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TOWARD A TAXONOMY OF THE GNATHIIDAE (ISOPODA) USING JUVENILES: THE EXTERNAL ANATOMY OF *GNATHIA AUREAMACULOSA* ZUPHEA STAGES USING SCANNING ELECTRON MICROSCOPY

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ABSTRACT

The juvenile stages of the isopod family Gnathiidae have a large impact on marine ecosystems through their parasitic interactions with marine fishes, as well as being a substantial food source for the cleaner fish guild. However, the taxonomy of Gnathiidae is based on non-feeding adult males. Adults are difficult to obtain owing to their cryptic lifestyle in the sea floor, while the juveniles can be collected in abundance either from their hosts or using emergence traps. Given the known diversity of the hosts, and assuming host partitioning among species, the diversity of gnathiids could be much greater than currently recognised. We argue that the systematics of this group should recognise this ecological role, and develop the taxonomy of the juvenile stages. We provide a detailed external anatomy of one species, *Gnathia aureamaculosa*, that has been used extensively in recent ecological studies. Images from scanning electron microscopy of two juvenile instars are compared with two other species, and the literature surveyed to illustrate a range of taxonomically useful traits. The expansion cuticle of the pereion shows how gnathiids are able to expand enormously during a blood meal and also lends caution on using total body length for comparative purposes. A range of morphometric and categorical features are available on the non-expandable sections of the body, such as the head, pleotelson and uropods. Of the three species illustrated, the shape of the eyes and head, and the shape and setation of the pleotelson uropods are diagnostically useful. Evidence from the literature shows that the free-living juveniles are not mancas; the manca instars are passed during the internal developmental period. Anatomical observations cast doubt on the classification of Gnathiidae as Cymothoida, in agreement with recent phylogenetic studies.

KEY WORDS: anatomy, Cymothoida, fish parasite, Gnathiidae, Isopoda, SEM

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INTRODUCTION

Gnathiid isopods are well known as ectoparasites of fishes (Monod, 1926; Grutter and Poulin, 1998), and their impact on the health of their hosts (Honma et al., 1991; Marino et al., 2004; Jones and Grutter, 2005; Paperna and Por, 1977; Mugridge and Stallybrass, 1983; Penfold et al., 2008; Grutter, 2008; Grutter et al., 2010, in press) and their role in coral reef ecology (Grutter and Poulin, 1998; Grutter, 2003) is becoming better known. Because of their high abundances as juveniles, gnathiids also perform a keystone role in coral reef cleaner-host symbiotic interactions in that gnathiids provide a large proportion of the cleaner's nutrition (Grutter, 1996, 1997a, b, 2008). Gnathiids also are intermediate hosts for fish blood parasites (Smit and Davies, 2004). Despite their important ecological roles, our ability to identify the unfed juvenile or ''zuphea'' stage (the active parasitic form) is impaired because taxonomic descriptions are based on the non-feeding benthic adults, or the third juvenile stage that have fed or ''praniza'' stage (Smit and Davies, 2004; Nagel et al., 2008; Nagel, 2009; Ferreira et al., 2009). This lack of taxonomic information is a challenge for understanding the ecology of parasite-host interactions on coral reefs. The zupheae of gnathiids, however, offer an opportunity to improve this situation because they can be collected in large numbers using emergence traps (Grutter et al., 2000a; Jones and Grutter, 2007). These stages have identifiable differences, even when using coarse descriptors like length, head width, and eye size (Nagel et al., 2008), and colour pattern (Grutter et al., 2000b).

Gnathiids also are abundant, and the group may be much more diverse than currently appreciated. Gnathiids emerge from the reef during the day and night and are common on the reef (Grutter et al., 2000a). On small patch reefs at Lizard Island, Great Barrier Reef, they emerge at a rate of 41.7 ± SE 6.9 m^2 day^{-1}; integrating this value over the area of these reefs (91-125 m^2) yields 4552 ± SE 2632 zupheae $reef^{-1}$ day^{-1}(Grutter, 2008). One species of gnathiid, *Gnathia aureamaculosa* Ferreira and Coetzee, 2009 has been found on 20 species of fishes from 8 families (Ferreira et al., 2009). Because the Great Barrier Reef (GBR) system has at least 1,700 species of teleost and elasmobranch fishes (Randall et al., 1997), the potential for host resource partitioning among gnathiid species is substantial. The diversity of gnathiids on the GBR, currently known to be 20 species (Table 1), is therefore likely to be much higher. For most described species, however, their hosts are unknown because the taxonomy has been based on adult males only. The gains for coral reef ecosystem research would be substantial if these active and

Table 1. Species of Gnathiidae known from the Great Barrier Reef region of Queensland Australia. [1] Ferreira et al., 2009; [2] Jones et al., 2007; [3] Coetzee et al., 2008; [4] Coetzee, 2006; [5] Coetzee et al., 2009; [6] Nagel, 2009; [7] Ferreira et al., 2010; [8] Jones and Grutter, 2008; [9] Farquharson et al., 2009, [10] Grutter et al., in press.

