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LARVAL DEVELOPMENT OF *CARIBEOPSYLLUS AMPHIODIAE* (THAUMATOPSYLLIDAE: COPEPODA), AN ENTEROZOIC PARASITE OF THE BRITTLE STAR *AMPHIODIA URTICA*

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ABSTRACT

Although most parasitic copepods produce free-living larvae, *Caribeopsyllus amphiodiae* and other thaumatopsyllid copepods have a parasitic larval stage (metanauplius) that inhabits an ophiuroid stomach. Metanauplii of *C. amphiodiae* collected from its burrowing host, *Amphiodia urtica*, and reared in the laboratory gave rise to ovigerous females that released free-swimming first nauplii (NI). We provide the first full descriptions for a thaumatopsyllid of the NI, parasitic metanauplii, and free-living copepodid I to copepodid VI of the female and male based on developmental stages obtained. Extraordinary features of *C. amphiodiae* NI are the presence of one pair of setae on the labrum, a character unique among Copepoda, and the presence of a maxillule represented by one seta, making the larva a metanauplius by definition. In most other copepods, the one-seta or one-spine maxillule appears no earlier than NII. The mandible of the metanauplius becomes massive, and a spiniform process from the first endopodal segment forms a chelate complex with the distinctly curved, clawlike second endopodal segment. Transient vestiges of the antenna and mandible remain in the CI, but maxillule, maxilla, and maxilliped are absent during the copepodid phase. Other body structures appear earlier than reported for other copepods: pediger 5 and the bud of swimming leg 4 are present at CI, and pediger 6 and the buds of legs 5 and 6 are present at CII. During leg development setal development is accelerated, but ramal segmentation is delayed. In addition, the major body articulation of the copepodid and adult stages occurs between the third and fourth pedigers, unlike the tagmosis of most gymnoplean and podoplean copepods. Such shifts in the timing of ontogenesis, atypical naupliar morphology, and unique adult body set apart the thaumatopsyllids from other copepods.

KEY WORDS: Copepoda, copepodid, development, heterochrony, larva, life-cycle, metanauplius, ontogenesis, Ophiuroidea, parasitism, paedomorphosis, protelean, symbiosis, tagmosis, thaumatopsyllid

INTRODUCTION

Approximately 100 years ago, Georg Ossian Sars sampled plankton from deep in Oslo Fjord and brought to light the first example of a new and profoundly enigmatic copepod taxon. Having only a single specimen, and unable to refer the "most peculiar" animal to any recognized group, he hesitated many years before naming the new species *Thaumatopsyllus paradoxus* Sars, 1913 and erecting Thaumatopsyllidae to accommodate it (Sars, 1913: 3). Due to its lack of mouthparts and second antennae, deficiencies shared with the bizarre adults of Monstrillidae, Sars placed both families in his Suborder Monstrilloida. Perhaps reflecting on the protelean life cycle of monstrillids, in which nauplius and copepodid stages are parasitic, he envisioned *T. paradoxus* also "... spending the far greater part of its existence as a true endoparasite within the blood of some invertebrate host, whereas the adult, free-living stage is solely devoted to the propagation and in all probability is of very short duration" (Sars, 1913: 4). Although Sars's conjecture proved prescient, empirical information about the development of Thaumatopsyllidae has accrued only slowly and incrementally.

Nearly 50 years after Sars's discovery, Bresciani and Lützen (1962) and Fosshagen (1970) detected the metanauplius of *T. paradoxus* within the stomachs of several ophiuroid species, partially vindicating Sars's predictions. The larvae were isolated and then reared through six copepodid stages to adulthood, a developmental process taking only 4-5 days. The largest metanauplii became adult females, and since they somewhat differed in color from smaller larvae, it was suggested the latter might be males. Publications describing other thaumatopsyllid species including *Orientopsyllus investigatoris* Sewell, 1949 and *Australopsyllus fallax* McKinnon, 1994, were based on extremely small numbers of preserved female specimens, and revealed little regarding the biology of the group. However, numerous individuals of *Caribeopsyllus chawayi* Suárez-Morales and Castellanos, 1998, were obtained from plankton samples by Suárez-Morales and Tovar (2004), who described the copepodid stages in some detail. Their report added considerably to the understanding of thaumatopsyllid ontogenesis, but some of their conclusions, such as the assertions that the life cycle lacks a C4 stage and the copepodids lack eyes, are not consistent with results of earlier studies of *T. paradoxus* and the present study.

The fifth and most recently recognized species, *Caribeopsyllus amphiodiae* Ho et al., 2003, is only the second thaumatopsyllid for which both sexes and a host have been described. Ho et al. (2003) found that the larva of

C. amphiodiae lives in the stomach of a burrowing ophiuroid, *Amphiodia urtica* Lyman, 1860, and provided detailed descriptions of laboratory-reared adults. The present contribution presents detailed descriptions of the first nauplius, metanauplius, and copepodid stages. It also contrasts the morphology of developmental stages of *C. amphiodiae* with that of other thaumatopsyllid species and of other copepods. The appraisal casts light on the long-disputed phylogenetic relationships of Thaumatopsyllidae, which previously have been analyzed using adult characters exclusively (Ho et al., 2003; Boxshall and Halsey, 2004). Additionally, this study complements an associated contribution treating the ecology, internal morphology, sexual dimorphism, and timing of development of *C. amphiodiae*, and the evolutionary implications of its life cycle (Hendler and Dojiri, submitted).

Materials and Methods

Between April 1991, and July 1993, the ophiuroid *A. urtica* was periodically sampled with a 0.1 m^2 Van Veen grab, from Santa Monica Bay near Los Angeles, at station C3 (33°59.383′N, 118°36.033′W) established by the City of Los Angeles Environmental Monitoring Division (EMD). Sediment samples were washed on a 0.1-mm mesh sieve and ophiuroids retained on the sieve were transported to the laboratory in bottles of seawater cooled on ice, in an insulated container. Living ophiuroids were dissected by inducing autotomy, by stimulating the radial shields to disengage the disk from the arms and oral frame. The stomach of each ophiuroid was examined, using a stereomicroscope, by inverting the disk and retracting the oral opening.

The parasites were transferred using glass Pasteur pipettes to 0.2 μm-filtered seawater in 90 × 50 mm Pyrex crystallizing dishes. Dishes were covered with polyethylene film, partially immersed in a 15°C water bath that approximated ambient temperature of the natural habitat, and maintained in a 16:8 h light:dark cycle. Food was not provided throughout the rearing period. Dishes were checked on alternate days, daily on occasion. Water in each dish was decanted, replaced with new seawater, and all exuviae were collected. Individuals that successfully transformed into adults (copepodid VI) were maintained until they died or were sacrificed. Pairs of adult males and females were held in the same crystallizing dish to facilitate mating. Adult specimens that died in culture were preserved in 70% ethanol, along with representative specimens of developmental stages.

Preserved specimens were cleared in lactic acid for approximately 1 hour, measured with an ocular micrometer, and dissected on wooden slides (Humes and Gooding, 1964). Drawings were made with the aid of a drawing tube attached to a Nikon Optiphot, HFX-II, using Koehler illumination. Living individuals were immobilized with dilute ethanol and photographed with an Olympus PM-10AD photomicrographic system on a Wild M5 Apo stereomicroscope. Individuals studied with scanning electron microscopy (SEM) were fixed in formalin, dehydrated in an ethanol series, transferred to hexamethyldisilazane, and air-dried. The desiccated specimens were sputter coated with gold-palladium and examined at 10 KV with a Hitachi S-3000N microscope. Vouchers of developmental stages are deposited in the Crustacea Collection, Natural History Museum of Los Angeles County (LACM).

Only those features that differ from those of the preceding stage are noted in the descriptions of the larval stages. Abbreviations used to denote developmental stages are NI to NVI for nauplii I to VI, and CI to CVI for copepodids I toVI (CVI being the adult).

Results

Nauplius I
Fig. 1

Description.—Body (Fig. 1A, B) oval, weakly divided by shallow lateral groove into dorsal shield and ventral part. Dorsal shield weakly constricted near mid-length. Mean body length 72 (70-74) μm and width 42 (41-43) μm , based on 12 specimens. Anterior margin of body with minute notch slightly to right or left of apex, in about 50% of examined specimens (Fig. 1B). Caudal region bearing 2 caudal setae. Labrum triangular, posterior margin indistinct, bearing posterolateral pair of naked setae.

Antennule (Fig. 1C) 3-segmented. First segment with 1 pinnate posterodistal seta; posterior border bearing 4 spinules, anterodistal one largest. Second segment with 1 smaller naked and 1 larger distal pinnate setae, and 4 small spinules. Third segment with 3 unequal terminal setae, largest one pinnate, and 6 spinules. Spinules on all segments stiff and transparent.

Antenna (Fig. 1D) consisting of coxa, basis, exopod and endopod. Coxa broad and short, with 1 posterior pinnate seta, and 2 small and 1 larger spinules. Basis with 2 spinules. Exopod 4-segmented. First segment completely fused to basis, with 1 small naked seta on inner side, 1 long distal pinnate seta, and 5 spinules. Second and third segments each armed with 1 long, distal, pinnate seta. Fourth segment with 1 smaller inner seta, 2 terminal setae (smaller naked, and longer pinnate), and 2 spinules. Endopod 1-segmented with 3 smaller inner and 2 longer distal naked setae, one mounted on papilliform process, and 1 proximal and 2 subdistal spinules.

Mandible (Fig. 1E) with unarmed coxa. Basis with 7 spinules of unequal size on anterior surface. Exopod 4-segmented, each segment armed with 1 large, distal plumose seta, additionally 1 spinule on second segment, and 2 spinules on first, third, and terminal segments. Endopod 2-segmented. First segment short, with 2 unequal spinules. Second segment indistinctly demarcated from first segment, elongate, tapering distally, terminating in robust, recurved claw, and armed with 1 subdistal pinnate seta.

Maxillule represented by 1 pinnate seta (Fig. 1B).

Color.—Apart from the minute, brilliant red nauplius eye, the newly released, free-swimming nauplius is a pale grayish-green; the pigmentation presumably imparted by stored yolk.

Metanauplius
Figs. 2, 3, 4A, 5, 7A, C

Description.—Body (Fig. 2A, B) with truncate anterior end, expanded midregion, and tapered posterior end. Dorsal shield of large metanauplius (Fig. 5A) smaller relative to total body than in small metanauplius (Fig. 5D), and more restricted to midline. Mean body length 0.79 (0.62-1.04) mm and width 0.59 (0.49-0.72) mm based on 14 specimens. Caudal region with 2 groups of caudal setae; 1 short and 1 longer setae observed with the light microscope in each group, however 3 small setae, 1 medium-size seta, and 1 large seta arising from a papilla, associated with two rows of spinules, and 2 circular pores in each group observed with SEM (Fig. 5G). Delicate, sinuous, ridge and groove micro-ornamentation covering most of body, except dorsal shield (Figs. 5E-G, 7A, C). Posterolateral portion of body with dorsolateral sensillum on each side (Fig. 5E, F).

Antennule (Fig. 2C) appearing unsegmented with 3 possible segmental sutures, and bearing total of 20 setae; first suture distal to first anterior seta, second distal to third anterior seta, and third distal to small anterior seta near tip.

Antenna (Fig. 2D) biramous. Exopod consisting of 4 parts (segments?); first part with 1 inner seta and 1 stout spine with bifid tip; second part with 1 stout spine with bifid tip;

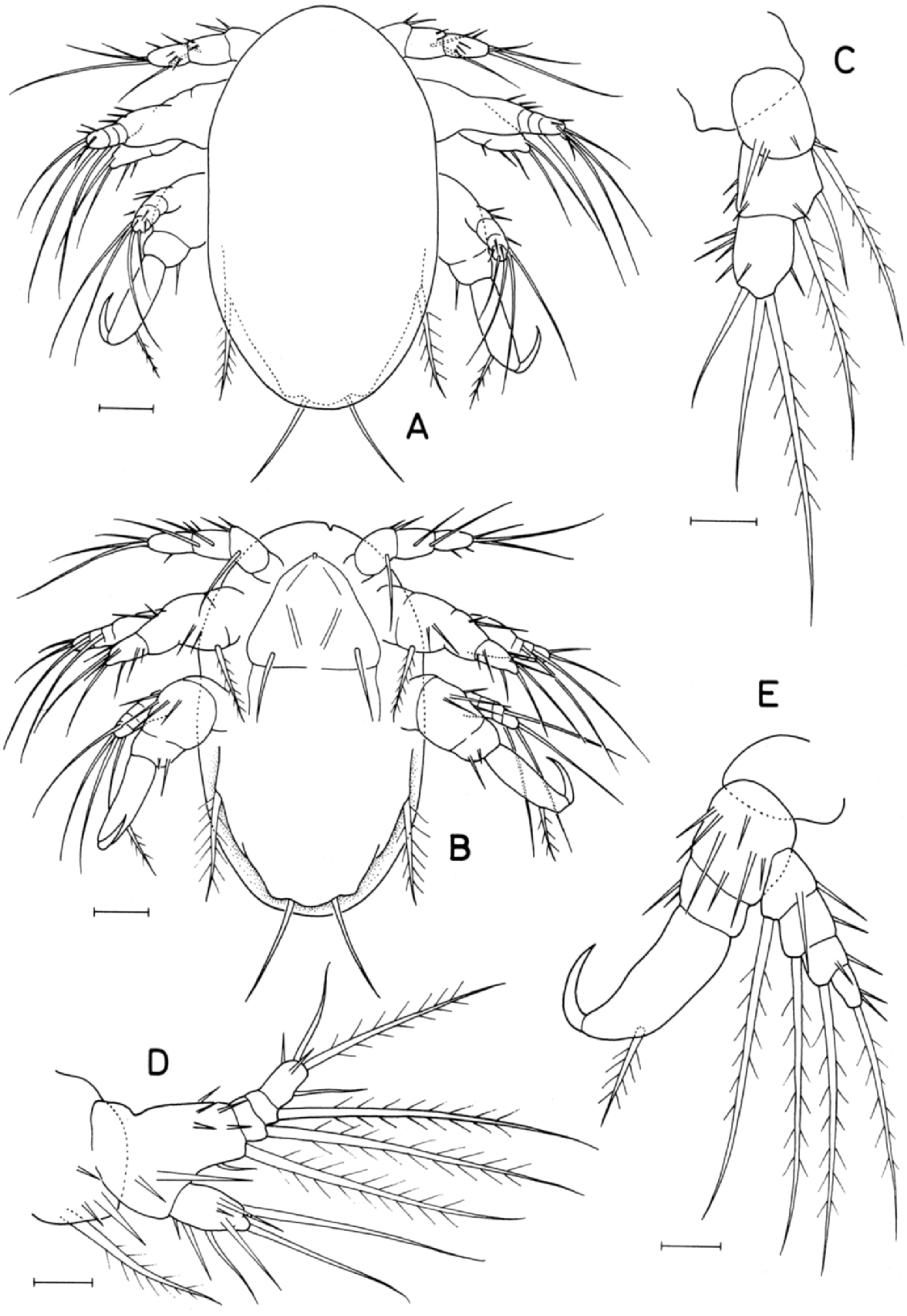

Fig. 1. Nauplius I of *Caribeopsyllus amphiodiae*. A, habitus, dorsal; B, habitus, ventral; C, antennule; D, antenna; E, mandible. Scales: A, B, 10 μm; C-E, 6 μm.

