament falling short of or slightly overreaching end of body. Body largely filled with uncolored oil droplets except for clear area posteriorly.

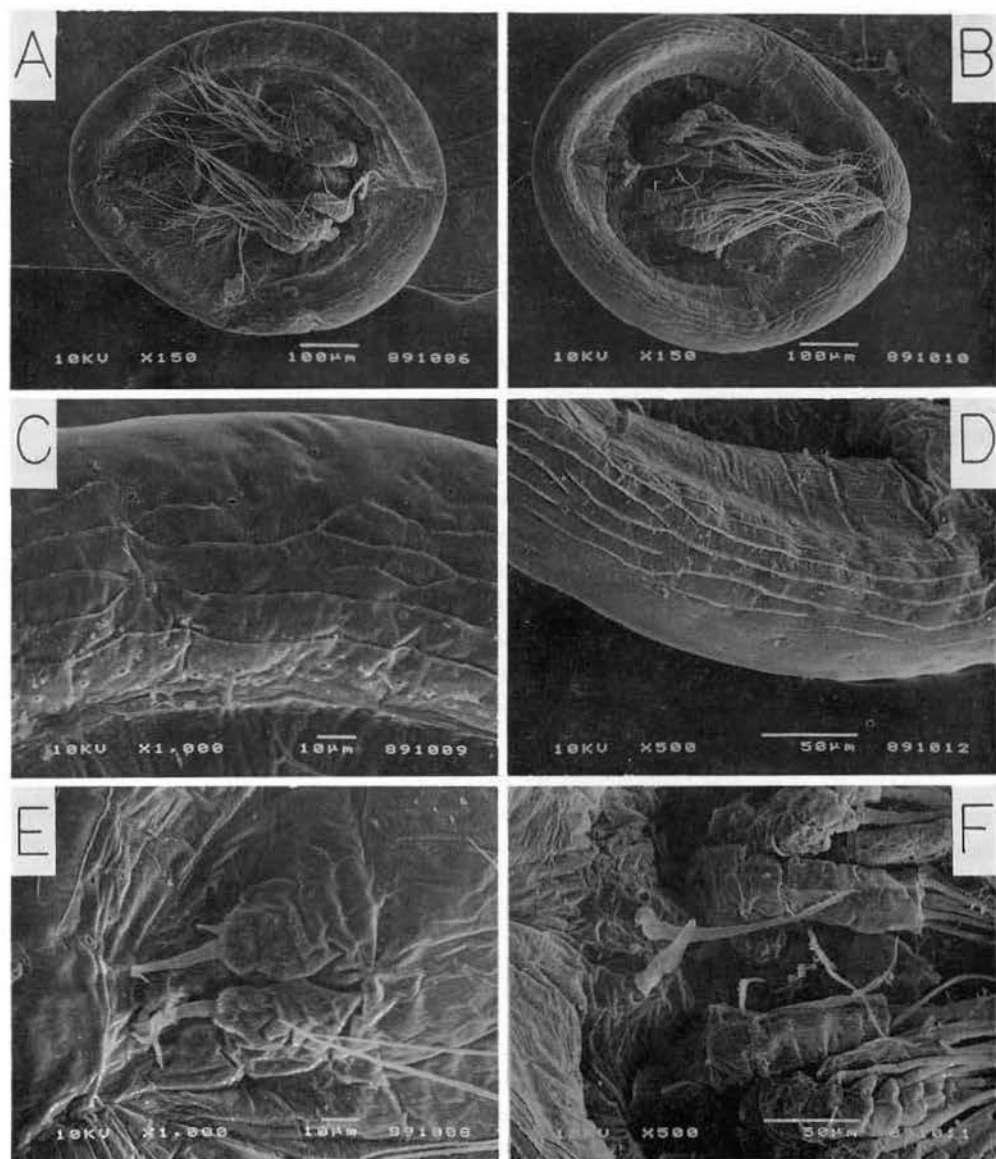


Fig. 1. Instar IV nauplii, scanning electron micrographs, left column D1, right column D3. A, B. Habitus ventral views; C, D. Marginal cuticular ridges of dorsal shield and, best seen in C, equatorial and dorsal pores; E. Caudal armament; F. Frontal filaments, antennules, and labrum.

*Instar II*

D5 measuring  $0.62 \times 0.49$  mm, body swollen. Antennule 5-segmented (Fig. 2A), proximal three segments short, fourth one long, fifth very small and not clearly articulated, second through fourth segments spinulose. First segment unarmed, second with setulate "a" seta medially, third with longer but simple "b" seta medially, fourth with long, setulate "d" seta and hair-like "e" seta medially and medium-long, simple "f" seta laterally, fifth segment with 3 unequal "g" setae, setulate central one longest, others simple and medial one shortest.

Antenna (Fig. 3A) with distinct coxa, basis, 3-segmented endopod (second segment longest, third smallest and not distinctly articulated), and 9-segmented exopod almost twice as long as endopod (in D7, fifth segment of one exopod partly divided: Fig. 3A, arrow). Coxa with small enditic spine and spinule, lateral hairs not observed. Enditic knob on basis with 2 short, equal, hairlike setae or spines. First segment of endopod with 2 short, unequal, hairlike setae, second segment with moderately long, setulate seta, third with short, hairlike seta and 2 long, setulate ones apically. Exopod with 7 setae, 1 each on segments 4-8, 2 on apical segment; lateral apical setae short, thin, and perhaps simple, other setae long and setulate. Row of spinules (or possibly pleats) along distal edge of many or perhaps all exopod segments, patches of spinules on all endopod segments.

Mandible (Fig. 3E) with distinct coxa, basis, 3-segmented endopod (segmental proportions as in antenna, articulation of third segment indistinct), and 7-segmented exopod 50% longer than endopod. Coxa with spine larger than that on antenna, usually seen accompanied by spinule. Basis with short, setulate seta and very short, hairlike one. First endopod segment with medium-long, setulate seta and short, hairlike one, second segment with long, setulate seta, third segment as in antenna. Exopod with 6 setae, one each on segments 3-6, 2 on apical segment, most if not all setulate. Spinules on first 2 endopod segments and along distal rims of at least some exopod segments (latter may be pleats).

Maxillules (Fig. 4A) represented by pair of long, simple setae. Terminal spine naked in D6, basally spinulose in D7, furcal spines naked in both, about half as long as terminal spine, preceded by scale-like arrays of spinules (Fig. 4A). Caudal armament barely reaching end of body.