Genus	Species	Host: Teleost (T) or Elasmobranch (E)	General locality
Bathygnathia Dollfus, 1901	*adlerzia* Cohen and Poore, 1994	?	Coral Sea
Elaphognathia Monod, 1926	*bifurcilla* (Holdich and Harrison, 1980)	?	Bowling Green Bay
	forceps (Holdich and Harrison, 1980)	?	Rowes Bay
	rimifrons (Holdich and Harrison, 1980)	?	Halifax Bay
Gnathia Leach, 1814	*asperifrons* Holdich and Harrison, 1980	?	Lizard Island
	aureamaculosa Ferreira and Smit, 2009	T[1, 3, 6, 10]	Lizard Island
	biorbis Holdich and Harrison, 1980	?	Magnetic Island, Townsville Harbour, Heron Island
	calmani Monod, 1926	?	Victoria; Heron Island
	cornuta Holdich and Harrison, 1980	?	Pallarenda
	falcipenis Holdich and Harrison, 1980	T[2, 6, 7, 8]	Magnetic Island, Lizard Island
	grandilaris Coetzee, Smit, Grutter and Davies, 2008	E[3, 4]	Lizard Island
	G. grutterae Ferreira, Smit & Davies, 2010	T[7]	Lizard Island
	halei Cals, 1973	?	Moreton Island
	latidens (Beddard, 1886)	?	Northern Australia (Flinders Passage)
	sp. nov. Farquharson & Smit	T[9]	Lizard Island
	meticola Holdich and Harrison, 1980	?	Magnetic Island, Townsville Harbour
	rhytidoponera Cohen and Poore, 1994	?	Coral Sea
	trimaculata Coetzee, Smit, Grutter and Davies, 2009	E[4, 5]	Lizard Island
	variobranchia Holdich and Harrison, 1980	?	Heron Island
	sp. in Coetzee, 2006	E[4]	Heron Island, Moreton Bay and Lizard Island

abundant ectoparasites were identifiable while still associated with their host or from emergence traps.

To address this gap in our taxonomic knowledge of the gnathiid zuphea, we evaluated the external anatomy of a species described recently in Ferreira et al. (2009), *G. aureamaculosa*, using scanning electron microscopy (SEM). This species is a potential model for evolutionary and ecological studies on gnathiid ectoparasites, owing to its use in ecological studies (reviewed in Ferreira et al., 2009), and the ease with which it is kept in culture.

Our study adds to a growing body of knowledge on the anatomy of gnathiid juveniles. Monod's (1926) monographic examination of the family provided detailed external and internal anatomical data for *Paragnathia formica* (Hesse, 1864) and other species. The review by Smit and Davies (2004) provided SEM data on several key parts of the anatomy, in particular the pleotelson plus uropods and setae/spines, and discussed the value of various features for identification. Other SEM studies of limited parts of gnathiid anatomy include: Davies (1981), the praniza and adult mouthparts of *Gnathia maxillaris* (Montagu, 1804); Wägele (1987), adults of *Gnathia calva* Vanhöffen, 1914; Charmantier et al. (1987), *Paragnathia formica* adult male, zuphea, praniza, and mouthparts of the juveniles; Svavarsson (1999), adults and praniza of *Caecognathia bicolor* (Hansen, 1916); Smit et al. (1999), adult male and praniza of *Gnathia africana* Barnard, 1914; Smit and Basson (2002), male, female and praniza of *Gnathia pantherina* Smit and Basson, 2002; Giannetto et al. (2003), external anatomy *Gnathia vorax* (Lucas, 1849); Coetzee et al. (2008), the male, female, and praniza of *Gnathia grandilaris* Coetzee, Smit, Grutter, and Davies, 2008; and most recently, Coetzee et al. (2009), the male, female and praniza of *Gnathia trimaculata* Coetzee, Smit, Grutter, and Davies, 2009. Here, we focus on the detailed external anatomy of the zupheae of *G. aureamaculosa*, a species from Great Barrier Reef where we expect the overall diversity of gnathiids to be high (see Table 1). To highlight taxonomically useful features, we compare this species with zupheae of other species (cf. *G. falcipenis* Holdich and Harrison, 1980 and an undescribed species) that occur at Lizard Island (northern GBR). We also discuss data from the literature on the pranizae of other species. This anatomical study also has phylogenetic relevance for the relationships of gnathiids to other isopods, which we briefly consider.