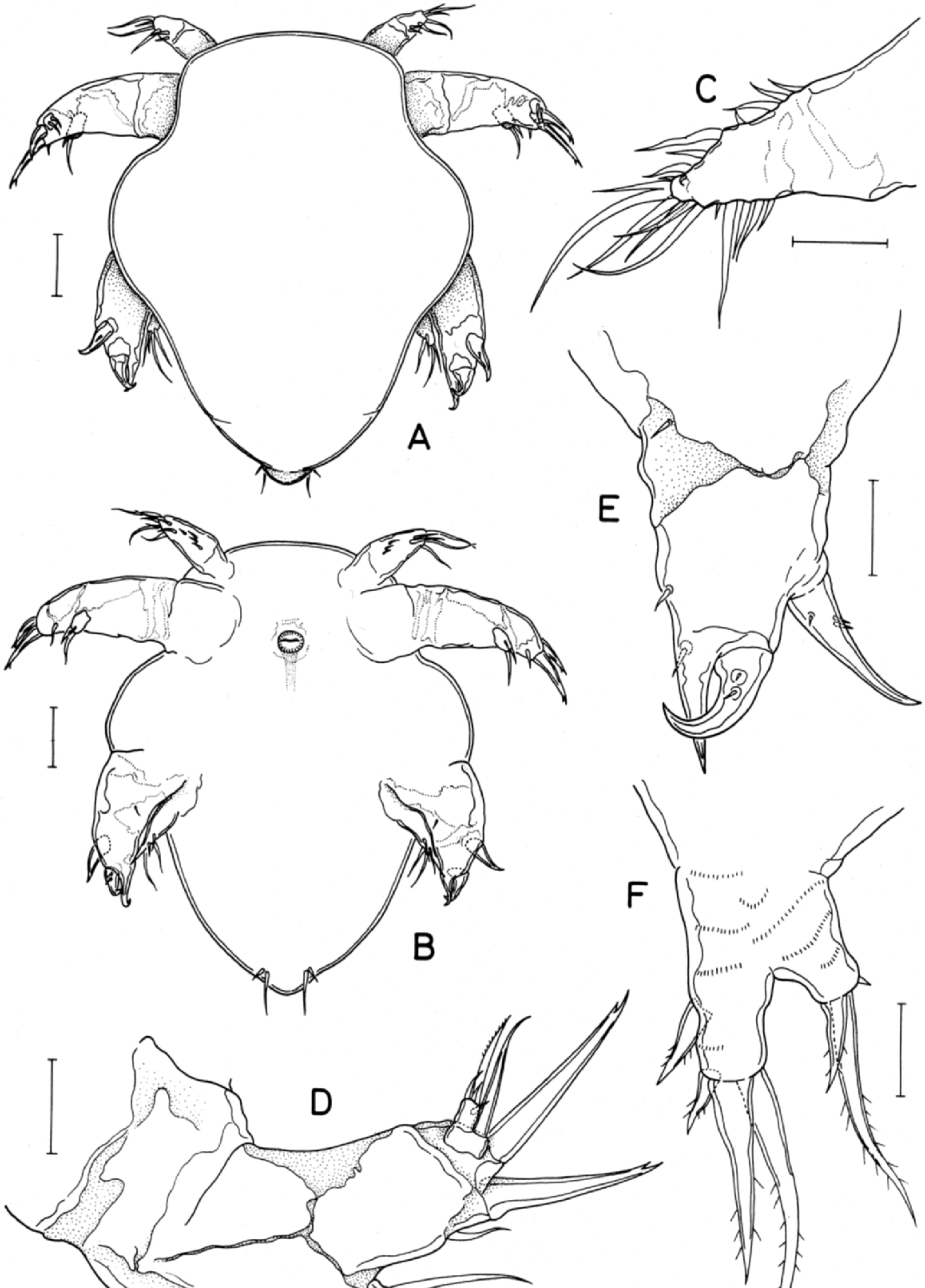

Fig. 2. Metanauplius of *Caribeopsyllus amphiodiae* of moderate body size. A, habitus, dorsal; B, habitus, ventral; C, antennule; D, antenna; E, mandible; F, maxillule. Scales: A, B, 100 µm; C-E, 50 µm; F, 20 µm.

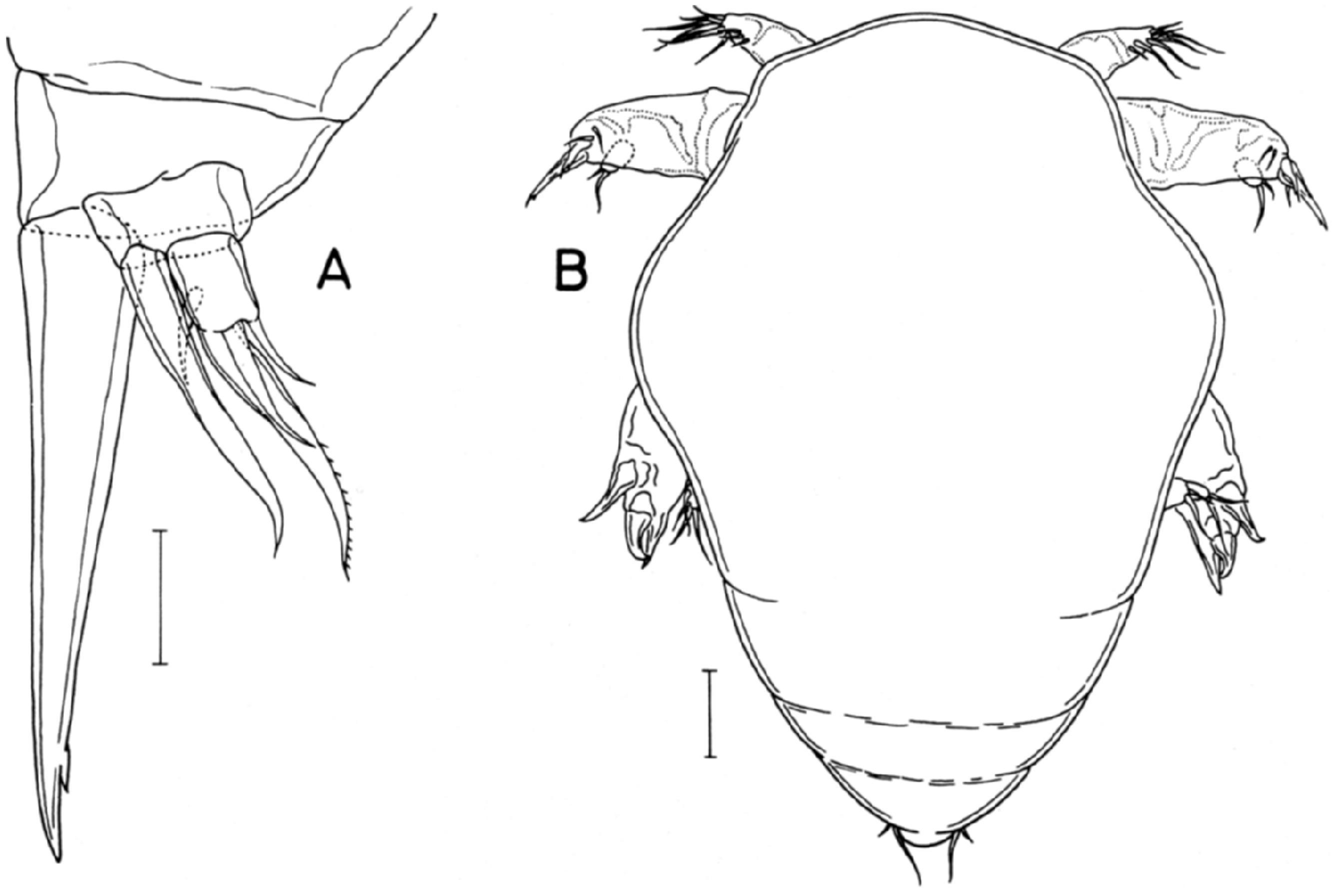

Fig. 3. Metanauplius of *Caribeopsyllus amphiodiae* of large body size. A, distal part of mandibular exopod. B, habitus, dorsal. Scales: A, 0.02 mm; B, 0.1 mm.

third part with large naked seta; fourth part (Fig. 3A) a papilla bearing 1 large semipinnate seta and 3 small naked setae. Endopod with 3 small, inner, naked setae, 1 terminal spine, and 1 long, inner, naked seta. Mouth (Figs. 2B, 5B, C) located between labrum and labium, posteromedially to bases of antenna; 2 lips composed of labral and labial folds, each fold associated with paired papilliform processes supporting a circular pore; additional medial pore on labrum (Fig. 7A, C).

Mandible (Fig. 2E) biramous. Coxa with small inner seta. Exopod an outwardly curved spiniform process bearing 2 outer setae and 1 small inner seta. Endopod with first segment indistinguishably fused to basis; complex bearing 2 small inner setae; large spiniform projection, most likely arising from first endopodal segment, forming chelate complex with strongly curved clawlike second segment.

Maxillule (Fig. 2F) bilobate, situated close to mandible (see Fig. 2B). Base with several rows of minute spinules; neither lobe clearly demarcated from base. Outer lobe small with 1 small naked seta and 2 stout pinnate setae. Inner lobe longer than outer lobe, bearing 2 stout, inner, pinnate, setae and 2 terminal, pinnate setae.

Size of cephalic appendages decreases relative to body size during development; negative allometry of maxillule particularly evident (compare Fig. 5B, C). Limb buds of legs 1 and 2, in metanauplius of intermediate size, represented by 2 pairs of circular patches bearing spinules (Figs. 5C, 7A). Large metanauplius (Fig. 5B) with several parallel ridges in limb bud area of legs 1 and 2 indicating presence of spines and setae on buds beneath the cuticle. Posterolateral suture lines (Fig. 5A) demarcate developing pedigers.

Color.—Metanauplii are translucent. Their body cavity is filled with masses of microscopic spherules that presumably contain lipid stores. They overlay an opaque, yellow or white, elongated, medial structure that probably is the digestive tract (Fig. 4A). Within the body of the smallest individuals, there are regions of greenish or brownish-orange pigment. Intermediate-sized larvae are more green or blue-green, and may have patches of orange pigmentation. The largest larvae are either predominantly blue-green, or a dull-colored cream, grayish, or brownish green, and may have transverse posteroventral bands of greenish-brown or reddish-brown patches or spots. Regions of the body contain green, turquoise, brown, or reddish-brown pigmentation, and the appendages contain yellow, orange, brown, or yellow-brown material. The red naupliar eyes (Fig. 4A), which consist of three separated ocelli, are relatively much larger than in the NI. Morphogenesis of metanauplii, including development of the eyes and reproductive organs and significance of the size-classes, are described elsewhere (Hendler and Dojiri, submitted).

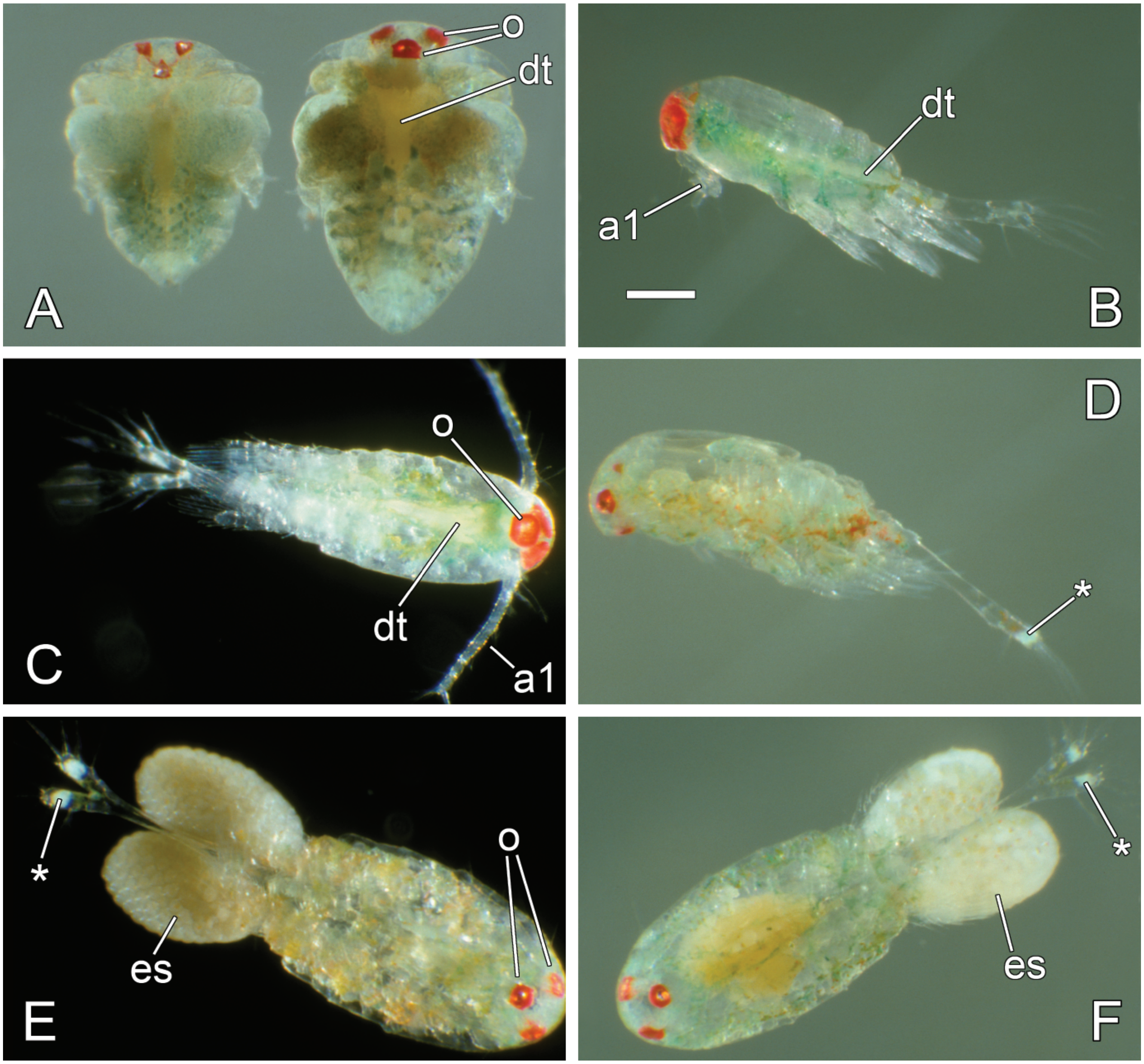

Fig. 4. *Caribeopsyllus amphiodiae* metanauplii (A) and adults (B-F): A, parasitic larvae of moderate and large body size, ventral with prominent ocelli (o); B, adult male, lateral, with flexed geniculate antennules (a1)[presumed digestive tract (dt) visible in larval and adult stages]; C, adult male, ventral; D, adult female, lateral; E, ovigerous female, ventral, with recently extruded egg sacs (es); F, ovigerous female, ventral, with mature egg sacs from which nauplii are emerging. Three separated red ocelli (o) comprise the naupliar eye of larvae and adults; those of males are noticeably larger than females (compare C and E). In comparison with the female (E) carrying new egg sacs (es), one with advanced embryos (F) has the body relatively devoid of stored nutrient; red flecks in its egg sacs are naupliar eyes of the embryos. Both sexes have caudal rami with a distinctive white patch (*). Scale: 200 μm.