*Instar III*

Antennules 4-segmented (Fig. 2B); former fifth segment reduced to small apical lobe of fourth. Seta "a" setulate in D3, setules not seen in D6 and D7. Seta "e" minute in D3 and D7, but longer in D6. Medial "g" seta setulate, longer than lateral one. Newly arisen lateral seta "h<sub>1</sub>" about as long as "f".

In antennae (Fig. 3B), coxa with lateral hairs, enditic spinule not seen in some preparations. Basis with hairlike seta and short spine, or 2 unequal short spines. First endopod segment with 2 short, unequal, hairlike setae; second with 1 medium-long, simple seta and, in D3 only, 1 very short seta on both antennae; medial apical seta as long as seta of second segment. Exopod probably usually 10-segmented, but

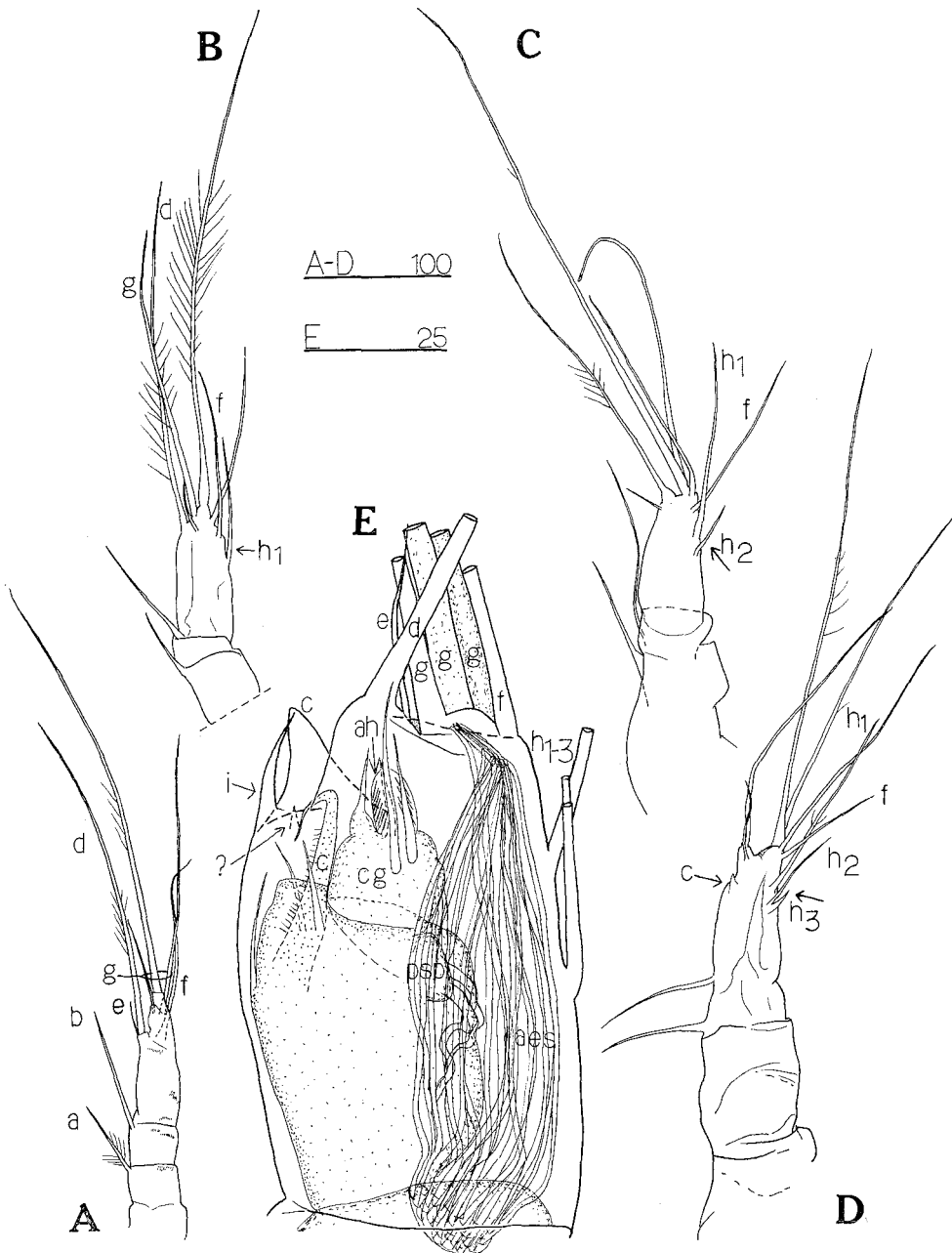


Fig. 2. Naupliar antennules, A-D based on exuviae. A. Instar II of D7; B. Instar III of D6; C. Instar IV of D2; D. Instar V of D2; E. Fourth segment of D4 instar VI and developing sixth segment of ascothoracid-larva. Setal designations (a-h<sub>1-3</sub>) following Itô & Grygier (1990), new structures at each instar marked by arrows, only clearly seen setules illustrated. aes, aesthetascs; ah, apical hood; c, claw; cg, claw guard; psp, proximal sensory process; ?, possible posterior setal rudiment. Scale bars in μm.