Materials and Methods

Laboratory maintenance of gnathiid juveniles to adults and culturing gnathiids is well known (see Smit and Basson, 2002). Specimens were obtained from a culture maintained at the Lizard Island Research Station (14 °40.91′S, 145 °27.895′E; Grutter, 2003; Grutter et al., 2000b; Ferreira et al., 2009) and were preserved in 100% ethanol. Two other species, one specimen each, were taken from a mixed-species formalin fixed assemblage collected near Lizard Island using emergence traps. These species, used in comparison with *G. aureamaculosa*, are not treated in detail because their species identification was not confirmed and their poorer preservation. All specimens were examined using light microscopy and SEM. For SEM preparation, specimens in ethanol were submitted to sonication for 3-5 seconds to remove debris and some epibionts. The specimens were dehydrated through a graded series of ethanol ending in 100%, critical point dried, mounted on pins attached to SEM stubs, gold-palladium sputter coated. Pin mounting was accomplished by using a tiny drop of adhesive on the pin tip to attach to the ventrolateral or dorsolateral surface of specimens, approximately in the centre of the body. This attachment method allowed specimens to be viewed from multiple angles (lateral, dorsal and ventral; method available from GDFW on request), but still retained on a stable SEM stub. Specimens were imaged using a Evo LS15 Carl Zeiss microscope (http://www.zeiss.de). The SEM stubs are retained at the Australian Museum under these registration numbers: *G. aureamaculosa*, adult male, P.81101, adult female, P.81102, zuphea 3,

P.81103, P.81396; zuphea 2, P.81394, P.81395, P.81397; *G.* cf. *falcipenis* zuphea, P.81398; *G.* sp. zuphea, P.81399.

The anatomical terminology, including numbering of pereionites and pereiopods, follows Monod (1926) with standardisations in Wilson (2009): somites and podomeres are indicated by Arabic numerals, and limbs are labelled with Roman numerals. Although the term ''gnathopod'' found on the zuphea/praniza stages is frequently used in the literature, the term pereiopod I is used here, as it is not as fully transformed as in the adults, where it is named ''pylopod.'' Some publications have used the term ''labrum'' for the dorsal part of the oral cone (Coetzee et al., 2008), but Monod (1926) showed that the labrum is not present and used the term ''clypeus'' for this part, a usage that we follow here. Monod (1926) and later authors have referred to the head as the ''cephalon'' or ''cephalosome'' (see Holdich and Harrison, 1980). Because this structure includes the first 2 thoracic segments, it is more properly ''cephalothorax'' but we have used here the simpler term ''head.'' We also introduce the term ''expansion cuticle'' for the highly folded articular cuticle found on pereionite 5 and elsewhere. The three zuphea instars are abbreviated Z1, Z2 and Z3; similarly, praniza instars are indicated P1, P2, and P3, although they are the same instar but having fed.

Measurements from SEM photographs were conducted using a graphics tablet (Intuos3; http://www.wacom.com) and a pixel analysis application (ImageJ; http://rsbweb.nih.gov/ij/); lengths of flexed structures (whole body, limbs) were measured using summed line segments. Each value reported is the median of 3 measurements, converted to millimetres. Total body length, from tip of the labrum/clypeus to the pleotelson, was measured in lateral view along a midline arc by summing line segments. In cases where the specimen had expanded at pereionite 5 (AM P.81394), the anterior length was taken to the posterior margin of pereionite 4 and starting from posterior margin of the coxa V where it intersects pereionite 6; these two points are approximately adjacent in an unexpanded specimen because the dorsal shield of pereionite 4 covers the expandable part of pereionite 5. Values are reported as smallest-largest (n = number of observations).

Figures were prepared either using GIMP (ver.2.6.6, http://Gimp.org) or Photoshop (ver.7.01, http://www.adobe.com). Additional observations on other species are taken from the literature or from our own observations on live specimens.