Copepodid I
Figs. 8, 9

Female.—Body length 1.33 mm (another specimen 1.36 mm) and width 0.45 mm (another specimen 0.54 mm). Major body articulation between 4th and 5th thoracic somites (3rd and 4th pedigers). Prosome ovoid, approximately 2.2 times longer than broad; urosome relatively stout (Fig. 8A, B). Cephalothorax about 1.5 times longer than broad. Rostrum broad and rounded. Fourth pediger (Fig. 8A) much smaller than 3rd pediger, 2.4 times broader than long. Urosome indistinctly 3-segmented, with anterior somite (5th pediger) distinctly narrower than posterior quandrangular somite (anal somite). Caudal ramus (Fig. 8D) 1.3 times longer than wide, bearing 5 pinnate setae and 1 naked seta and inner distal rows of setules. Mouth and esophagus present (Fig. 8C). Digestive tract could not be observed beyond the esophagus in cleared specimens. Anus most likely represented by indentation at midpoint of posterior margin of last abdominal (anal) somite.

Antennule (Figs. 8E, 9A) indistinctly 2-segmented: first segment with 3 pinnate and 3 small naked setae; second segment with 8 pinnate and 8 naked setae. Antenna (Fig. 9B) and mandible (Fig. 9C) vestigial, thin, and translucent

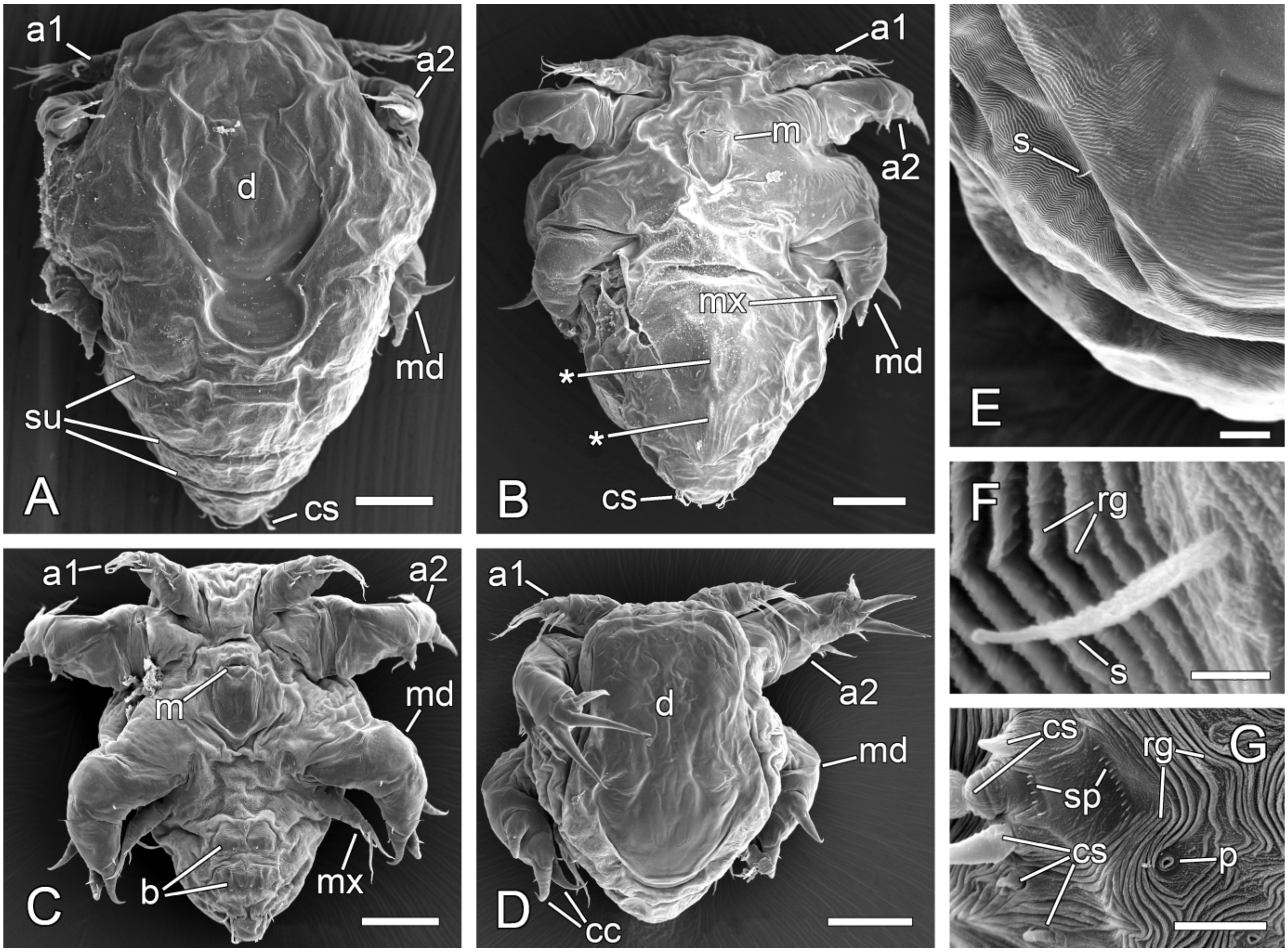

Fig. 5. *Caribeopsyllus amphiodiae* metanauplii: A, large metanauplius, dorsal, body length 0.70 mm; B, large metanauplius, ventral, body length 0.68 mm, with spines and setae of swimming legs (*) visible beneath cuticle; C, intermediate size metanauplius, ventral, body length 0.47 mm, with rudimentary limb buds (b); D, small metanauplius, dorsal, body length 0.20 mm; E, intermediate size metanauplius, posterolateral portion of dorsal shield showing ridge and groove integumental microstructure and sensillum, body length 0.50 mm; F, detail of structures shown in E; G, detail of intermediate size metanauplius, body length 0.57 mm, lateral aspect showing caudal setae, ridges and grooves, setules, and circular pores. Abbreviations: a1, antennule; a2, antenna; cc, chelate complex; cs, caudal setae; d, dorsal shield; rg, integumental ridges and grooves; b, limb bud; md, mandible; mx, maxillule; m, mouth; p, circular pore; s, sensillum; sp, spinules; su, suture line. Scales: A, B, C, D = 100 µm; E = 20 µm; F = 2 µm; G = 10 µm.

with vestiges of rami and setae; other oral appendages absent (Fig. 8C).

Legs 1-3 (Fig. 9D-F) biramous, with 1-segmented rami. Formulae for leg armature provided in Table 1. Medial margin of basis of legs 1 and 2 with small patch of setules. Lateral spiniform processes near bases of exopodal spines of legs 1 and 2 (Fig. 9D, E). All exopodal spines with membranous flanges; terminal exopodal spines of legs 1 and 2 with lateral margin flanged and medial margin pinnate. Endopod of legs 1 and 2 with lateral border bearing rows of setules and spiniform processes next to outermost setae (Fig. 9D, E). Leg 3 with lobiform rami; exopod with 1 pinnate outer seta and 2 small setae; endopod with 2 small setae (Fig. 9F). Leg 4 rudimentary, with 2 indistinct papilliform lobes (Fig. 8B). Legs 5 and 6 apparently absent.

Color.—Copepodid phase larvae of both sexes have a prominent red naupliar eye consisting of one ventral and two dorsolateral ocelli that are sexually dimorphic (as noted for the adult stage). There is greenish or bluish pigment behind the ocelli. Within the cephalothorax are masses of small tan spherules, and larger translucent structures that are delineated by greenish or bluish pigment. Patches of red or orange pigment occur at the posterior end of the cephalothorax. Red or orange strands traverse the urosome, extending into the caudal rami. Each caudal ramus has a prominent, reflective whitish patch.

Male.—Body length 1.15 mm (another specimen 1.14 mm) and width 0.39 mm (another specimen 0.36 mm); cleared male and female indistinguishable except for smaller size of former.

Copepodid II
Figs. 10, 11

Female.—Body length 1.45 mm and width 0.51 mm. Prosome ovoid and about 2 times longer than broad (Fig. 10A). Rostrum broad and rounded. Urosome indistinctly 3-segmented; indistinct segmentation present between 4th and 5th pediger. Anal somite (Fig. 10B) about 1.3 times

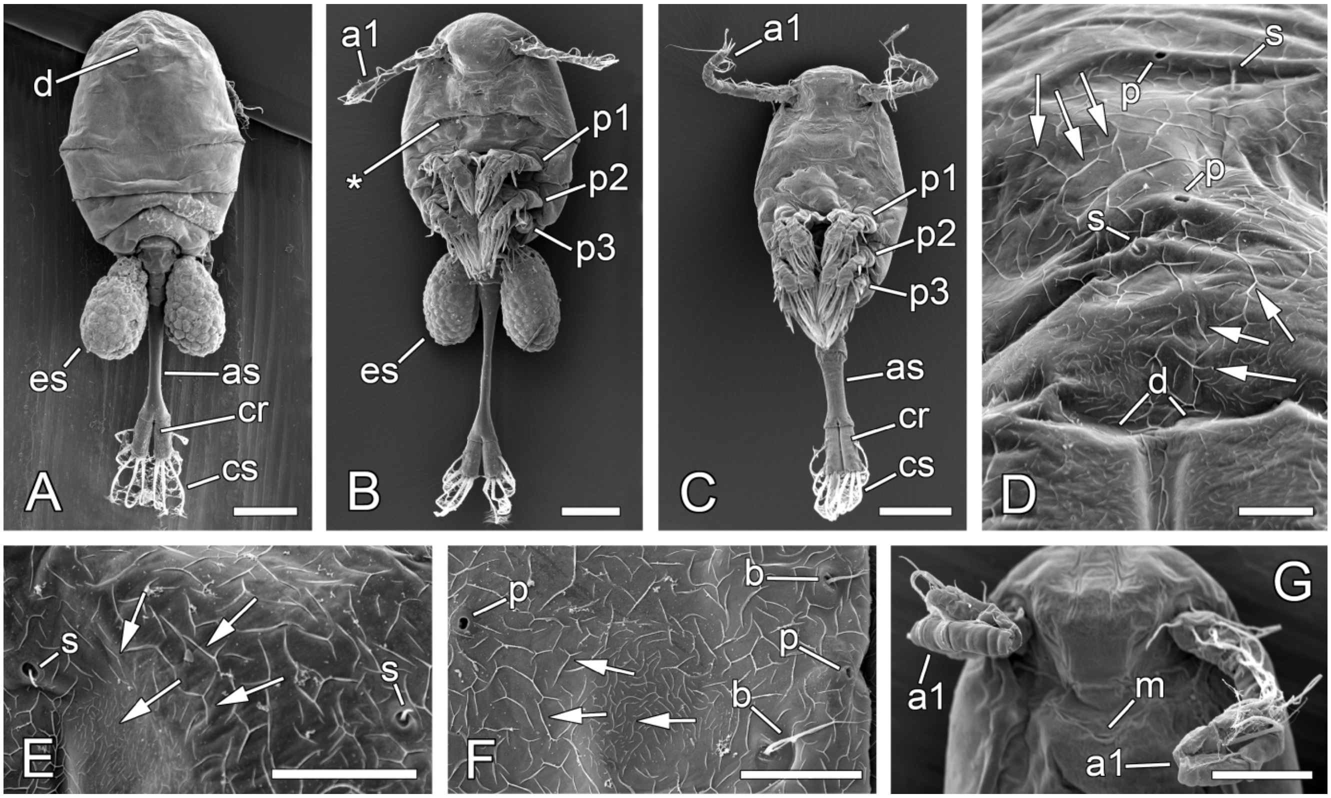

Fig. 6. *Caribeopsyllus amphiodiae* adult females (A, B, D), and males (C, E, F, G): A, ovigerous female, dorsal, body length 1.19 mm; B, ovigerous female, ventral, body length 1.63 mm, with transverse crease (*) across cephalothorax; C, male, ventral, body length 1.19 mm; D, detail of A showing edge of dorsal shield (d), single hair sensilla (s), and circular pores (p) on flexible anteromedial portion of cephalic region; E, male, body length 1.23 mm, detail of lateral cephalic region with single hair sensilla; F, male, body length 1.26 mm, detail of lateral edge of cephalothorax with circular pores (p) and bifid hair sensilla (b); G, male, ventral, body length 1.09 mm, detail of cephalic region showing flexed geniculate antennules (a1) and mouth (m). Note divaricate integumental wrinkles (arrows) of integument in D-F. Abbreviations as in Fig. 5; as, anal somite; cr, caudal ramus; cs, caudal setae; es, egg sac; p1-p3, legs 1-3. Scales: A-C = 200 μm; D = 10 μm; E, F = 20 μm; G = 100 μm.

longer than broad. Caudal ramus (Fig. 10B) 1.6 times longer than wide, bearing 6 pinnate setae.

Antennule (Fig. 10C) indistinctly 3-segmented; first segment with 6 pinnate setae; second segment with 5 pinnate and 1 naked setae; third segment with 3 pinnate setae and 11 naked setae. Antenna and oral appendages absent. Mouth present.

Legs 1-3 (Fig. 10D-F) biramous, with 1-segmented rami. Formulae for leg armature provided in Table 1. Medial margin of basis of legs 1-3 with small patch of setules. All exopodal spines distally serrated, some of them proximally pinnate. Endopod of legs 1-3 with lateral border bearing rows of setules and spiniform processes next to outermost setae (Fig. 10D-F). Leg 4 (Fig. 10G) apparently biramous; sympod with outer pinnate seta; exopod with 1 long and 4 shorter pinnate setae; endopod a small lobe with a terminal pinnate seta. Leg 5 (Fig. 10H) consisting of a lobe with 1 long pinnate seta and a very small process. Leg 6 (Fig. 10H) represented by 2 very small processes.

Male.—Body length 1.13 mm and width 0.33 mm. Urosome slender (Fig. 11A). Combined genital and anal somites about 2 times longer than broad. Antennule (Fig. 11B) with no clear-cut segmental sutures, bearing 29 setae. Leg 4 (Fig. 11C) with second and third outer exopodal setae longer than in female. Leg 5 (Fig. 11D) lobiform, bearing 1 naked and 1 pinnate setae. Leg 6 represented by 2 very small processes.

Copepodid III
Fig. 12

Female.—Body length 1.55 mm and width 0.54 mm. Prosome pyriform, about 2 times longer than broad; urosome about 1.7 times longer than broad, indistinctly 3-segmented (Fig. 12A). Rostrum broad and rounded. Fourth pediger (Fig. 12A) much smaller than 3rd pediger, 3 times broader than long. Fifth pediger (Fig. 12B) narrower than 4th pediger, about 1.7 times broader than long. Combined genital and anal somites (Fig. 12A) approximately 1.7 times longer than broad, with slightly concave lateral borders. Caudal ramus (Fig. 12A) 1.8 times longer than broad, bearing 6 pinnate setae and inner distal rows of setules.

Antennule (Fig. 12C) indistinctly 3-segmented (presumed segmental sutures may be cuticular wrinkles), with total of 26 setae as in CII.