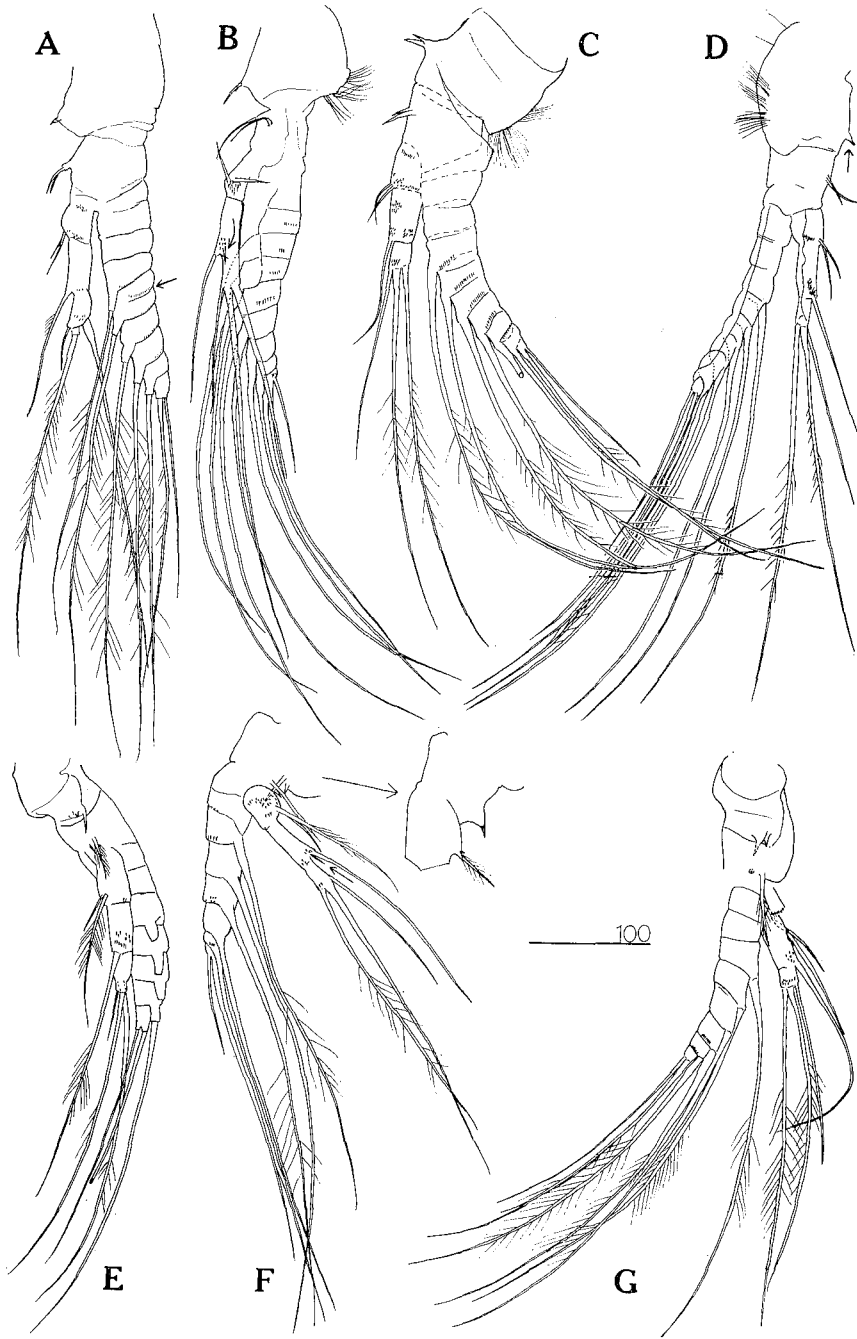


Fig. 3. Naupliar antennae (A-D) and mandibles (E-G) based on exuviae, only clearly seen setules illustrated. A. Instar II of D7, arrow pointing to extra partial segmental division; B. Instar III of D3, arrow pointing to possibly atavistic seta; C. Instar IV of D2; D. Instar V of D6, arrow pointing to unusually small coxal spine (usually as in C); E. Instar II of D7; F. Instar III of D3 with inset of protopod of D6 instar III, arrow pointing to possibly atavistic seta; G. Instar V of D2, composite drawing, propod and rami from different members of pair. Spinules illustrated on far side of limbs. Scale bar in  $\mu\text{m}$ .

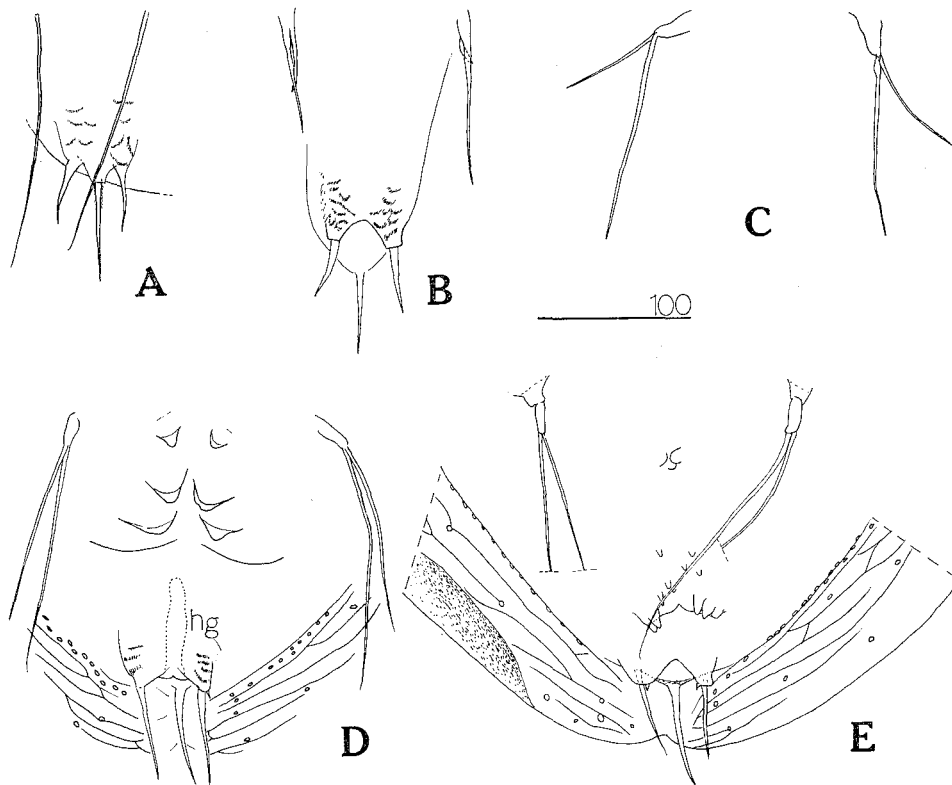


Fig. 4. Naupliar maxillules and caudal armament based on exuviae. A. Instar II of D7; B. Instar III of D6; C. Maxillules only of D2 instar IV; D. Instar V of D6 showing hindgut (hg); E. Instar VI of D6 (lengths of maxillular setae and extent of spinule beds on furcal lobes unclear). Scale bar in  $\mu\text{m}$ .

most preparations unclear, one in D6 11-segmented due to intercalary sixth segment; 8 setae, 2 on apical segment relatively short and unequal, 1 long seta each on 6 preceding segments, skipping intercalary one in D6, all but lateral apical seta setulate.

In mandible (Fig. 3F), coxa with small spine and spinule, short seta on basis reduced to spinule, and not always seen. Setulate setae of first 2 endopod segments longer than before, that of second segment accompanied by tiny seta on one mandible in D3 only. Medial apical seta of third segment simple, half as long as other 2. Exopod 8-segmented with 7 setae, 1 each on segments 3-7, 2 on apical segment, lateral apical seta short and simple, all others long and setulate.