Results

SEM

Five ethanol preserved specimens of two zuphea stages (Z2, Z3)) were clean and provided good SEM images. In our experience, small specimens that have been recently preserved in pure (95%) ethanol provide satisfactory SEM specimens, provided that that any seawater is rinsed away beforehand and the specimens are sufficiently calcified that osmotic differences don't collapse the cuticle. This proved to be the case with the zupheae, which were surprisingly tough. The two adults, female and male (AM P.81101, P.81102), were covered with detritus and microscopic filaments, possibly fungal in nature. These latter two stages were described in detail in Ferreira et al. (2009) and are not discussed further. The two specimens of *Gnathia* cf. *falcipenis* and *G.* sp., which had been stored in a formaldehyde solution, had partially collapsed cuticles having a parchment paper appearance (Fig. 6A-E). These specimens had been stored in formaldehyde-seawater solution for around 4 years without adjusting the buffer during that period. As a consequence, the degradation products from the formaldehyde (formic acid and methanol) caused the specimens to lose calcium carbonate in their cuticle, resulting in their degraded appearance. These images are included here for comparison, although fresh specimens would provide better results.

External Anatomy of Zuphea of *Gnathia aureamaculosa*

Head (Figs. 2, 3).—The head in lateral view is smooth and only weakly curved with indentations at the clypeal and posterior margin. If held with the dorsal surface in the plane of observation (plan view), the head has a linear posterior margin, adjacent to the inarticulate thin pereionite 1. The dorsal cuticle has several pairs of tiny setae, and shallow indentations that can be seen only in SEM images, and no other projections or tubercles. At higher magnifications, an ''X''-shaped pattern of tiny pores can be seen in the dorsal surface (Fig. 2C), which surround central bulbous sensillae that sit flush inside the pores. Although the eyes are large, they do not occupy the entire lateral margin of the head as in some species, e.g., *G.* cf. *falcipenis*, Fig. 6D. The head thus has abbreviated curved lateral margins anterior to and behind the eyes. Each lateral eye has approximately 46 ocelli in 7 horizontal rows, and has a nearly perfect oval outline in lateral view; this outline is retained in the adults, although the shape of the head in males and females is considerably transformed by the maturation moult. The eye is proportionally larger on the head in the smaller individuals: eye diameter/head length ratio for Z2 is 0.57-0.62, ($n = 3$), and for Z3 it is 0.48-0.56 ($n = 2$). In ventral view, the eyes have well-developed, ventrally facing elements, suggesting that vision in the actively swimming zuphea is omnidirectional. The ventromedial margin of the eye is concave with an anterior obtuse angle lateral to the merus of pereiopod I (Fig. 3A). The anterior margin of the head where it articulates with the clypeus is approximately linear. The antennulae articulate in arc-like indentations on the anterior margin of the head and on the posterolateral margin of the clypeus. The anterior limb of the arc (on the clypeus) is longer than the lateral limb (on the head).

Unlike the dorsal surface, the sternal cuticle of the head appears to be flexible, especially between the first pereiopods. In *Gnathia* sp. (Fig. 3E), the head ventral surface has a large marginal carina (''carène marginale'' in Monod, 1926) (see discussion) that covers the lateral margin of merus and carpus. In *G. aureamaculosa*, this carina is only weakly expressed.

Pereionites (Figs. 1, 5).—All pereionites of *G. aureamaculosa*, similar to the pleonites, lack any ridges or tubercles, and have a smooth unembellished surface. In dorsal view, pereionite 1 is a thin transverse bar that lacks articulation with the head and is part of the cephalisation of pereiopod I that characterises Gnathiidae in general. The thinness of the bar suggests that the extrinsic musculature of the first pereiopod may insert on the inner surface of head. Pereionites 2-3 are approximately equal sized and curve anteriorly on the lateral margin. Pereionite 4 has the longest zupheal tergite owing to a posterior projection that covers the expandable cuticle of pereionite 5. This extension on pereionite 4 is visible in lateral view as an angular posterior projection that extends as far as the posterior margin of coxae IV. Often the posterior arc of pereionite 4 is pigmented so that it remains visible in the praniza.