Legs 1-3 (Fig. 12D, E) biramous, with incompletely 2-segmented rami. Leg 3 identical to leg 2 in shape and

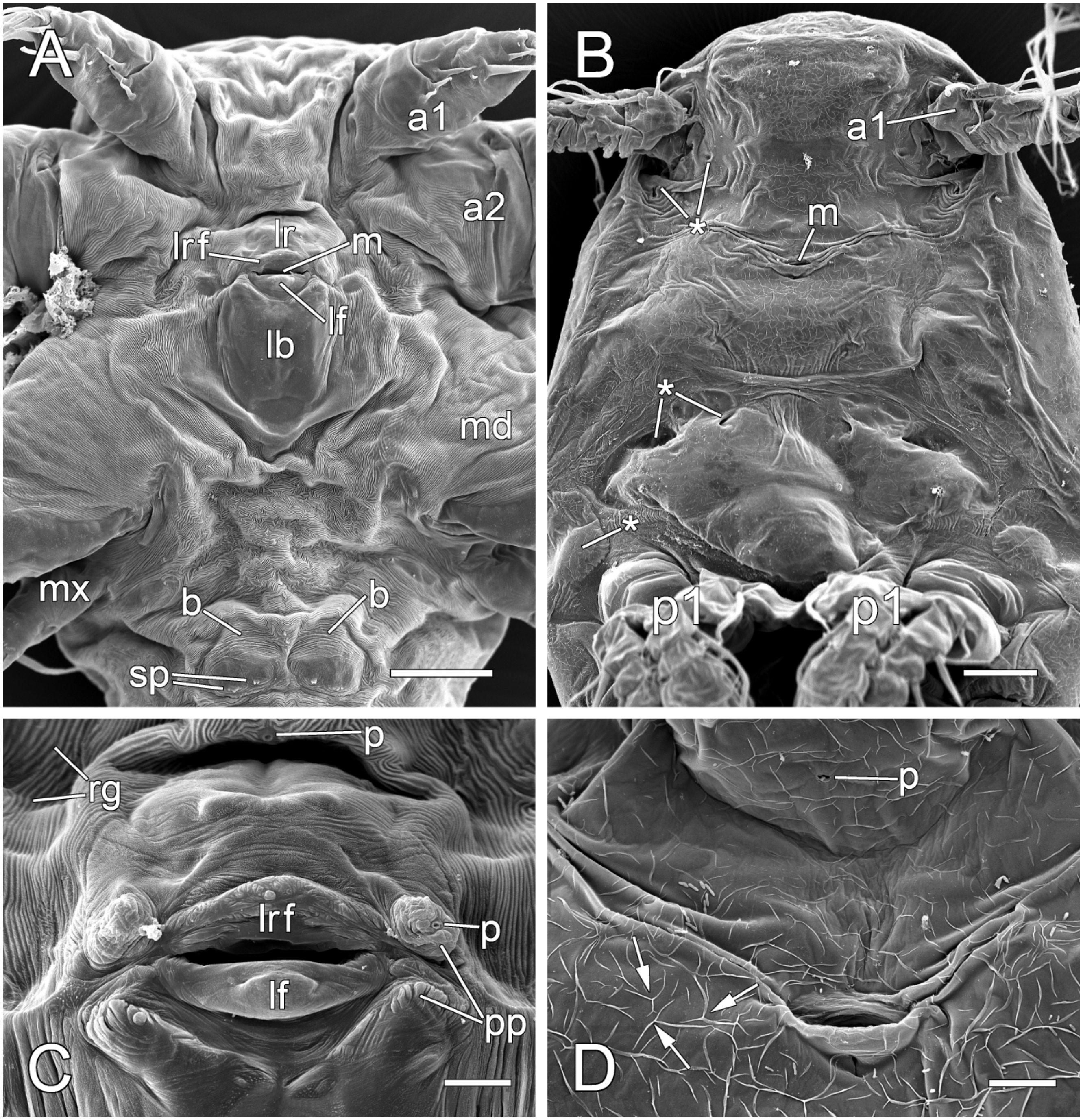

Fig. 7. *Caribeopsyllus amphiodiae* metanauplius (A, C) and adult male (B, D): A, metanauplius, ventral, body length 0.47 mm; B, adult male, ventral, body length 1.19 mm, showing integumental pits, protrusions and folds (*) that may be related to the loss of cephalic appendages; C, oral region of A; D, oral region of B, arrows indicating divaricate integumental wrinkles. Abbreviations: a1, antennule; a2, antenna; b, leg bud; lb, labium; lf, labial fold; lr, labrum; lrf, labral fold; m, mouth; md, mandible; mx, maxillule; p, pore; pp, pore-bearing papilliform process; p1, leg 1; rg, integumental ridges and grooves; sp, spinules. Scales: A, B = 50 μm ; C, D = 10 μm.

setation. Formulae for leg armature provided in Table 1. Legs 4 (Fig. 12B) and 5 (Fig. 12B) as in CII. Leg 6 represented by 2 small spiniform setae (Fig. 12B).

Male.—Body length 1.12 mm and width 0.43 mm based on damaged exuvium. Antennule (Fig. 12G) indistinctly segmented bearing a total of 36 setae; segmental sutures difficult to discern but armature formula appears to be 8, 12, and 16.

Legs 1-3 as in female. Leg 3 identical to leg 2. Leg 4 (Fig. 12H) with second and third outer exopodal setae longer than those of female. Leg 5 (Fig. 12F) with larger inner naked seta than in female. Leg 6 (Fig. 12F) lobiform, bearing 1 short and 1 longer spiniform setae.

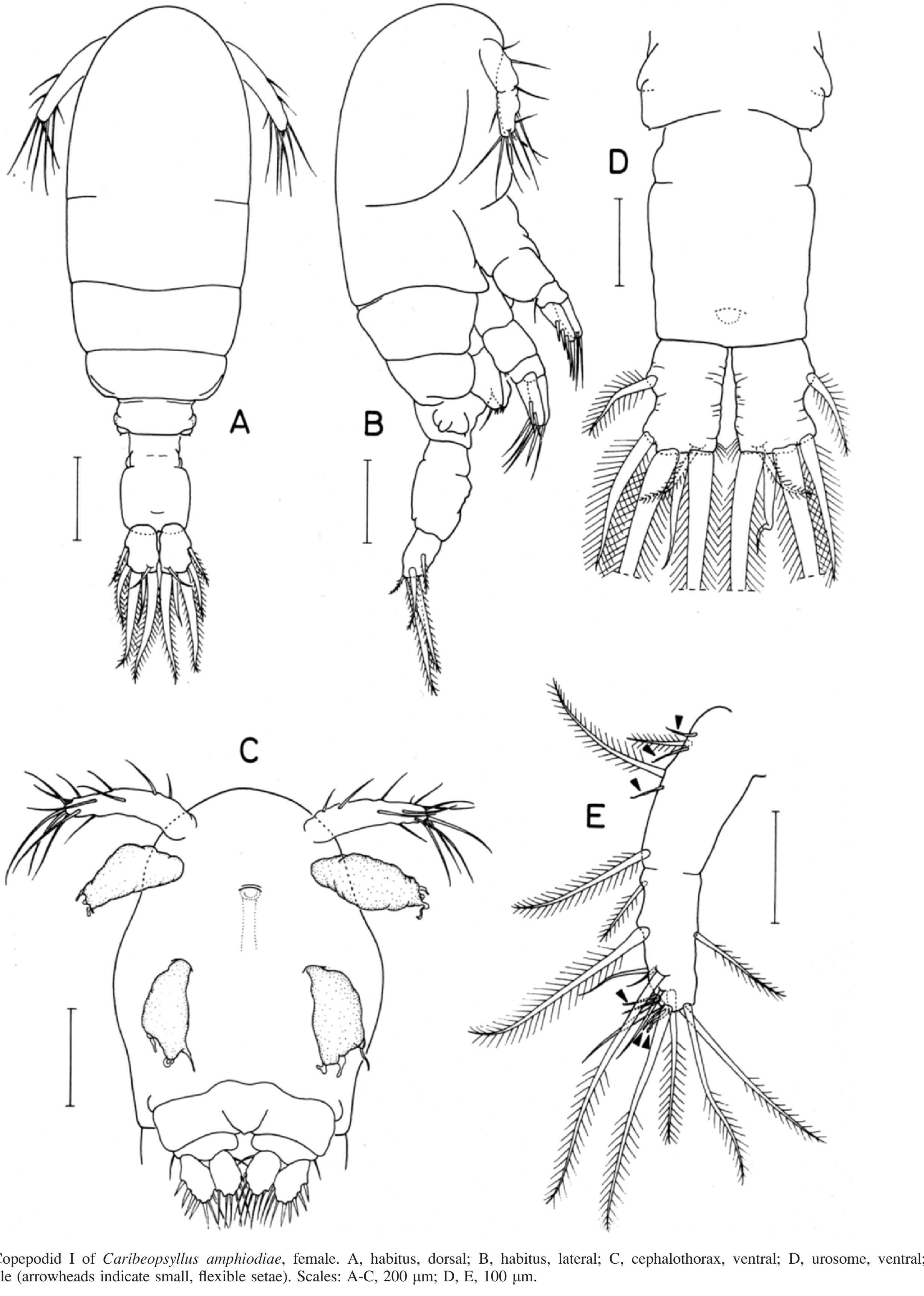

Fig. 8. Copepodid I of *Caribeopsyllus amphiodiae*, female. A, habitus, dorsal; B, habitus, lateral; C, cephalothorax, ventral; D, urosome, ventral; E, antennule (arrowheads indicate small, flexible setae). Scales: A-C, 200 μm; D, E, 100 μm.

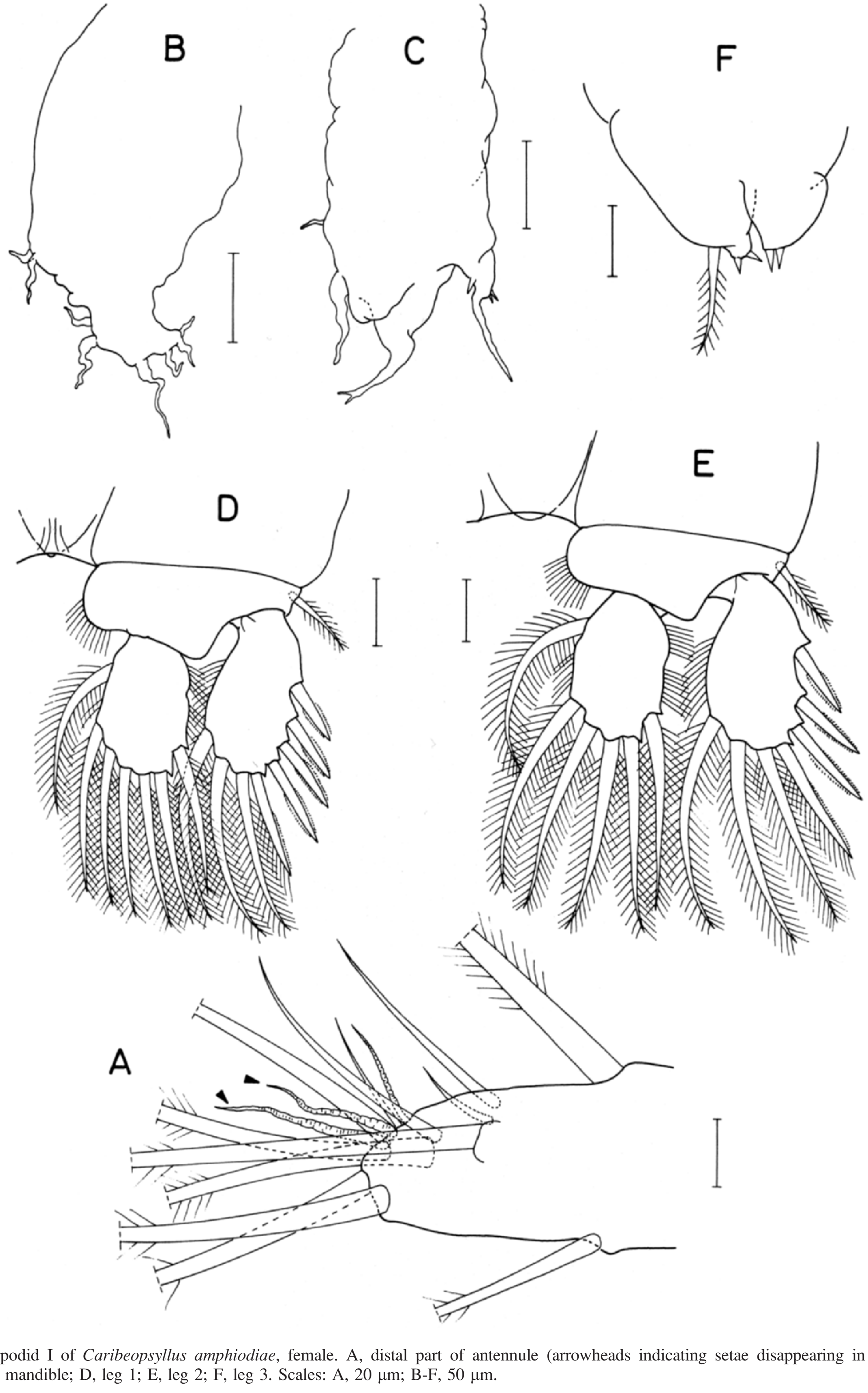

Fig. 9. Copepodid I of *Caribeopsyllus amphiodiae*, female. A, distal part of antennule (arrowheads indicating setae disappearing in copepodid II); B, antenna; C, mandible; D, leg 1; E, leg 2; F, leg 3. Scales: A, 20 μm; B-F, 50 μm.

Table 1. Comparison of armature formulae of legs 1-6 of copepodid I-VI of *Caribeopsyllus amphiodiae*. Armature formulae for legs are presented as outer margin first, with Roman numerals for the number of spines and Arabic numerals for setae. Dash indicates coxa not distinguishable from basis in leg 3 of CI.

Legs	Copepodid I	Copepodid II	Copepodid III	Copepodid IV	Copepodid V	Copepodid VI
Leg 1						
Coxa	0-0	0-1	0-1	0-1	0-1	0-1
Basis	1-0	1-0	1-0	1-0	1-0	1-0
Exopod	IV, I, 3	IV, I, 5	I-1; III, I, 4	I-1; III, I, 4	I-1; III, I, 4	I-1; I-1; II, I, 3
Endopod	7	8	0-1; 7	0-1; 7	0-1; 7	0-1; 0-1; 6
Leg 2						
Coxa	0-0	0-1	0-1	0-1	0-1	0-1
Basis	1-0	1-0	1-0	1-0	1-0	1-0
Exopod	III, I, 3	IV, I, 6	I-1; III, I, 5	I-1; III, I, 5	I-1; III, I, 5	I-1; I-1; II, I, 4
Endopod	6	8	0-1; 7	0-1; 7	0-1; 7	0-1; 0-1; 6
Leg 3						
Coxa	-	0-1	0-1	0-1	0-1	0-1
Basis	0-0	1-0	1-0	1-0	1-0	1-0
Exopod	3	IV, I, 6	I-1; III, I, 5	I-1; III, I, 5	I-1; III, I, 5	I-1; I-1; II, 1, 4
Endopod	2	8	0-1; 7	0-1; 7	0-1; 7	0-1; 0-1; 6
Leg 4						
Sympod	0-0	1-0	1-0	1-0	1-0	1-0
Exopod	0	5	5	5	5	5 (♀) or
Endopod	0	1	1	1	1	1, I, 3 (♂) 1
Leg 5						
	Absent	Lobe with 1 pinnate and 1 naked seta (♂) or spinule (♀)	Lobe with 1 pinnate and 1 naked seta (♂) or spinule (♀)	Lobe with 1 pinnate and 1 naked seta (♂) or spinule (♀)	Lobe with 1 pinnate and 1 naked seta (♂) or spinule (♀)	Lobe with 1 pinnate and 1 naked seta (♀) or 3 setae (♂)
Leg 6						
	Absent	2 small processes	Lobe with 2 small spinules	Lobe with 2 small spinules	Lobe with 2 small spinules	Lobe with 2 small spinules

Copepodid IV
Fig. 13

Female.—Body length 1.52 mm and width 0.47 mm. Prosome ovoid (Fig. 13A), about 2 times longer than broad. Rostrum broad and rounded. Urosome indistinctly 4-segmented, more slender than CIII. Anal somite (Fig. 13B) long and moderately slender, at least 1.5 times longer than broad, with concave lateral borders. Caudal ramus (Fig. 13A) 2.5 times longer than broad, bearing 6 pinnate setae and inner distal rows of setules.