Maxillules (Fig. 4B) represented by pair of long simple setae on raised bases in D3, by pair of similar setae each accompanied by shorter or very short seta in D6 and D7. Terminal and furcal spines similar in size, naked, latter preceded by several beds of small spinules (Fig. 4B).

#### *Instar IV*

D1 measuring  $0.62 \times 0.50$  mm, D3  $0.65 \times 0.57$  mm, D7  $0.65 \times 0.51$  mm. Dor-



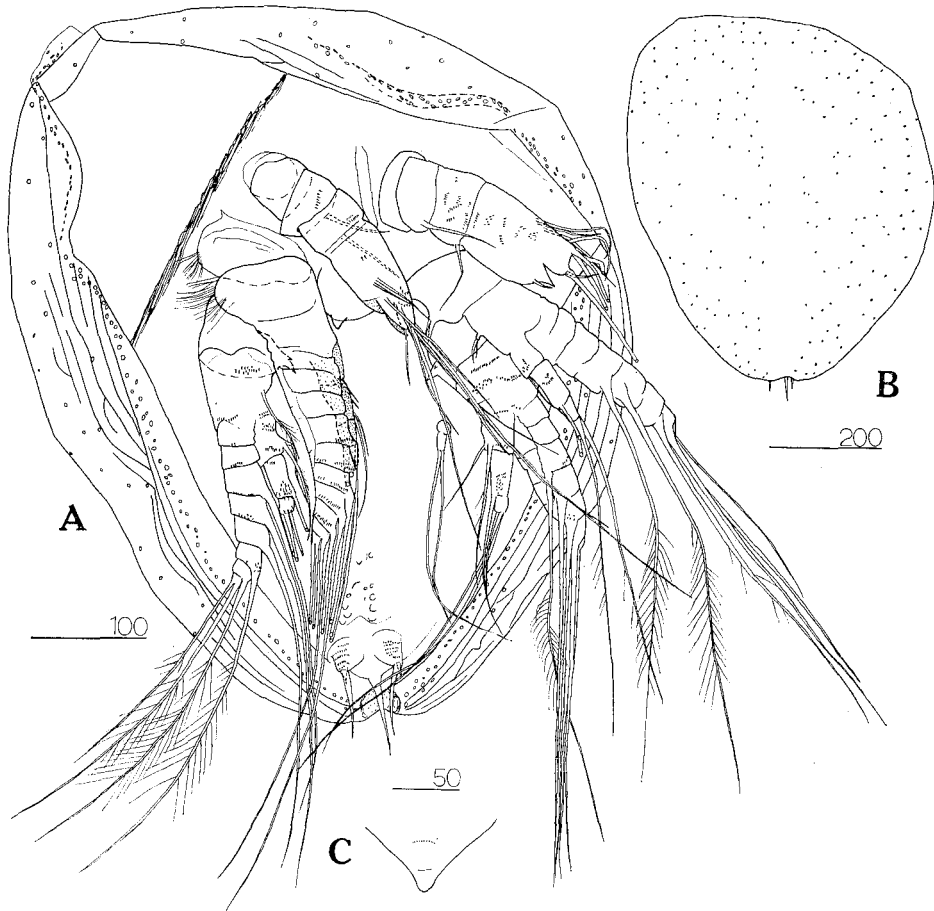


Fig. 5. Instar VI nauplius, exuvia of D2. A. Complete exuvia mounted on slide, ventral view, some setae cut short for clarity, only one frontal filament clearly seen, ventral cuticle detached from dorsal shield along inner margin of latter except at posterior end; B. Dorsal pore pattern of same exuvia, as seen from ventral side; C. Outline of labrum as seen in exuvial preparations at all instars. Scale bars in  $\mu\text{m}$ .

sal shield forming shallow bowl with broad, rounded, incurved rim (Fig. 1A, B) ornamented with 5 concentric, sometimes connected, cuticular ridges, better expressed in D3 than D1 (cf. Fig. 1C, D), and ring of equatorial pores (Fig. 1C). Frontal filaments about  $90 \mu\text{m}$  long, pore-bearing papilla between them and pair of pores or pits (artifacts?) just lateral to them (Fig. 1F). Four pairs of posterior mesodermal blocks visible internally in D7.

Remnant of fifth antennular segment more fully incorporated into fourth (Fig. 2C). Setulation displayed very poorly in D2, but seta "a" apparently setulate in D1 and D3 (Fig. 1F). Short "h<sub>2</sub>" seta newly arisen just proximal to "h<sub>1</sub>".

In antenna (Fig. 3C), coxal spinule larger than in previous instar. Enditic spines on basis equal or not. Hairlike setae on first endopod segment equal; medial apical seta of third segment longer than previous instar, half as long as other 2 apical

setae. Exopod 10-segmented (third seta missing on one of them), lateral apical seta longer than in instar III.

Mandible poorly observed and not illustrated, seemingly unchanged from usual situation in instar III, no tiny seta on second endopod segment.

Maxillules as pair of lobes bearing 2 unequal, simple setae, medial one longer (Fig. 4C). Small accessory spine next to left furcal spine in D3, next to both in D7; beds of small spinules anterior to furcal spines clearly shown in Fig. 1E. Caudal armament reaching end of body in D3, not in D1 or D7.

#### *Instar V*

Antennule (Fig. 2D) with weak claw rudiment ("c") subapically on medial side, but not seen in D6. Three "g" setae and "d" seta on two separate, apical lobes, all but lateral "g" seta setulate; "a" seta simple. Seta "e" almost twice as long as in previous instar except in D4. Seta "h<sub>1</sub>" twice as long as "f", "h<sub>2</sub>" slightly longer than in previous instar, accompanied by newly arisen, very short "h<sub>3</sub>", except latter absent on one side in D6.

No notable change in antenna (Fig. 3D).

Mandible basically unchanged (Fig. 3G), but lateral apical seta of exopod setulate (not confirmed in D6).

Bases of maxillules longer than before, cylindrical but probably non-articulated (Fig. 4D). Four pairs of large, rounded bumps on ventral body surface beginning between maxillules, and beds of spinules restricted to furcal lobes proper (Fig. 4D). Weak accessory spine observed next to both furcal spines in D4 and left one in D6. Chitinous hindgut observed in D6 (Fig. 4D), extending forward from anus located between furcal lobes.

#### *Instar VI*

D4 measuring  $0.61 \times 0.51$  mm; exuvia of D2 distorted, but approximately  $0.63 \times 0.55$  mm (Fig. 5A). Dorsal shield thicker than in previous instars, many pores scattered over it except along midline (Fig. 5B), no dorsal setae observed except possibly in D4. Caudal armament extending past end of body. In D4, thoracopods and abdomen of developing ascothoracid larva occupying oil droplet-free, posterior region.