The central dorsal surface of pereionites 1-4 have a pair of tiny setae. Because the dorsal surface of pereionite 5 consists entirely of the expansion cuticle (Fig. 5B-C), this segment lacks a dorsal tergite, and the plates associated

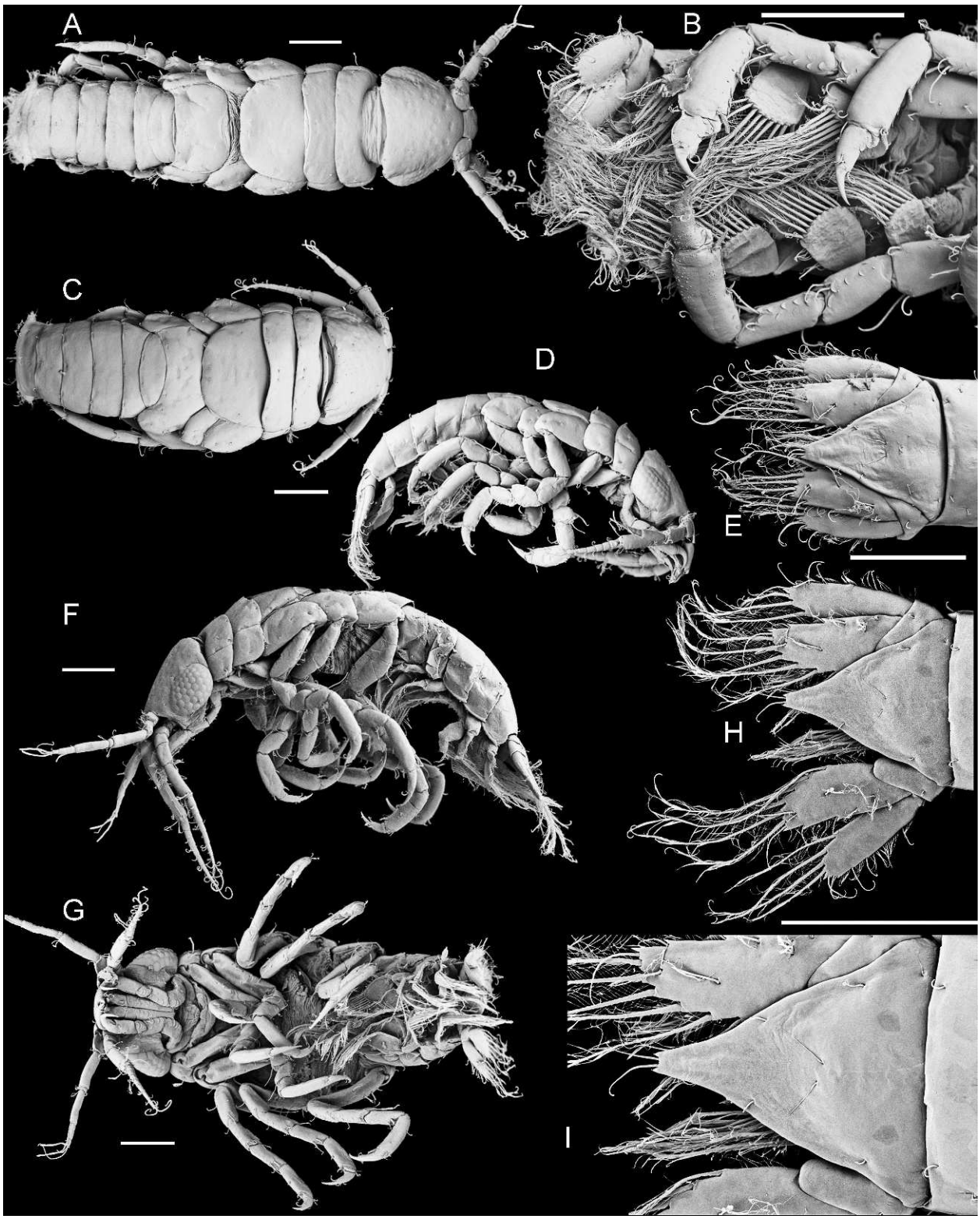

Fig. 1. General body views, *Gnathia aureamaculosa*. A-B, AM P.81394 (Z2). C-D, AM P.81397 (Z2). E, AM P.81395 (Z2). F-I, AM P.81103 (Z3). A, C, dorsal habitus, showing somewhat expanded (A) and not expanded and curled specimens (C). A, pereionites and pleonites in approximately dorsal view. B, pleon with distal parts of pereiopods V-VI, ventral view. D, F, lateral habitus, Z2 and Z3, respectively. G, ventral habitus. E (Z2), H (Z3), pleotelson and uropods, Z2 and Z3, respectively, dorsal view. I, enlargement of pleotelson. Scale bars 100 μm, A, and C-D, E-H, F-G at same scale for comparison.