Antennule (Fig. 13C) indistinctly 4-segmented, with armature formula 6, 2, 4, and 14; setae sparsely pinnate or naked.

Legs 1-3 (Fig. 13D, E) biramous, with distinctly 2-segmented rami. Formulae for leg armature provided in Table 1. Legs 2 and 3 identical. Leg 4 (Fig. 13B) with single segment on exopod and endopod. Leg 5 (Fig. 13B) represented by two lobes, each bearing 1 small naked and 1 large pinnate terminal setae. Leg 6 represented by 2 small spiniform setae.

Male.—Body length 1.31 mm and width 0.38 mm. Antennule (Fig. 13G) indistinctly segmented, bearing a total of 36 setae; segmental sutures difficult to discern, but armature formula appears to be 6, 2, 4, 2, 2, 4, and 16; some setae sparsely pinnate.

Legs 1-3 as in female. Leg 4 (Fig. 13F) with 2nd and 3rd outer exopodal setae longer than those of female. Leg 5 (Fig. 13F) consisting of two large lobes, each bearing 1 pinnate and 1 naked setae. Leg 6 (Fig. 13F) lobiform, bearing 1 short and 1 longer spiniform setae.

Copepodid V
Fig. 14

Female.—Body length 1.57 mm and width 0.51 mm. Prosome ovoid, 2 times longer than broad (Fig. 14A). Rostrum broad and rounded. Fifth pediger, genital double-somite, and anal somite indistinctly segmented.

Antennule (Fig. 14C) indistinctly 4-segmented, with armature formula 6, 6, 2, and 12; setae sparsely pinnate or naked.

Legs 1-3 (Fig. 14D, E) biramous, with 2-segmented rami; second segment with lateral indentations indicating partial articulating suture for 3rd segment. Formulae for leg armature provided in Table 1. Legs 2 and 3 identical. Leg 4 with single segment on exopod and endopod.

Male.—Mean body length 1.27 (1.22-1.32) mm and width 0.33 (0.32-0.34) mm based on 3 cleared specimens. Similar to female, but first abdominal somite distinct from genital somite and anal somite (Fig. 14F). Antennule (Fig. 10G) 8-segmented with armature formula of 6, 2, 4, 2, 2, 4, 3, and 13; some setae sparsely pinnate.

Legs 1-3 as in female. Leg 4 with 2nd and 3rd outer setae longer than those of female. Leg 5 distinctly longer than that of female. Leg 6 lobiform, with 2 terminal, spiniform setae of unequal sizes.

Copepodid VI (adult)
Figs. 4B-F, 6A-G, 7B, D, 15, 16

Female.—Mean body length 1.64 (1.51-1.89) mm and width 0.51 (0.49-0.55) mm, based on 10 specimens. Prosome ovoid, 1.9 times longer than broad; urosome slender (Figs.

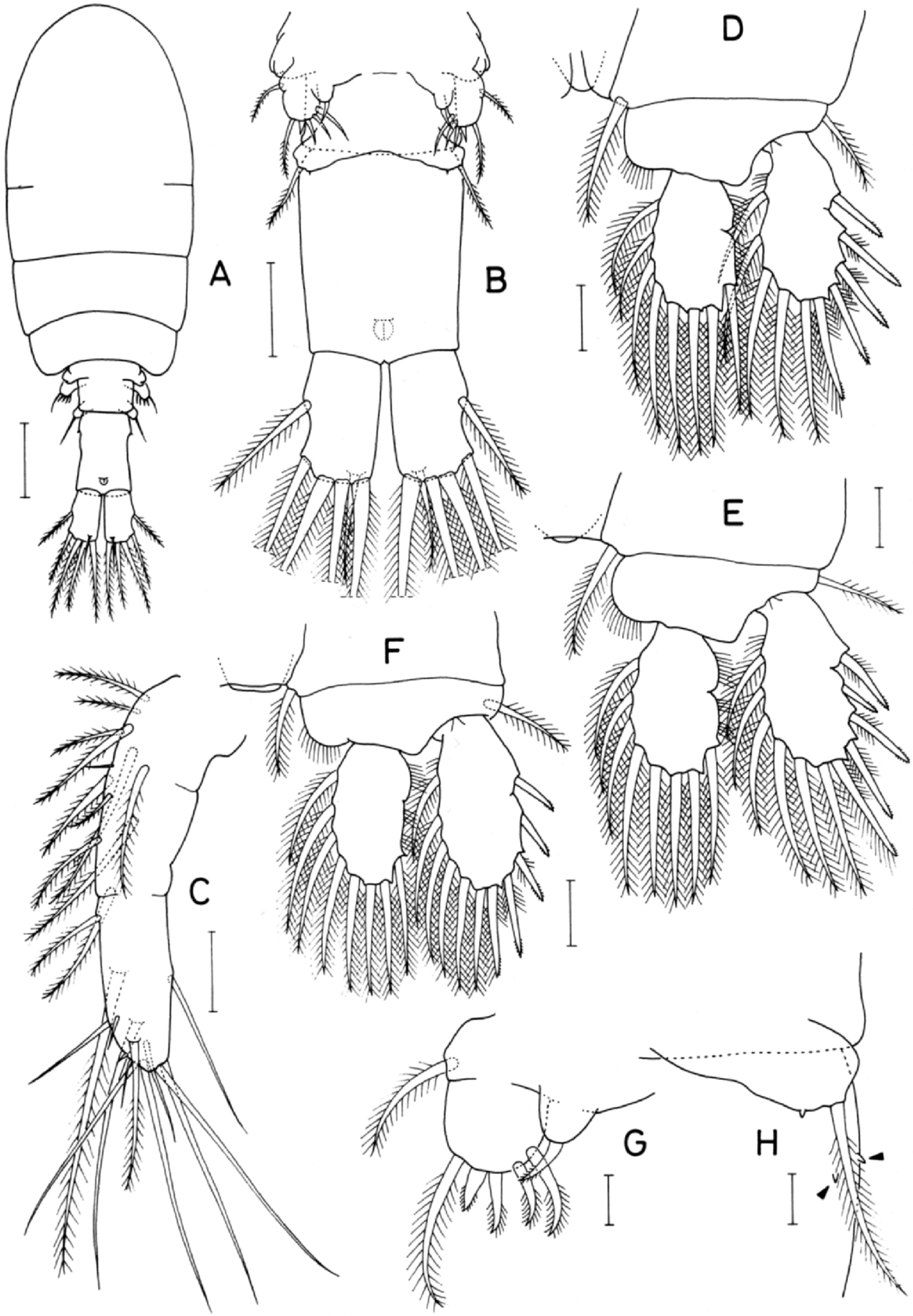

Fig. 10. Copepodid II of *Caribeopsyllus amphiodiae*, female. A, habitus, dorsal; B, urosome, ventral; C, antennule; D, leg 1; E, leg 2; F, leg 3; G, leg 4; H, left leg 5, ventral (arrowheads indicate two processes representing primodial leg 6). Scales: A, 200 μm; B, 100 μm; C-F, 50 μm; G, H, 20 μm.

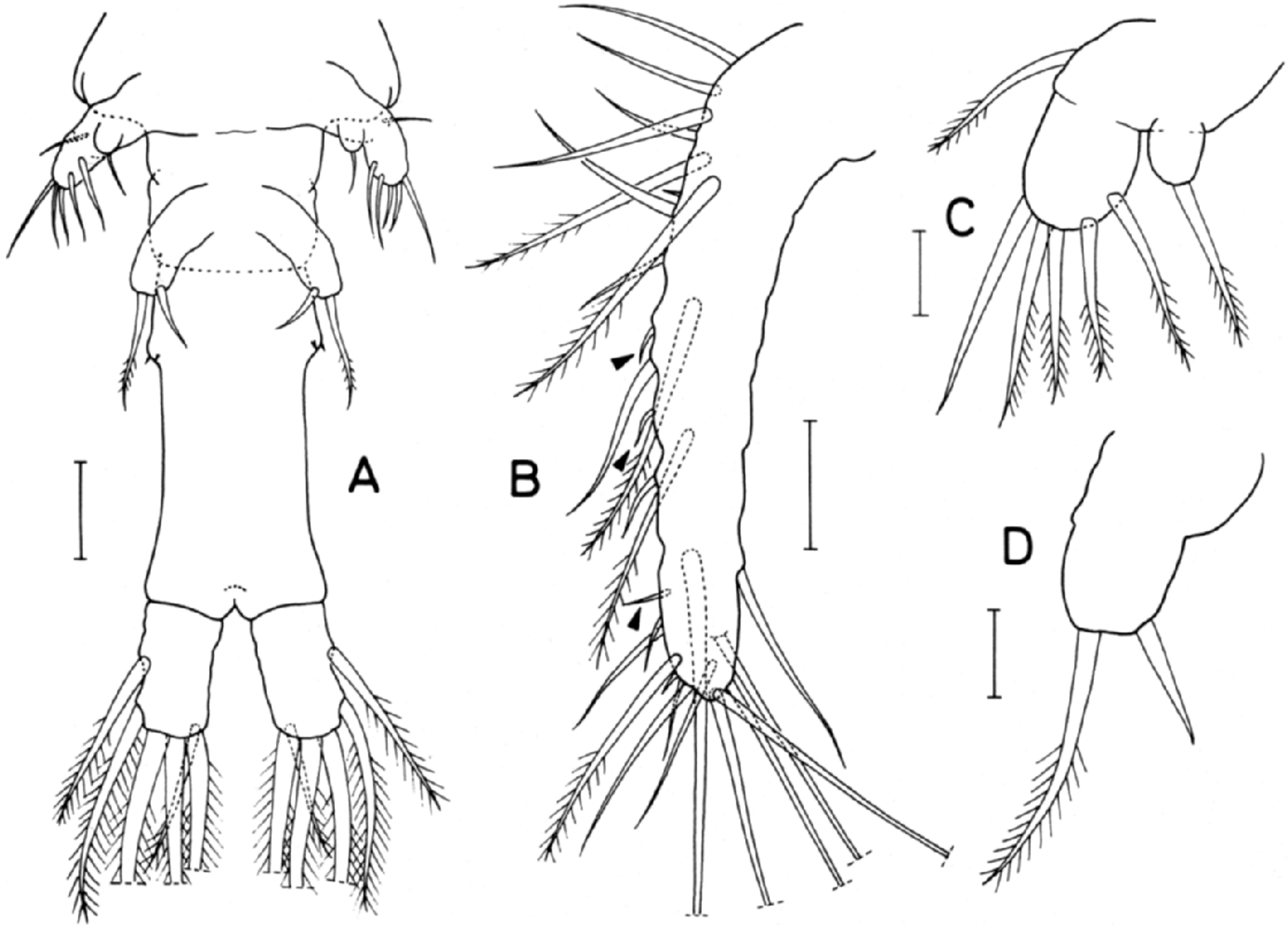

Fig. 11. Copepodid II of *Caribeopsyllus amphiodiae*, male. A, urosome, ventral; B, antennule (arrowheads indicate sexually dimorphic addition of setae); C, leg 4; D, leg 5. Scales: A, B, 50 μm; C, D, 20 μm.

6A, B, 15A). Rostrum broad, rounded, extending posteriad to dorsal shield (Fig. 6A, D). Integument ornamented with fine divaricate wrinkles (possibly artifactual?), and folded or pleated in places on ventral surface of cephalothorax (Figs. 6D, 7B, D). Cephalothorax bearing transverse ventral crease possibly delineating juncture between cephalon and thorax (Fig. 6B). Numerous circular pores and sensilla noted on cephalothorax (Fig. 6D). Urosome distinctly 4-segmented, long and slender. Fourth pediger (Fig. 15A) much smaller than 3rd pediger, 3.2 times broader than long. Fifth pediger (Fig. 15B) narrower than 4th pediger, about 1.6 times broader than long. Genital somite broadest anteriorly, about 1.5 times broader than long, carrying 2 small setae (spines?) representing leg 6 (Fig. 16A) in area of egg sac attachment. Egg sacs multiseriate (Figs. 4E, F, 6A, B). Anal somite (Fig. 15A) slender, at least 4.8 times longer than broad, with concave lateral borders. Caudal ramus (Fig. 15A) 2.8 times longer than broad, bearing 6 pinnate setae and inner distal rows of setules. Vestigial mouth present, but examination with SEM reveals orifice sealed by tissue.

Antennule (Fig. 15C) 5-segmented, with armature formula 6, 2, 4, 2, and 12 (segmentation differs from that described by Ho et al., 2003); setae sparsely pinnate or naked. Antenna and oral appendages lacking.

Legs 1-3 (Fig. 15D, E) biramous, with 3-segmented rami. Leg 4 (Fig. 16B) exopod and endopod each consisting of 1 segment. Formulae for leg armature provided in Table 1. Medial margin of basis of legs 1-3 with small patch of setules. Second and third exopodal segments of leg 2 (Fig. 15E) and 1st to 3rd exopodal segments of leg 3 with lateral spiniform processes near bases of spines. All exopodal spines with membranous flanges; terminal exopodal spines of legs 1-3 with lateral margin flanged and medial margin pinnate. Endopod of legs 1-3 with lateral border bearing rows of setules; terminal segment of legs 1 and 2 (Fig. 15D, E) with spiniform processes next to outermost setae; spiniform process next to outermost seta on terminal segment of leg 3 endopod. Leg 4 (Fig. 16B) as in CV. Leg 5 (Fig. 15B) originating near ventromedial surface of somite; each member broad at base, about 1.8 times longer than broad, and bearing 1 small naked and 1 large pinnate terminal setae (Fig. 16C).