Antennule clearly 4-segmented (Figs 2E, 5A), third and fourth segments heavily spinulose, setae "a" and "b" on second and third segments, with tiny setal rudiment "b<sub>1</sub>" accompanying latter on one antennule of D6 and at least one of D4. Fourth segment with large, triangular, medial claw rudiment ("c") subapically, accompanied by short, hairlike seta "i" anteriorly and possibly by much tinier one posteriorly (latter never seen clearly); seta "e" always short. Seta "h<sub>3</sub>" longer than before, but apparently still absent on one antennule in D6. Setulation not well observed, "d", medial and central "g", and "h<sub>1</sub>" clearly setulate in D4.

No notable change in antenna, except 2 unequal setae seen on second endopod segment of one antenna in D2 (Fig. 5A).

In mandible (Fig. 5A), coxa with large spine and spinule, basis apparently with

1 simple seta. Short seta of first endopod segment very short in D6 but its length unchanged from previous instars in D2. Lateral apical seta of exopod observed to be setulate on 1 mandible in D2.

Each maxillule consisting of articulated, cylindrical segment on short base, with 2 long, simple, unequal setae (Figs 4E, 5A), "segment" extremely reduced on right side in D4. Each furcal spine accompanied dorsomedially by weak accessory spine in D6, dorsally by longer, stronger accessory spine in D2 and D4. D2 with 5 pairs of small bumps or double bumps just anterior to furcal lobes (Fig. 5A); D6 with 3 bumps close to midline just behind level of maxillules, 7 irregularly placed small bumps posteriorly, and 2-3 small bumps just before each furcal lobe (Fig. 4E).

### Remarks

The agreement in timing of molts was not very great. Instar II lasted at least 3-5 days, instar III from 1 to at least 5 days, instar IV at least 6-7 days, instar V 2-5 days (but in D6, instars IV+V took only 5 days), and instar VI at least 8-9 days. D2 took 19 days from instar IV to the ascothoracid-larva, D6 between 12 and 23 days (probably closer to 23, since the instar VI exuvia was still in good condition). Naupliar development therefore probably lasts approximately 3-4 weeks, assuming instar I is short-lived, and ascothoracid-larvae can live at least 4 weeks afterwards.

The differences between the present specimens and the nauplii of *Baccalaureus falsiramus* are minor, and some of them may be due to difficulties in observing small structures in certain preparations.

The nauplii of *B. falsiramus* are slightly smaller,  $0.59-0.60 \times 0.45-0.52$  mm in instars II and VI, and instar VI lasted longer, 14-15 days, perhaps due to the lower incubation temperature of 19°C. The rim of the dorsal shield has 1 or 2 fewer major cuticular ridges, and 4 pairs of dorsal setae were present at instar VI. Instar II rather than IV was examined by SEM in that species, and at that stage no medial process or lateral pores or pits are associated with the frontal filaments. Labral pores were not specifically noted in the present study, though *B. falsiramus* has 4 of them.

Antennular features seeming to differ from *B. falsiramus* include: many setules on seta "a" in instar II and at least sometimes later; no distal spinulation of lateral "g" seta in instar III; a weak claw rudiment usually detectable in instar V; length changes in seta "e"; only 2 instances of a rudimentary seta accompanying seta "b" at instar VI rather than universally accompanying "a" and "b", and possibly a tiny posterior setal rudiment accompanying the claw rudiment at instar VI.

Antennal differences seem to include: a lack of lateral coxal hairs in instar II (perhaps actually present, but not visible in preparation); sometimes a short, additional seta on the second endopod segment in instar III and maybe VI (perhaps an atavism, since this seta is lost after instar I in *B. falsiramus*); variability in the length of the short setae or spines on the basis and first endopod segment; and a non-setulate, lateral apical seta on the exopod in instar VI.

Apparent mandibular differences from *B. falsiramus* include: a short seta rather

than a spinule on the basis in instar II; the medial apical seta of the endopod never becoming setulate; and the lateral apical seta of the exopod becoming setulate in instar V.

The posteroventral armament differs in having: naked furcal spines and sometimes also a naked terminal spine in instar II and the latter longer than the former; 4 rather than 2–3 pairs of ventral bumps in instar V; and the irregular timing of appearance and strength of the accessory furcal spines.

Dr. Itô's and my initial observations of the nauplii of *B. falsiramus* did not always agree and took concerted effort to reconcile; furthermore, only three larval series were available for the later instars. This and the lack of uniformity of some features on the present specimens mean that the differences compiled above do not have enough weight to force us to consider the Okinawan nauplii as a distinct species (but see below). Moreover, this morphological closeness reaffirms Itô & Grygier's (1990) suggestion that the nauplii of *B. falsiramus* are probably free in the plankton by instar II, since the present ones are.

### Ascothoracid-larvae

(Figs 6, 7)

A Tessmann's larva *sensu* Grygier (1988). At rest, body wholly enclosed within carapace (Figs 6A, 7A). Swimming position upside down, propulsive force provided by powerful strokes of thoracopods and furcal rami at a rate of a few strokes per second.

#### Description

Carapace (Fig. 6). Bivalved, D2 and D6 both 575  $\mu\text{m}$  long, 349  $\mu\text{m}$  high, planktonic specimen smaller, 556  $\mu\text{m}$  long, 333  $\mu\text{m}$  high, all with straight or slightly convex dorsal margin and slightly recessed hinge line, oblique posterodorsal margin straight or very slightly concave, remainder of margin curved but nearly straight ventrally (Fig. 6A). Outer surface of carapace ornamented with polygonal meshwork of low cuticular ridges (Fig. 6B), meshes usually 12–18  $\mu\text{m}$  across (longer along a-p axis, 12–14  $\mu\text{m}$  in planktonic specimen), with much smaller, round, pore- or seta-bearing meshes at vertices or along segments of main meshwork except over adductor muscle. Several meshes, mostly in arc below adductor muscle attachment, with one-quarter to half of area as circular region of thin cuticle (Fig. 6B). Each valve usually with 5 cardiac organs (*sensu* Itô & Grygier, 1990), 2 anterodorsally, 3 posterodorsally, each a narrow mesh near hinge with thick, anteriorly originating, cylindrical structure lying along bottom (Fig. 6C, D); 4 posterior cardiac organs on left valve of D6 (Fig. 6E). One valve of D2 with peculiar anterior mesh with 6 pores (Fig. 6C). Inner surface of carapace with scattered pores and complex armament of hairs. Concentric cuticular ridges near margins lined with short, fine hairs. Innermost anterior ridges with reflexed ends and with shorter, thicker hairs in feather-like arrays (Fig. 6F) similar to but half as long as arrays illustrated in another Tessmann's larva

by Grygier (1988). Posterior end with more complex pattern of intersecting cuticular ridges and many fringes of fine hairs, as well as several ranks of much longer, stouter guard setae (Fig. 6G).