Color.—Pigmentation of the adult and copepodid is similar, although the adult cephalothorax may be more transparent and the opaque medial structure within it more evident, possibly due to the depletion of stored nutrient. As in the naupliar and CI-CV stages, the prominent red naupliar eye

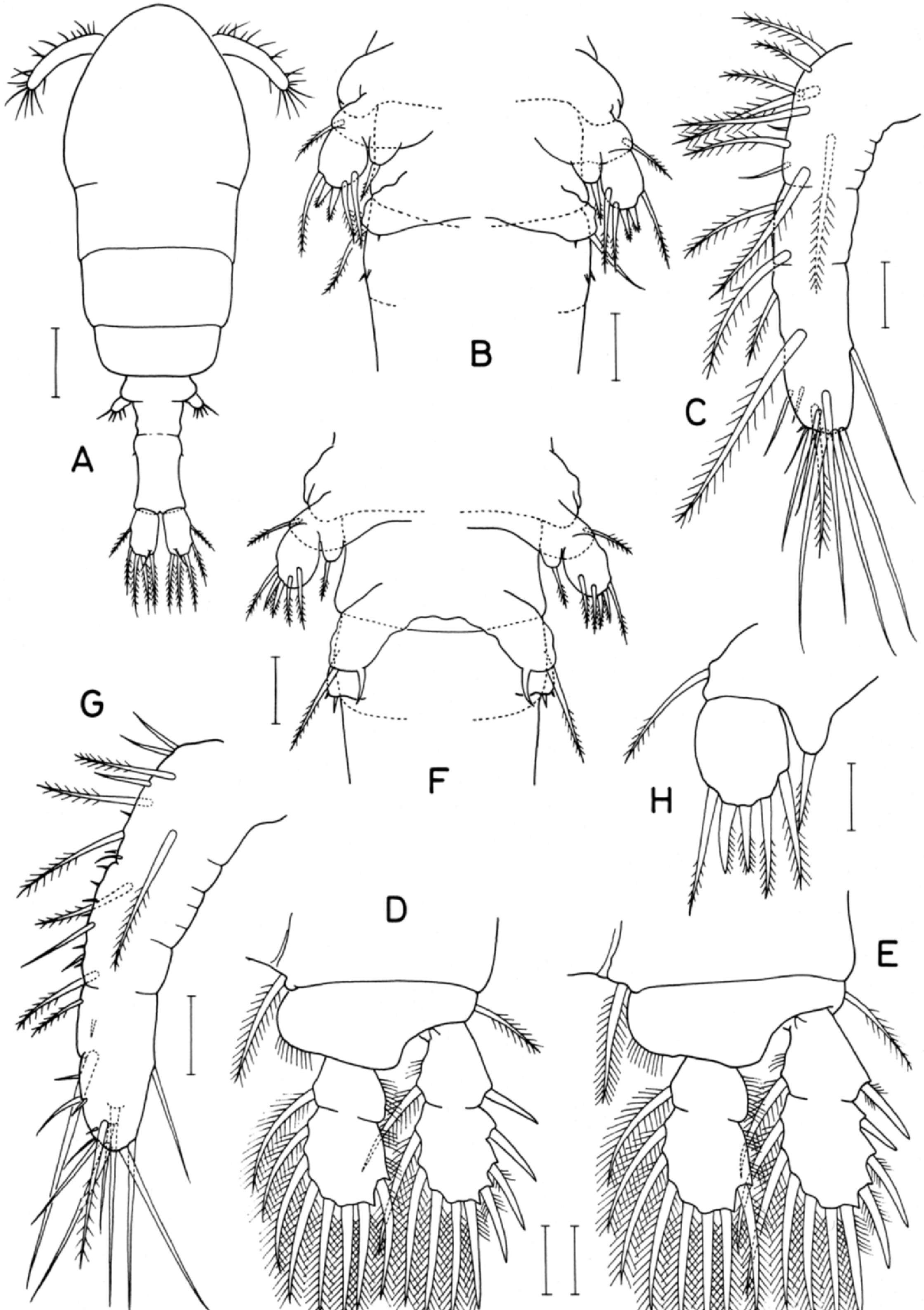

Fig. 12. Copepodid III of *Caribeopsyllus amphiodiae*. Female: A, habitus, dorsal; B, anterior part of urosome; C, antennule; D, leg 1; E, leg 2. Male: F, anterior part of urosome, ventral; G, antennule; H, leg 4. Scales: A, 200 μm; B-G, 50 μm; H, 20 μm.

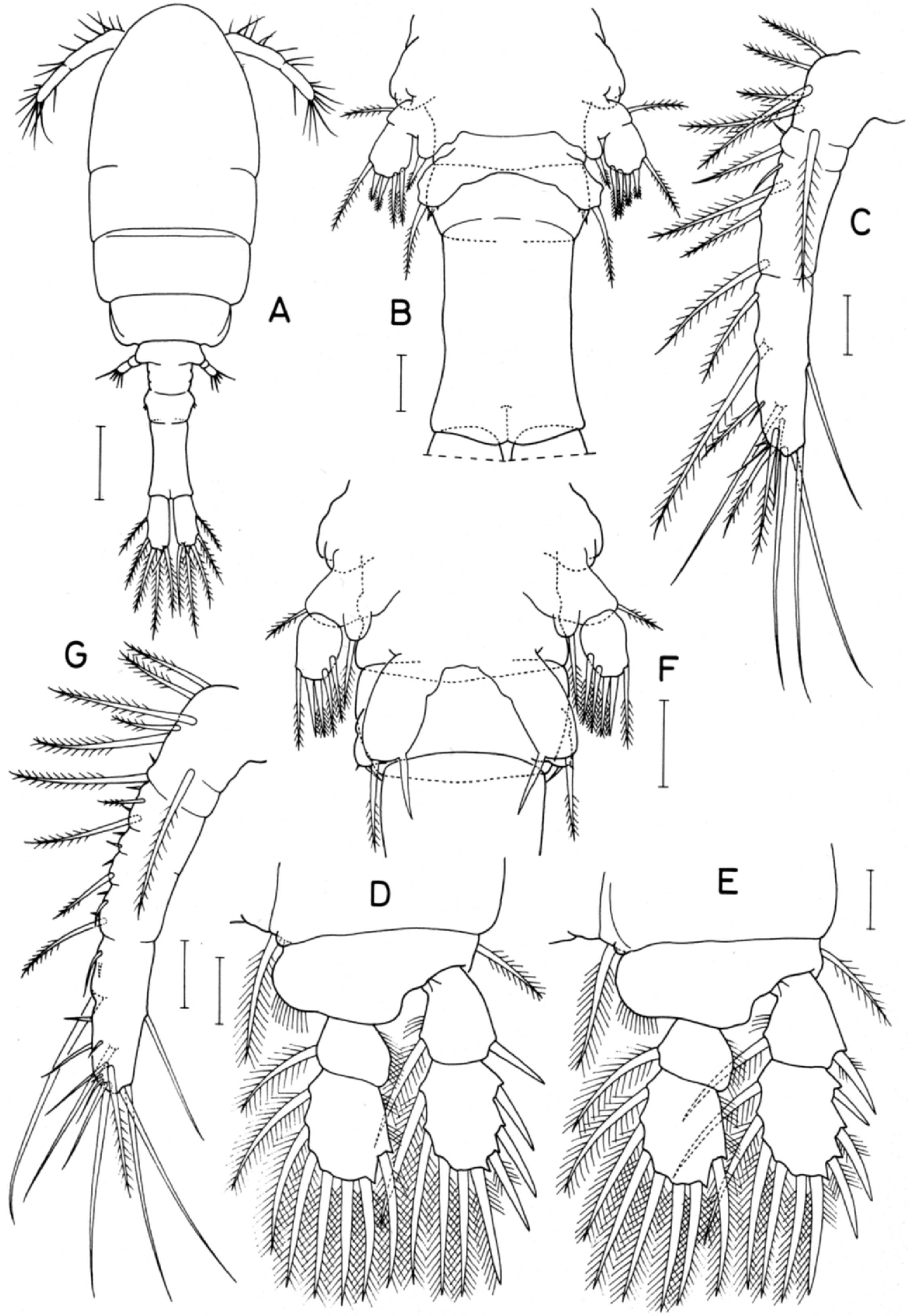

Fig. 13. Copepodid IV of *Caribeopsyllus amphiodiae*. Female: A, habitus, dorsal; B, urosome, ventral; C, antennule; D, leg 1; E, leg 2. Male: F, anterior part of urosome; G, antennule. Scales: A, 200 μm; B-G, 50 μm.

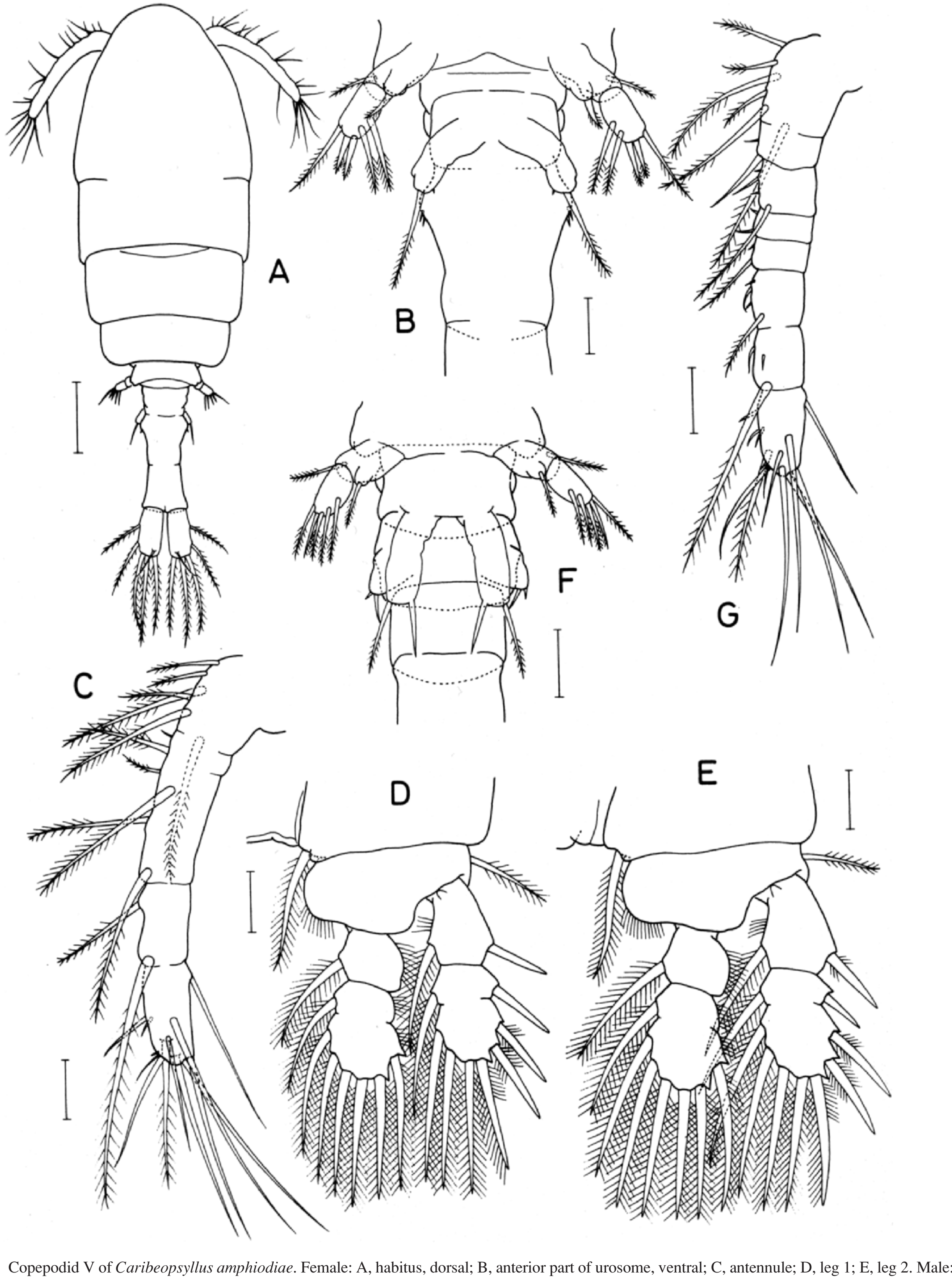

Fig. 14. Copepodid V of *Caribeopsyllus amphiodiae*. Female: A, habitus, dorsal; B, anterior part of urosome, ventral; C, antennule; D, leg 1; E, leg 2. Male: F, anterior part of urosome, ventral; G, antennule. Scales: A, 200 µm; B-G, 50 µm.

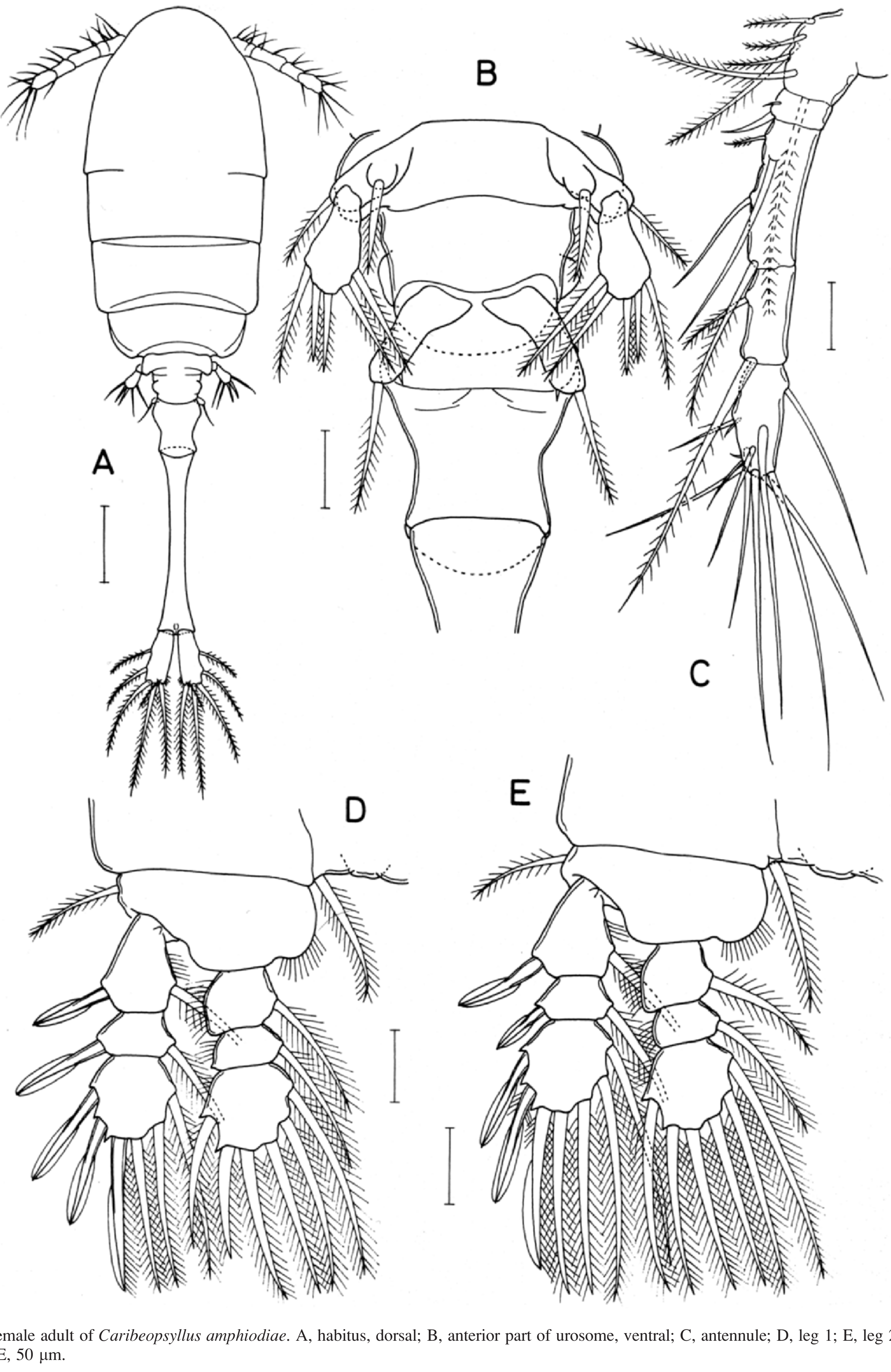

Fig. 15. Female adult of *Caribeopsyllus amphiodiae*. A, habitus, dorsal; B, anterior part of urosome, ventral; C, antennule; D, leg 1; E, leg 2. Scales: A, 200 µm; B-E, 50 µm.

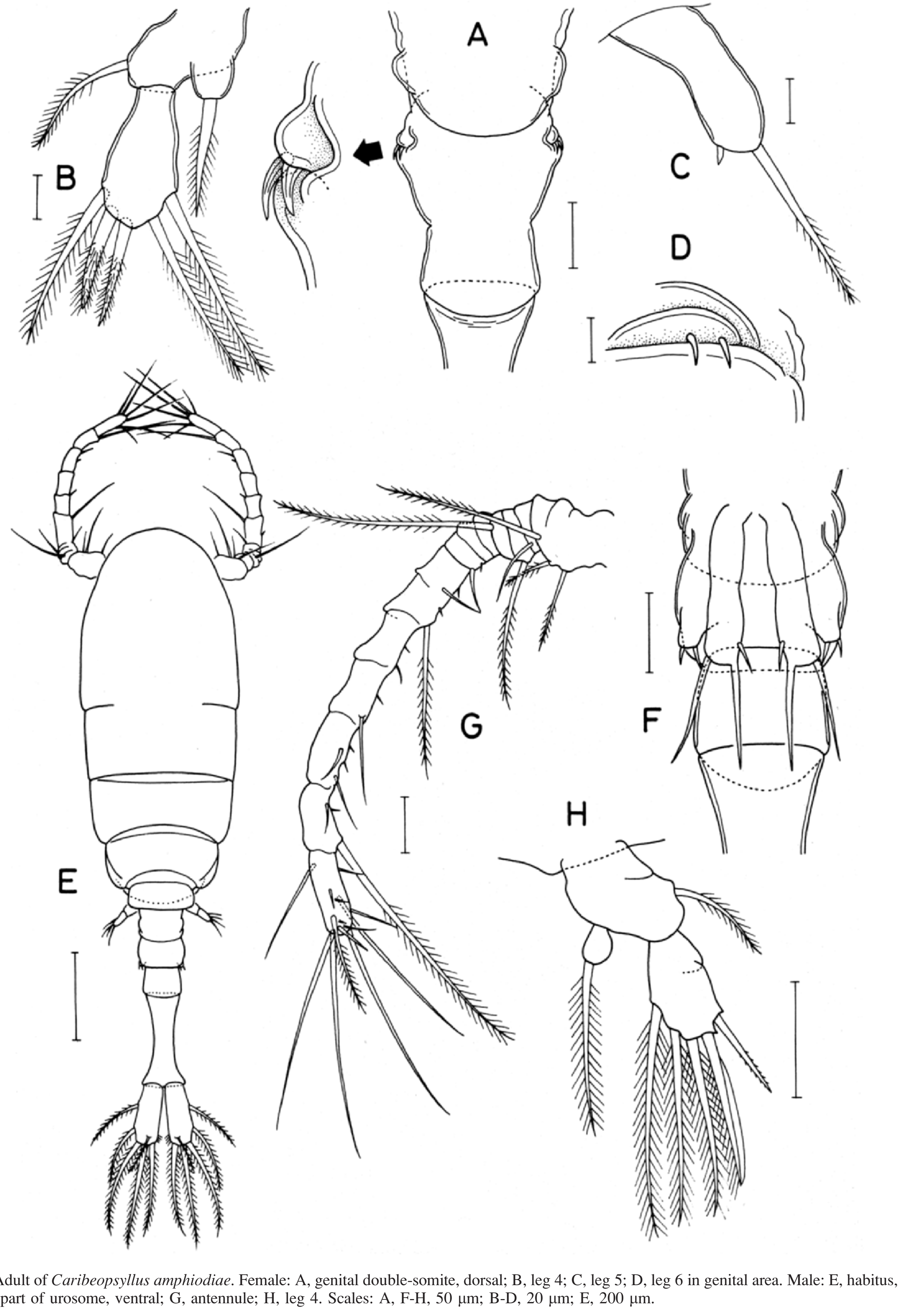

Fig. 16. Adult of *Caribeopsyllus amphiodiae*. Female: A, genital double-somite, dorsal; B, leg 4; C, leg 5; D, leg 6 in genital area. Male: E, habitus, dorsal; F, anterior part of urosome, ventral; G, antennule; H, leg 4. Scales: A, F-H, 50 µm; B-D, 20 µm; E, 200 µm.

(Fig. 4B-F) consists of a ventral ocellus and two dorsolateral ocelli (referred to as ''conspicillae'' by Ho et al., 2003). Red pigmentation within the urosome is less conspicuous than in early copepodid stages, and strands and patches of green and red-brown pigment are concentrated at the posterior end of the cephalothorax. There is a prominent, reflective whitish patch on each caudal ramus, and green or blue pigmentation behind the red naupliar eyes and in the swimming legs (Fig. 4B-F). The female (Fig. 4D) has smaller ocelli and less intense blue pigmentation around the eye than the male (Fig. 4B), and more gray-green and brown pigmentation at the juncture of cephalothorax and urosome. After extrusion of the greenish egg sacs, the female's cephalothorax appears transluscent; within it, strands of red-brown pigment are replaced by blue-green, and lipid globules (adipocytes?) are fewer. In comparison to an individual carrying new egg sacs (Fig. 4E), one with advanced embryos (Fig. 4F) appears to have a body relatively devoid of stored nutrient; red flecks in its egg sacs are naupliar eyes of the embryos.

Male.—Body (Figs. 6C, 16E) differs conspicuously from that of female (Figs. 6A, B, 15A) in having less elongate anal somite and markedly narrower cephalosome. Mean body length 1.31 (1.23-1.33) mm and width at broadest point 0.37 (0.32-0.42) mm, based on 10 specimens. As in female, integument of cephalosome folded and pleated in places, with delicate divaricate wrinkles, and endowed with circular pores and with sensilla having single or divergent hairs (Fig. 6E, F). Rostrum broad and rounded. Urosome distinctly 5-segmented. First abdominal somite (Fig. 16E) short, about 1.3 times broader than long. Anal somite (Fig. 16E) elongate, almost 3 times longer than broad, with concave lateral margins. Some depressions and folds in ventral surface of cephalothorax (Fig. 7B) possibly representing vestiges of structures such as apodemes associated with cephalic appendages that were present in naupliar stages. Mouth of adult (Figs. 6G, 7B, D) markedly reduced compared to metanauplius (Fig. 7A, C). Morphogenetic changes associated with reduction may produce transverse folds of integument lateral to mouth. Medial pore on labrum of metanauplius (Fig. 7C) is retained in adult (Fig. 7D), but pores associated with the labial and labral folds are lost. Digestive tract not observed in cleared specimens; however, longitudinal cream-colored structure along midline of living individuals likely representing digestive tract (Fig. 4B, C). Anus most likely represented by indentation at midpoint of posterior margin of last abdominal (anal) somite.

Antennule (Figs. 6C, G, 16G) geniculate, 13-segmented with armature formula as follows: 3, 1, 1, 1, 0, 0, 2, 4, 2, 2, 4, 3, and 13 (somewhat different from count in Ho et al., 2003); some setae sparsely pinnate. Segments XV-XVI fused, lacking sheath; geniculation occurring between fused ancestral segments XIX-XX and XXI-XXIII; terminal (13th) segment representing fused ancestral somites XXIV-XXVII. When living specimens are exposed to dilute ethanol, the antennules coil by bending at the base and flexing the geniculate segments (Figs. 4B, 6G). All other cephalic appendages present in metanauplius (Fig. 7A) lacking in adult (Fig. 7B).

Legs 1-3 as in female. Leg 4 (Fig. 16H) essentially as in female, but of smaller size. Leg 5 (Fig. 16F) situated on ventromedial surface of somite at posterior margin of pediger, with 2 members of pair fused medially at bases, intercoxal sclerite lacking; each leg elongate, about 4.3 times longer than broad, bearing 1 small and 2 large terminal setae. Leg 6 (Fig. 16F) lobiform, bearing 2 short setae.

Discussion

Comparisons Among Larval Stages of Thaumatopsyllid Species

Nauplii.—*Caribeopsyllus amphiodiae* is the only thaumatopsyllid whose first naupliar stage has been observed. Its NI, a swimming metanauplius, is the first of three major phases in the life cycle, and it is compared with the nauplii of other copepods in the discussion below. It escapes from the egg sac and presumably enters the ophiuroid host, *A. urtica* (Fig. 17). The second phase consists of parasitic metanauplius larvae that live in the host's stomach, and the final phase consists of free-living, non-feeding copepodid and adult stages (Fig. 17).

The parasitic metanauplius of *C. amphiodiae* can be compared with that of *T. paradoxus*, which was cursorily described by Bresciani and Lützen (1962). Although metanauplii of the two species resemble one another, their appendages are different in proportion to the body; for example, the maxillule is the smallest appendage of *C. amphiodiae* and the largest of *T. paradoxus* (compare Figs. 2, 5B herein, with 5A in Bresciani and Lützen, 1962).

Metanauplii of *C. amphiodiae* were seen, using light microscopy, to have two caudal setae (Fig. 2). However, five setae were observed using SEM (Fig. 5G), compared with three caudal setae reported in *T. paradoxus*. The antenna of *C. amphiodiae* is biramous with a 4-segmented exopod, and lacks the arthrite bearing two long setae and a gnathite present on the 5-segmented exopod of *T. paradoxus*, a disparity quite unexpected for confamilials. In *T. paradoxus*, the mandibular exopod is represented by a spiniform process with three large setae and a row of spinules near its base, and the maxillule has three additional setae on the proximal inner margin of the endopod. The maxillule of *C. amphiodiae* is much smaller than the mandible, and minuscule compared with the more robust structure in *T. paradoxus*. In the latter species the mandible and maxillule are of comparable size.

Copepodids.—The present study of *C. amphiodiae* is the only one for a thaumatopsyllid for which all copepodid stages have been described. As is typical of Copepoda, this species has five copepodid stages (CI-CV) before the adult stage (CVI) (Fig. 17). Their morphology is similar in many respects to that of copepodids of *C. chawayi* described by Suárez-Morales and Tovar (2004). As those authors appear not to have found CII and CIV stages in their plankton samples, we can only compare the CI, CIII, CV, and CVI stages of both species. In addition, we compare copepodid stages of the two species of *Caribeopsyllus* with *T. paradoxus*, using Fosshagen's previously unpublished observations that are provided by Suárez-Morales and Tovar (2004). The segmentation of the antennule of

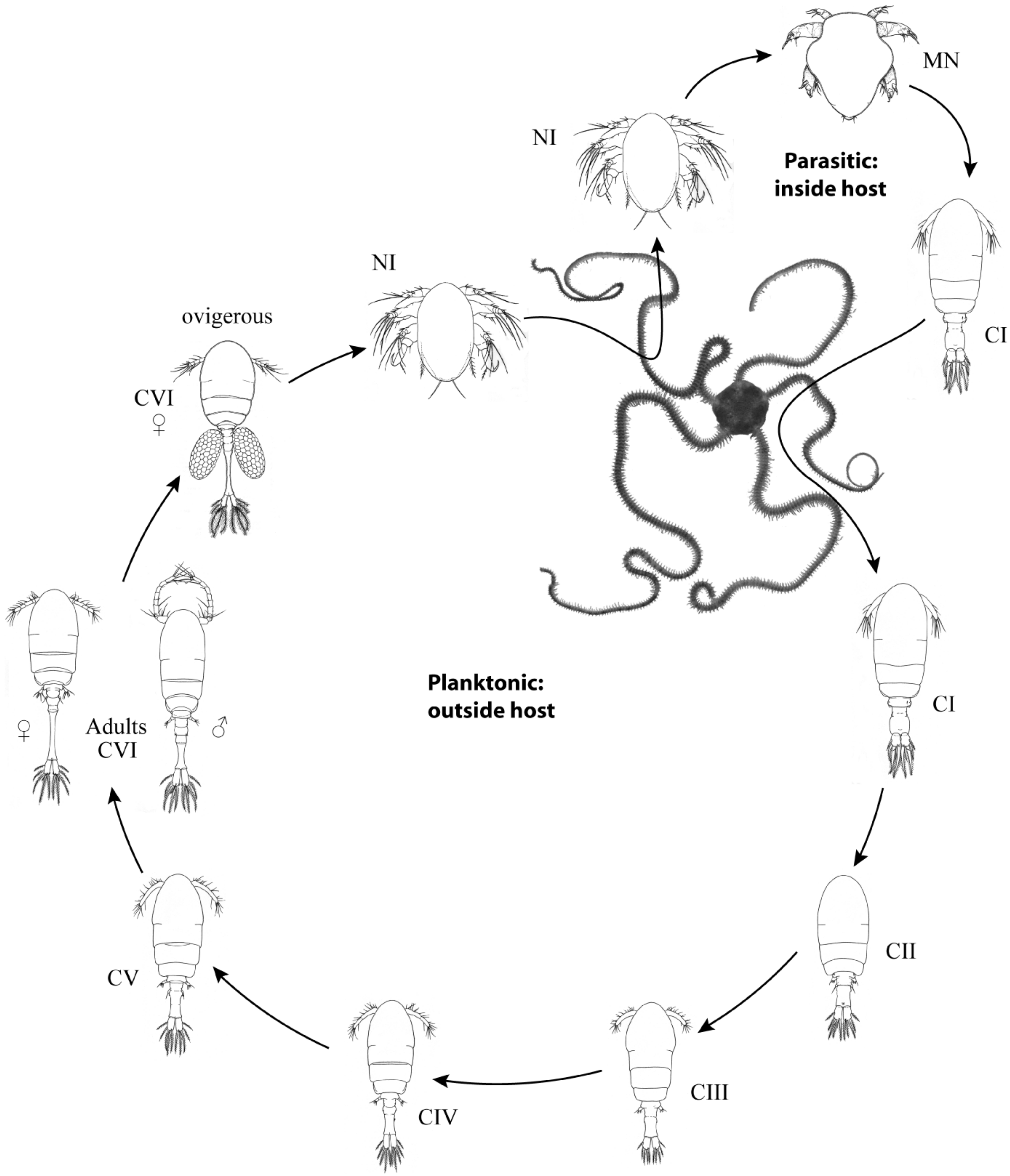

Fig. 17. Life cycle of *Caribeopsyllus amphiodiae*. Solid arrows indicate transitions from one larval stage to the next. Note that the parasitic stage is the metanauplius larva.

C. amphiodiae is difficult to determine, because the difference between cuticular wrinkles and developing segmental sutures is difficult to distinguish, but the number of antennular segments increases during larval development. The number of setae on the female antennule increases from six in NI to 20 in the metanauplius, 22 in CI, and reaches 26 in the CII to CVI (adult female) stages. The early loss of the antennular setae in CI and "progressive regain" of the setae in CV reported by Suárez-Morales and Tovar (2004: 240) for *T. paradoxus* is a common occurrence in calanoid copepods (Oberg, 1906; Hulsemann, 1991), but it was not observed for *C. amphiodiae*.

The maxillule, present in the metanauplius, is not expressed in the CI and more advanced stages of *C. amphiodiae* or *C. chawayi*. The CI of *C. amphiodiae* has lobiform vestiges of the antenna and the mandible, but to our knowledge, similar vestigial structures of entire appendages have not been reported for other parasitic copepods lacking feeding appendages, although transitory vestigial rami develop in several species (Dudley, 1966). They might represent vestiges of feeding structures produced by copepodids of ancestral species; if so, similar structures may occur in copepodid stages of other extant thaumatopsyllids. However, homologous vestiges were not described or illustrated for *C. chawayi*, although Suárez-Morales and Tovar (2004) mentioned "remnants" of "undetermined appendages" in CII and CIII.