Antennule (Fig. 7B, C). 6-segmented, Z-shaped, larger than oral cone; first

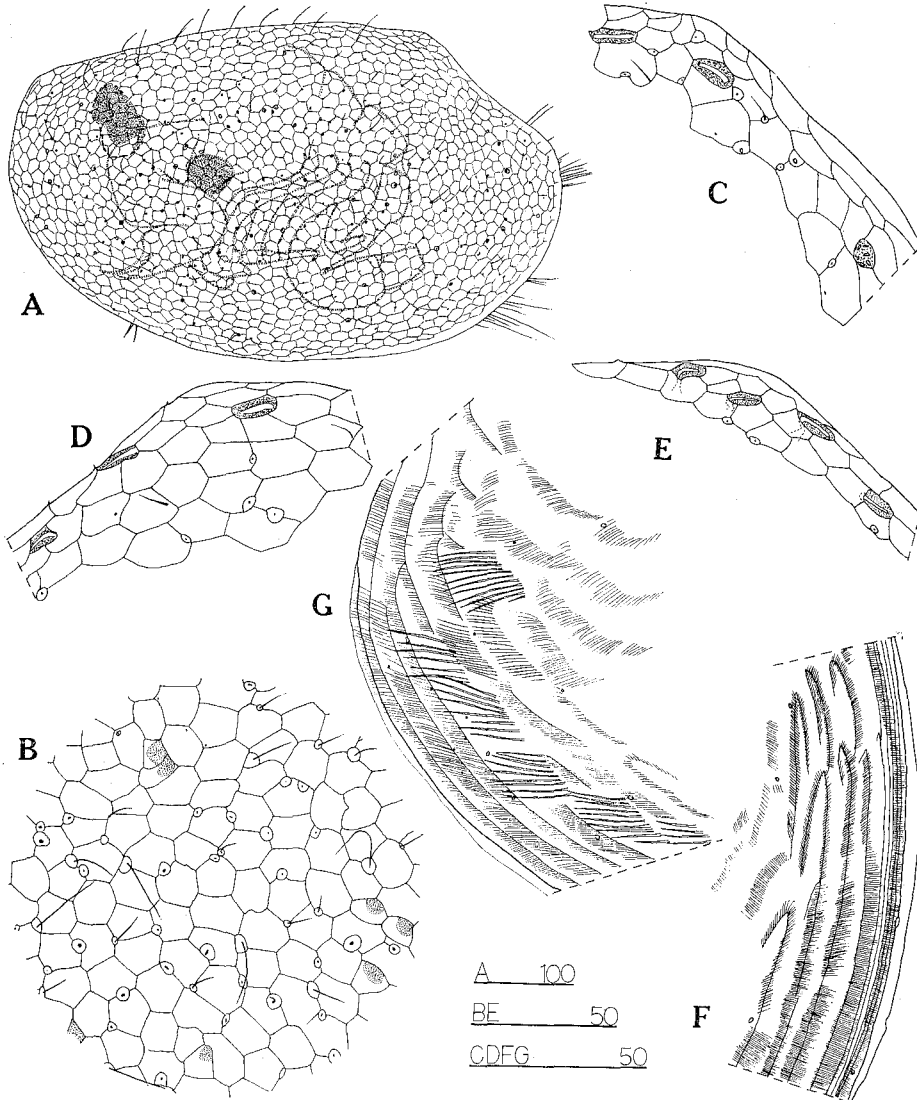


Fig. 6. Ascothoracid-larvae, carapace. A. Lateral view of plankton-caught specimen, anterior end left, with outline of body and positions of nauplius eye and pigmented lobe of frontal filament complex indicated, furcal and thoracopodal setae extending beyond rear margin; B. Surface ornamentation of D6, dorsal at top, anterior end left, upper left sector overlying adductor muscle, circular stippled areas with thin cuticle; C. Anterior cardiac organs and multiperforate mesh of D2 (stippled); D. Posterior cardiac organs of D2 (stippled); E. Posterior cardiac organs of left valve of D6 (stippled); F. Medial ornamentation of anterior end of valve of D2; G. Medial ornamentation of posterior end of valve of D2. Scale bars in  $\mu\text{m}$ .

segment short; second parallelogram-shaped; third triangular with long, thin hairs along anterior margin; fourth short with short hairs along posterodistal margin medially and 2 subequal setae on anterior margin; fifth segment longest, with 2 unequal basal setae and shallow longitudinal trough anteriorly. Sixth segment subrhomboidal with complex armament (Fig. 7C): movable claw with 30–40 denticles, distal ones longer, thicker, and more widely spaced; marginal and lateral seta at base of claw (also medial seta on 1 antennule in D2, both in D6, absent in planktonic specimen); laterally flanged claw guard similar in size to claw, with 2 apical and 1 suba-