The first appearance of the legs, and their segmentation and armature, differ from CI to CVI in *C. amphiodiae*. Legs 1 and 2 are present in CI and CII as biramous swimming legs with 1-segmented rami; the rami become indistinctly 2-segmented in CIII, distinctly 2-segmented in CIV and CV, and distinctly 3-segmented in CVI. Leg 3 is a bilobed structure in CI, then, as in legs 1 and 2, becomes a biramous 1-segmented swimming appendage with 1-segmented rami in CII, indistinctly 2-segmented rami in CIII, distinctly 2-segmented rami in CIV and CV, and distinctly 3-segmented rami in CVI. Leg 4 is a rudiment in CI, and a biramous appendage with final armature expressed as early as CII. However, leg 4 is only a small bud at CI in *C. chawayi* and *T. paradoxus*, with the final armature condition expressed at CV in *C. chawayi* and CIII in *T. paradoxus*. Legs 5 and 6 are absent in CI, but leg 5 is present in CII as a reduced, lobiform, non-swimming appendage and leg 6 as two very small processes in *C. amphiodiae*. Leg 5 is represented only as a small bud (lobe) at CII in *C. chawayi* and *T. paradoxus*.

The segmentation of the rami is the same in legs 1-4 for CI in *C. amphiodiae* and *C. chawayi*, and presumably in *T. paradoxus* for legs 1-3 (Suárez-Morales and Tovar, 2004). In CII, however, leg 3 is different (*C. amphiodiae* has indistinctly 2-segmented rami and *C. chawayi* has 1-segmented rami), as is leg 4 (*C. amphiodiae* has a biramous leg 4 and *C. chawayi* and *T. paradoxus* have a uniramous leg 4). In CV, legs 1-3 have 2-segmented rami in *C. amphiodiae*, but 3-segmented rami in *C. chawayi*. The 3-segmented ramal condition in legs 1-3 is attained at CVI in *C. amphiodiae*, but at CV in *C. chawayi*.

Setal and spinal elements of legs 1-4 increase in number from CI to CII, then remain constant through the adult stage of *C. amphiodiae*. This is in contrast to the pattern described by Suárez-Morales and Tovar (2004), who reported that in *C. chawayi* the leg armature does not stabilize until the CIII stage. However, their claim that the CI and CII stages are virtually identical in general habitus and in leg segmentation and spination suggests that their putative CII actually was a CI stage. Although they reported differences in the antennule of these two stages of *C. chawayi*, the differences can be attributed to either observational errors or damaged specimens. The first two copepodid stages of *C. amphiodiae* differ in many respects, and quite noticeably in that leg 3 develops from a bilobed structure with small setae in CI to a true biramous swimming leg with pinnate setae in CII.

Absence of the CIV stage in *C. chawayi*, reported by Suárez-Morales and Tovar (2004), is presumed to be in error. The authors base their interpretation on the absence of a CIV in their samples, although with the qualification that they may have failed to collect the stage. *Caribeopsyllus amphiodiae* clearly has a CIV larval stage that is distinguished from the CIII and CV stages by moults. According to Suárez-Morales and Tovar (2004), Fosshagen also found six copepodid stages in *T. paradoxus*, which further undermines the supposition that *C. chawayi* lacks a CIV stage. Just as its confamilials, *C. chawayi* most likely has six copepodid stages.

In summary, the metanauplius of *C. amphiodiae* differs from that of *T. paradoxus*, the only other thaumatopsyllid whose metanauplius has been described, in the size of the appendages relative to body size, number of caudal setae, absence of antennary arthrite and gnathite, and setal number in mandibular exopod and maxillule inner lobe. The copepodids of *C. amphiodiae* differ from those of *C. chawayi* and *T. paradoxus* in the number of setae on the antennule, presence of the vestiges of the antenna and mandible in CI, and the chronology of leg segmentation and armature.

Comparison of Thaumatopsyllid Larvae with Those of Other Copepods

Nauplii.—The most unusual feature of the *C. amphiodiae* NI is the presence of one pair of setae on the labrum. No other species of copepod is known to possess a similarly armed labrum in NI. Its NI antennule has the setation 1, 2, and 3, which distinguishes it from other copepods that have the general setation at NI of 1, 3, and 2 + 1 aesthetasc (Izawa, 1987).

It is notable, because of the relationship that has been posited (Ho et al., 2003) between thaumatopsyllids and monstrillids (a group recently placed within the Siphonostomatoida by Huys et al., 2007), that in advanced metanauplii of *C. amphiodiae*, the mandible becomes a massive structure, the exopod is transformed into a robust claw, and the inner distal portion of the first endopodal segment projects as a robust process forming a chelate complex with the distinctly curved, hook-like endopod. The NI mandibular endopod of *C. amphiodiae* is similar to that of *M. hamatapex* in which the distal segment is a robust claw. But the similarity could well be homoplasious, because harpacticoids such as Thalestridae, Harpacticidae, Metidae, and Miraciidae show a similar endopod at NVI (Dahms, 1991). Furthermore, the NI of *M. hamatapex* diverges from *C. amphiodiae*, as it has a three-segmented antennule with setation 0, 2, 3, a two-segmented antennary endopod with a terminal claw, a four-segmented antennary exopod, and an unsegmented mandibular exopod (Grygier and Ohtsuka, 1995). Nevertheless, sinuous ridge and groove microstructure of the naupliar integument of *C. amphiodiae* resembles the integumental ornamentation of CI-CIII *C. chawayi* shown by Suárez-Morales and Tovar (2004) and

Table 2. Comparison of absence/presence and segmentation of legs 1-6 of copepodid I-VI of *Caribeopsyllus amphiodiae*. Numbers in columns represent number of segments. Parentheses indicate indistinct segmentation.

Legs	Copepodid I	Copepodid II	Copepodid III	Copepodid IV	Copepodid V	Copepodid VI
Leg 1						
Exopod	1	1	(2)	2	2	3
Endopod	1	1	(2)	2	2	3
Leg 2						
Exopod	1	1	(2)	2	2	3
Endopod	1	1	(2)	2	2	3
Leg 3						
Exopod	Lobe	1	(2)	2	2	3
Endopod	Lobe	1	(2)	2	2	3
Leg 4						
Exopod	Bud	1	1	1	1	1
Endopod	Bud	1	1	1	1	1
Leg 5						
	Absent	Lobe	Lobe	Lobe	Lobe	Lobe
Leg 6						
	Absent	2 small processes	Lobe	Lobe	Lobe	Lobe

the NI of *M. hamatapex* shown by Grygier and Ohtsuka (1995), and there are similar fine, divaricate wrinkles on the adult integument of *C. amphiodiae* and *Monstrilla helgolandica* Claus, 1863 shown by Huys and Boxshall (1991).

Another distinguishing feature of *C. amphiodiae* is the presence of a maxillule that is represented by one seta in the NI. Such an NI maxillule has not been recorded in Cyclopoida, Siphonostomatoida, and *M. hamatapex* (Izawa, 1987; Grygier and Ohtsuka, 1995), and in many copepods, the one-seta or one-spine maxillule does not appear until NII or NIII (Izawa, 1987). Although the precocious formation of the maxillule might suggest that the putative NI is actually an NII that was preceded by a very short-lived and overlooked NI, the presence of a primordial maxillule at NI is also observed in harpacticoids such as *Longipedia*, *Microsetella*, *Sunaristes*, and *Canuella* (Izawa,1987), and in mystacocarids (Olesen, 2001). Therefore, unless the existence of a missing stage is confirmed, we propose that the species lacks an orthonauplius phase, and its NI, which has a maxillule bud, is by definition a metanauplius.

In the present study, the description of the parasitic metanauplius phase is based primarily on larvae ranging in length from 0.62-1.04 mm. They were readily distinguishable from the 0.07 mm long NI stage, but as best could be determined with light microscopy, these larger individuals all had identical appendage morphology, as do *T. paradoxus* metanauplii of similar size (Bresciani and Lützen, 1962). Individuals ranging from 0.62-0.70 mm in length usually had one incomplete posterolateral suture line (Fig. 2A), those 0.73-0.82 mm long had an incomplete posterolateral suture line and a complete posterior suture line, and those 0.88-1.04 mm long had an incomplete posterolateral suture line and two posterior suture lines (Fig. 3B).

These large metanauplii resemble the late naupliar stages of cyclopoids, harpacticoids, and calanoids, which have a bilobate maxillule as NIV larvae (Izawa, 1987), and setation of the maxillule is typical for NV stage copepods (Ferrari and Dahm, in press). A 0.47 mm-long metanauplius had ventral leg buds with projecting terminal setae (Figs. 5C, 7A), as generally found in NVI stage copepods (Ferrari and Dahms, in press), but larger *C. amphiodiae* metanauplii lacked leg buds (Fig. 5B). Although naupliar exuviae were found in only one culture, it seems likely that the parasitic metanauplii molt, since their body length increases more than ten-fold and their volume increases more than 1000 fold (up to 4000 fold approximating body shape as a prolate spheroid) between the NI stage and metamorphosis. However, it remains to be determined how many molts are passed and which naupliar stages occur in the life cycle of *C. amphiodiae*, or if the metanauplius could develop without repeatedly molting.

Copepodids.—The absence of fully developed antenna (= second antenna) and oral appendages in copepodids of *C. amphiodiae*, as in other thaumatopsyllids, restricts the scope of morphological comparisons that can be made with other copepods. Nevertheless, the CI of *C. amphiodiae* and *C. chawayi* possess a cephalon, five thoracic somites, the posterior abdominal somite, and two limb buds of swimming legs 3 and 4 (i.e., the 4th and 5th thoracopods). In comparison, the CI of siphonostomatoids, cyclopoids including poecilostomatoids, harpacticoids, misophrioids, calanoids, and platycopioids all differ in having only one limb bud, i.e., swimming leg 3 which represents the 4th thoracopod (Ferrari and Dahms, in press). The copepodids of *C. amphiodiae* display unusual features in leg ontogenesis, including an apparent delay of ramal segmentation and acceleration of setal development, and evidently develop arthrodial membranes differently than most other copepods. In *C. amphiodiae*, the rami of leg 1 are not two-segmented until CIV and become three-segmented as late as CVI (adult), and the same phenomenon occurs in legs 2 and 3 (Table 2). However, the predominant pattern of segment addition on the rami of leg 1, for example, is one segment at CI, two segments at CII-CIV, and three segments at CV and CVI (Ferrari, 1988, 1992).

In contrast to the delayed leg segmentation, leg setae develop precociously in *C. amphiodiae*, and the ultimate number of setae is unusually high. In most copepods, except for poecilostomatoids, legs 1-4 typically have one inner seta on the first exopodal segment, which first appears in legs 1 and 2 at CIII, and in leg 3 at CIV. However, the same seta of *C. amphiodiae* appears simultaneously in legs 1-3 as early as CII. The CI of *C. amphiodiae* has the same pattern of leg 1 setation that Ferrari (2000) described as the general case amongst Copepoda. However, leg 1 of the CII of most copepods have two-segmented exopods and endopods bearing 1, 8 and 1, 7 setae, respectively, but leg 1 of CII *C. amphiodiae* has exopod formula 2, 8 and endopod formula 1, 7; thus it has one more (medial) seta on the first exopodal segment. Leg 2 of the CII of most copepods has the exopod formula 1, 7 and the endopod formula 1, 6, but *C. amphiodiae* has a leg 2 exopod formula 2, 9 and the endopod formula 1, 7; thus it has three extra setae on the exopod and one more on the endopod, an armature formula that is unique among Copepoda. Leg 3 of the CII of most copepods has one-segmented rami with a maximum of 7 setae on the exopod and 6 on the endopod, but *C. amphiodiae* has 11 setae on the exopod and 8 setae on the endopod, which corresponds to the typical setation of the CIV in other copepods. Leg setae also arise precociously in *C. chawayi*. Although its CII stage has not been studied, its CIII has an inner seta on the first exopodal segment of leg 3 that does not arise until CIV in other copepods. Thus, comparatively rapid ontogenesis of leg setae seems to be a shared feature of the genus *Caribeopsyllus*.

The antennule also exhibits the phenomena of precocious setation and delayed segmentation. Although the segmentation of the antennule is weak or obscure throughout the copepodid stages of *C. amphiodiae*, the final number of setae on the antennule of *C. amphiodiae* is determined as early as CII in the female (26 setae) and CIII in the male (36 setae). This is contrasted to other copepods, in which the setae are continuously added to the antennule until CV or CVI (Boxshall and Huys, 1998).

Conclusions

Our study reveals characters of the naupliar and copepodid stages of *C. amphiodiae* that are unusual among Copepoda, and several that are unique. Some, but not all, of the distinctive features are shared by *C. chawayi* and *T. paradoxus*. Further studies of thaumatopsyllid ontogenesis are necessary to discriminate synapomorphies of the clade. Unique features of *C. amphiodiae* NI are the single pair of setae on the labrum (absent in other copepods) and the presence of seven setae on the NI antennary exopod (six in other copepods). Its NI maxillule is represented by one seta, a condition otherwise found in Mystacocarida and some Harpacticoida. Fusion of antennular segments in later naupliar stages, as occurs in *C. amphiodiae*, is otherwise known only for copepods with parasitic adults and the thalestrid harpacticoids. Another unusual feature of the parasitic metanaupliar larva is its chelate mandible composed of a spiniform process and a hooked endopod, which has counterparts only among some NVI harpacticoids and the *M. hamatapex* NI. Also noteworthy is the large, well-developed, and well-separated ocelli of the metanauplius, so unlike the typical, compact, copepodan naupliar eye, and the delicate, sinuous ridge and groove micro-ornamentation of the larval integument, which is replaced by fine, divaricate cuticular wrinkles in the adult.

The transient vestiges of the antenna and mandible occurring in the CI are, to our knowledge, a unique feature of *C. amphiodiae*, and the absence of the maxillule, maxilla, and maxilliped during the copepodid phase of development are atypical, but have been reported for non-feeding life stages of other copepods, such as the monstrillids. Other unusual features of *C. amphiodiae* and, as best can be determined, of *C. chawayi* and *T. paradoxus* copepodids are the presence of pedigers 3 and 4 (4th and 5th thoracomere) and the bud of swimming leg 4 at CI, and the presence of pedigers 5 and 6 (6th and 7th thoracomere) and the bud of leg 5 at CII. The initial appearance of these structures is earlier than reported for other copepods, with the exception of some parasites, e.g., *Nucellicola holmanae* Lamb et al., 1996. In addition, during leg development, setal development is accelerated and ramal segmentation is delayed with respect to other copepods. Finally, the major body articulation of the copepodid stages (CI-CVI) occurs between the third and fourth pedigers, as was first recognized by Kabata (1979), a pattern that contrasts with gymnoplean (between fifth and sixth pedigers) and podoplean (between fourth and fifth pedigers) tagmosis. Although a few siphonostomes, e.g., *Parartotrogus*, Caligidae, and other caligiform families (Dissonidae and Trebiidae) also exhibit the major body articulations between the third and fourth pedigers, the pattern of tagmosis of these copepods and that of thaumatopsyllids are likely homoplasious. Together with the unusual tagmosis of the adult, ontogenesis of legs in the copepodid stages and unique morphological features of the naupliar stages observed in the present study, help to distinguish the thaumatopsyllids as a distinctive group of copepods.

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