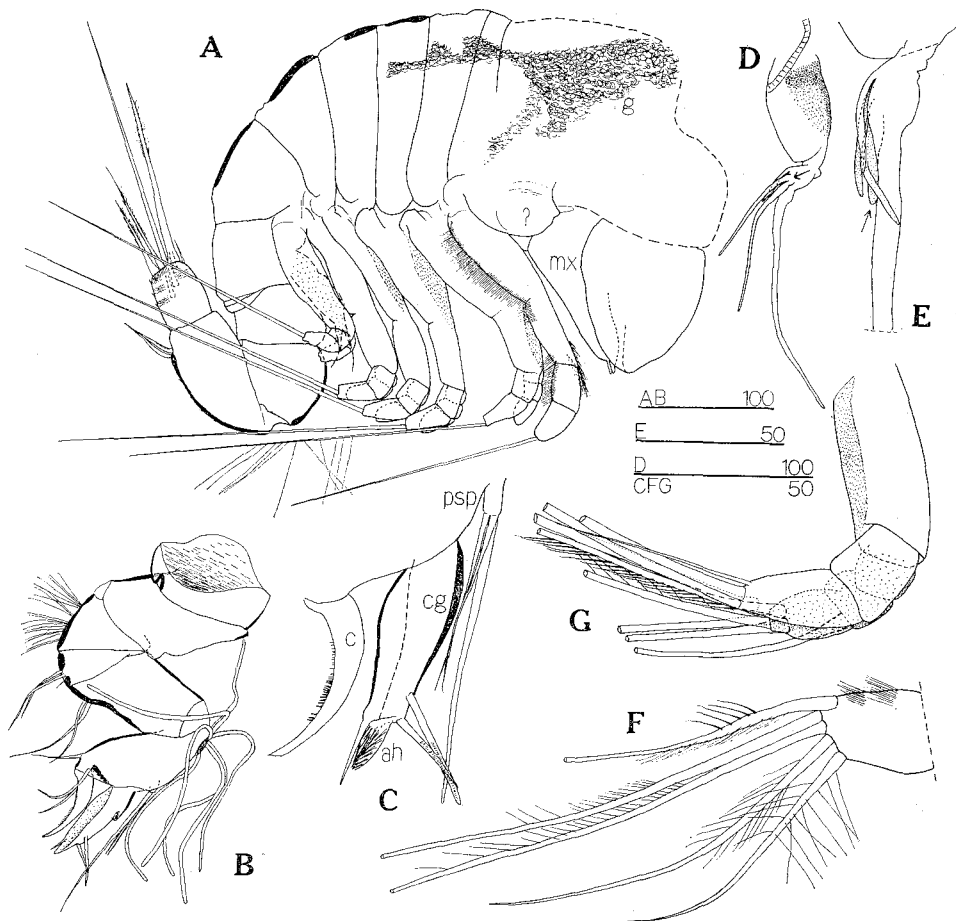


Fig. 7. Ascothoracid-larvae, body and appendages. A. Body of D2, antennules removed, representative exopod setae and setules shown, penis (between last thoracopods) stippled, only bases of medial furcal setae shown (as long as longest terminal seta); B. Right antennule of D2, medial view, only a few aesthetascs of proximal cluster shown; C. Distal armament of sixth segment of left antennule of D2, lateral view; D. Right frontal filament complex of D2; E. Left frontal filament of D6; F. Distal exopod segment of first thoracopod of D6; G. Basis and rami of fourth thoracopod of D2. ah, apical hood; c, claw; cg, claw guard; g, gut; mx, maxillae; ?, unidentified lobe; arrows in D and E pointing to bifid branches. Scale bars in  $\mu\text{m}$ .

pical setae, spinulose apical hood (no enclosed duct clearly seen), and in 5 of 6 cases deep invagination or shallow dimple midway along posterior margin (present in Fig. 7B, absent in Fig. 7C); knob-like proximal sensory process at midlength of segment, with long medial seta and 2 shorter, thinner, lateral ones; proximal cluster of 130  $\mu\text{m}$  long aesthetascs (about 25 in D2, 28–32 in D6 and planktonic specimen) arising within ridge-bounded oval.

Frontal filament complex (Fig. 7D, E). Oval basal lobe 65–70  $\mu\text{m}$  long containing extrinsic muscle and red pigment cup composed of 1  $\mu\text{m}$  granules (Fig. 7D). Frontal filament proper 165–180  $\mu\text{m}$  long in D2 with 2 basal side branches, first one very short, second immediately splitting into 2 subequal branches about half as long as main one (Fig. 7D); in D6 left frontal filament broken but with 3 short, thin basal branches, third one bifid (Fig. 7E) (right one not clearly seen); in planktonic specimen, right one shorter than in D2 but with similar branches, left one as long as in D2 but apparently lacking shortest side branch.

Oral cone (Fig. 7A). Shield-like labrum flanking maxillae laterally, latter with narrow, pointed, bifid tips; rudiments of mandibles and maxillae possibly present, but difficult to see. Red, 2-cupped nauplius eye located in "forehead" anterior to labrum and between antennules. Narrow midgut containing spherical, yellow-green particles 2.4–3.8  $\mu\text{m}$  in diameter beginning dorsally within head, extending posteriorly to at least third thoracomere in D2, sixth in D6, pair of short lateral branches entering carapace.

Thorax (Fig. 7A). Six-segmented, first segment short, distinguishable from head dorsally, next 3 segments equal, fifth and sixth longer dorsally; no thoracic ornamentation.

Thoracopods (Fig. 7F, G). Six pairs, first 5 decreasing slightly in length posteriorly, sixth markedly shorter; in D2 base of first preceded at least on right side by swelling (Fig. 7A; for maxillary glands?). Each thoracopod with long coxa, shorter basis, 2-segmented exopod; endopod variable, a thick, plumose seta in first pair, 3-segmented and shorter than exopod in middle 4 pairs, 2-segmented in last pair. First thoracopod lined with fine hairs along lateral margin (not second exopod segment and, in D2, part of basis), medial side also lined at least in D6, hairs on second exopod segment arranged in transverse rows (Fig. 7F). Natatory setae restricted to rami, up to about 270  $\mu\text{m}$  long. Second exopod segment of thoracopod I with 5 equally thick, apical setae, lateral 2 relatively short and basally plumose (Fig. 7F); 5 setae on this segment in pairs II–V as well (short, thin lateral seta, 2 long apical ones, 2 long medial ones; Fig. 7G), 3 setae in thoracopod VI (2 apical, 1 medially subapical). Proximal medial seta of each exopod with row of spine-like hairs along basal portion followed by long setules, other long setae with bare shaft before long setules; armament of short, lateral seta unclear. Endopods of thoracopods II–V with long seta on second segment, 1 subapical and 2 apical setae on distal one, setulation unclear, but lateral apical seta apparently bearing basal setules similar to but weaker than those on opposing exopod seta (Fig. 7G). Endopod of thoracopod VI with 2 long apical setae.

Abdomen (Fig. 7A). Four-segmented, third segment triangular, fourth twice as long as others. Penis rudiment on first segment nearly reaching second, with pair of pointed distal processes seen clearly in D2, less clearly in D6 (?rami, ?setae). Fourth segment with few ventral cuticular ctenae and pair of minutely spinulose posteroventral spines nearly as long as furcal rami. Furcal rami rectangular ( $55 \times 37 \mu\text{m}$  in D2), with distal ctenae on lateral face and several medial ctenae (ctenal pattern slightly variable), 3 long medial setae, and 4 distal setae plus dorsal spine. Uppermost distal seta long, thick, with short setules; ventral 2 setae similar, but shorter and thinner; third seta longest (about  $190 \mu\text{m}$ ), similar to medial setae, their setulation unclear.

#### Remarks

Specific identity. The ascothoracid-larva caught in plankton is similar enough to D2 and D6 to be treated as a representative of the same species in the following. The minor morphological differences, except perhaps the smaller mesh size, do not immediately force its recognition as distinct, but since it was not raised from a nauplius, this possibility does exist. Certainly none of them represents the ascothoracid-larva of the Okinawan species of *Baccalaureus* whose first two naupliar instars, the second of which is apparently planktotrophic, were described by Grygier (1990). The lecithotrophic instar II nauplius D6 is very different from the instar II nauplius of that species, and a settled ascothoracid-larva found associated with a female of the latter is clearly different, with much more prominent cuticular ridges separating pit-like meshes (Grygier, unpublished data).

These ascothoracid-larvae closely match those of *B. falsiramus* described by light and scanning electron microscopy (Itô & Grygier, 1990). The latter are smaller ( $0.51 \times 0.31 \times 0.30 \text{ mm}$ ), with a clear posterodorsal emargination and meshes averaging  $13 \mu\text{m}$  across. No "feathery" anterior ornamentation on the inside of the carapace valves was described, but this area was not visible under the SEM. The first antenna in *B. falsiramus* has 2 nearly equal setae on segment 5 and a tubule within the apical hood. The frontal filaments are shorter with only one side branch, and no pigment was observed in the basal lobe. There are large, vestigial, naupliar antennae and possibly vestigial mandibles; the swelling anterior to the first thoracopod in specimen D2 may represent such a structure, but otherwise, they are not evident in the present material. All the exopod setae of thoracopod VI are alike in *B. falsiramus*. Details of the penis are unknown. The fourth abdominal segment and furcal rami have a few more ctenae, the dorsal terminal seta inserts into a short process, and only the ventral seta closely resembles the dorsal one in form and armament.

The less distinct carapace emargination, lack of large naupliar antennae, more complex frontal filaments, and different furcal shape suggest that the Okinawan ascothoracid-larvae are distinct from *B. falsiramus*, while the nauplii did not provide firm evidence. *Zoanthus*, the host genus of *B. falsiramus*, is abundant at Sesoko Island, but at least intertidally it does not seem to be infested by *Baccalaureus*. One or perhaps two undescribed species of *Baccalaureus* in which brooded nauplii have not yet



been found parasitize *Palythoa* at Sesoko Island; perhaps one of these is the adult form of the present larvae.

Vestigial compound eyes. The red pigment in the basal lobe of the frontal filaments appears to be the same color as the pigment of the nauplius eye, and composed of similar particles. This strongly suggests that the lobe represents a vestigial compound eye and retains some light-receptive capacity. Grygier (1983) noted a well-developed compound eye in another ascothoracid-larva, with a simple frontal filament arising from the eyestalk. Whether the "basal appendix" of more complicated frontal filament complexes in many synagogid ascothoracidans is an even more reduced vestige of the compound eye, or a different structure involving part of the organ of Bellonci, will require ultrastructural investigation to decide.

Claw guard and proximal sensory process (Fig. 3E). Itô (1989) disputes Grygier's (1987) homology of the ascothoracidan claw guard with part of the antennular palp of facetotectan cyprids. He considers the former a specially developed protrusion of the claw-bearing segment that is homologous to a recess into which the facetotectan claw, when present, can be retracted. This hypothesis neglects a similar recess bounded by a pair of ridges between the claw and claw guard in many ascothoracidans, namely the site of insertion of the claw retractor muscles, and also the fact that the claw guard, though immotile, is at least sometimes bounded basally by a seam, suggesting a segmental fusion. A scanning electron micrograph of an antennule of a Tessmann's larva published by Grygier (1988: fig. 2D) shows these features clearly. Most importantly, Itô's hypothesis does not account for the setae that are usually found on the claw guard (3 in the present ascothoracid larvae, maximum of 4 in other ascothoracidans). While there is little doubt that the specialized form of the claw guard, especially of large, curved, laterally flanged ones, is an ascothoracidan apomorphy, it is possible in principal to show that its setae derive from certain naupliar setae.

Nauplius D4 instar VI, ready to molt to the ascothoracid-larva, provides such evidence. One dissected antennule shows very well the origins of many distal features of the ascothoracid-larval antennule (Fig. 2E). For example, the tip of the claw lies within the claw rudiment "c", and the lateral seta at its base apparently derives from seta "i". Also, the proximal aesthetasc cluster, occupying the rear third of the segment, leads directly to the apical lobe on which the "g" setae sit. The derivation of the three elements of the proximal sensory process is unclear. The presumptive claw guard with a spinulose apical hood is plainly visible, bearing 3 setae as expected and also a setiform structure within the apical hood. These setae, especially the longest one, are clearly associated with the naupliar apical lobe bearing setae "d" and "e". I conclude that the setae of the claw guard are positionally homologous with naupliar setae "d" and/or "e", and that my earlier hypothesis (Grygier, 1987), reformulated as follows, is correct: the claw guard is a hypertrophied modification of the medial side of the posteriorly migrated, penultimate antennular segment of generalized ascothoracidan nauplii (in the present case, of instar II nauplii), namely the segment that is homologous to the first segment of the facetotectan cyprid antennular

palp.

Itô (1989) also thinks that one or more aesthetascs that arise separately from the proximal sensory process in some ascothoracidans, including Tessmann's larvae, should be considered as distinct structures, while Grygier (1984) thought they were simply displaced from their primitive position on the process (e.g., in many Synagogidae). The present evidence cannot fully answer this dispute, since the ontogenetic origin of the setae of the process proper is not clear, but the basal aesthetasc cluster seems to have its origin in the naupliar "g" setae, which were originally apical. This supports Grygier's (1987) notion of a proximal migration of the apical armament in the Ascothoracida at metamorphosis, originally proposed on the basis of the similarity of the armament of the apical antennular segment in many facetotectan antennules to the armament of the proximal sensory process in certain ascothoracidans.

This type of study needs to be repeated with ascothoracidan metanauplii in which the apical segments remain distinct, such as the "metanauplius *incertae sedis*" of Grygier (1987) and "metanauplius type I" of Boxshall & Böttger-Schnack (1988).

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Note added in proof: Dr. J. Moyses (pers. comm.) has questioned an inconsistency between the text and an illustration in Itô & Grygier (1990: 509, Fig. 18A), concerning the cardiac organs of the ascothoracid-larvae of *Bacallareus falsiramus*. There are in fact two cardiac organs anteriorly and three posteriorly on each carapace valve.

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