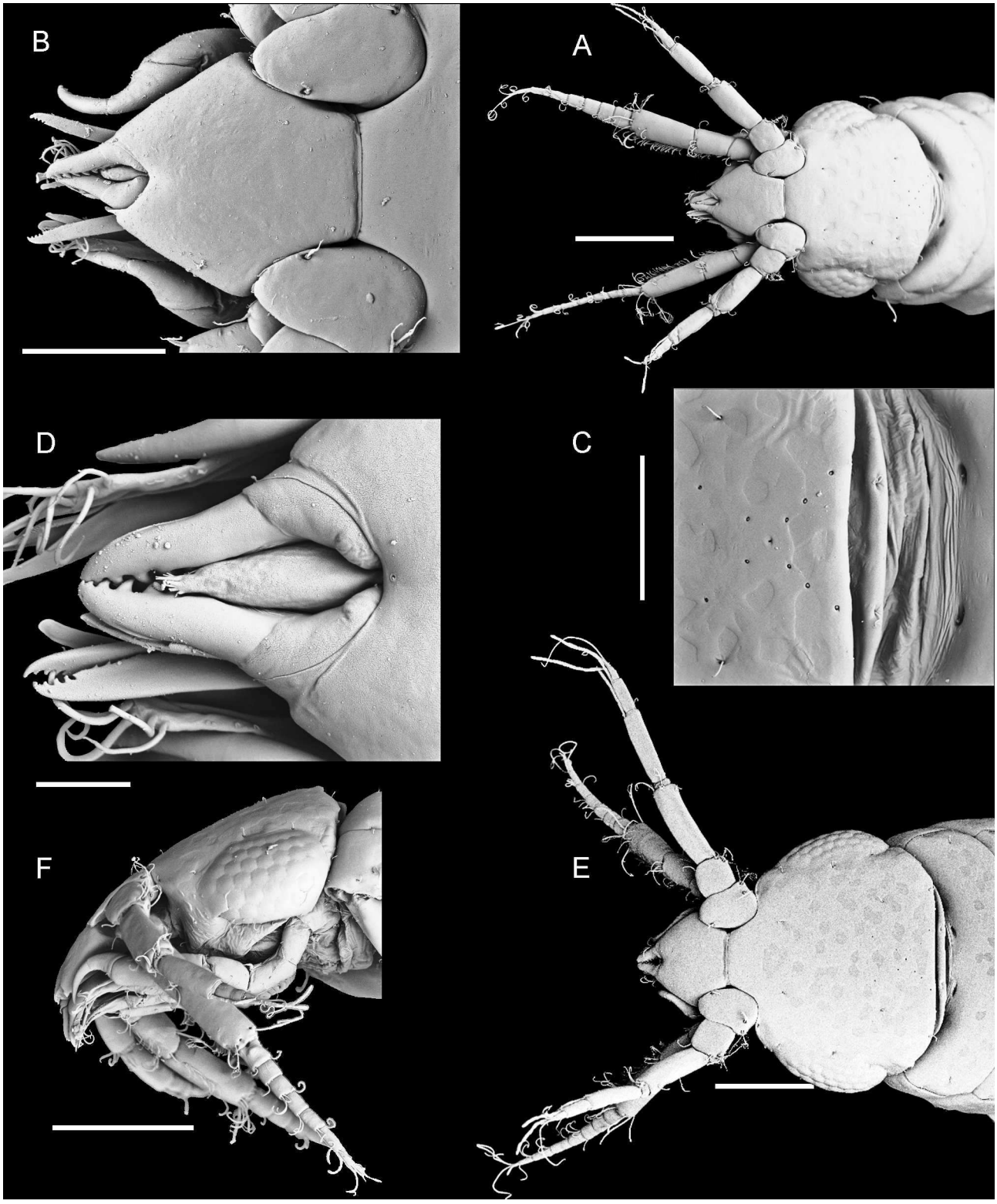

Fig. 2. Dorsal head and mouthparts, *Gnathia aureamaculosa*. A, C-D, AM P.81394 (Z2). B, AM P.81395 (Z2). E, P81103 (Z3). F, AM P.81396 (Z3). A, E entire head. B, clypeus, mouthparts, antennular base; D, enlargement of mouthparts. C, enlargement of posterior head surface showing sensory pores in an ''X'' pattern. E-F, dorsal and lateral view of the head and associated limbs. Scale bars 100 μm, except for B, 50 μm.

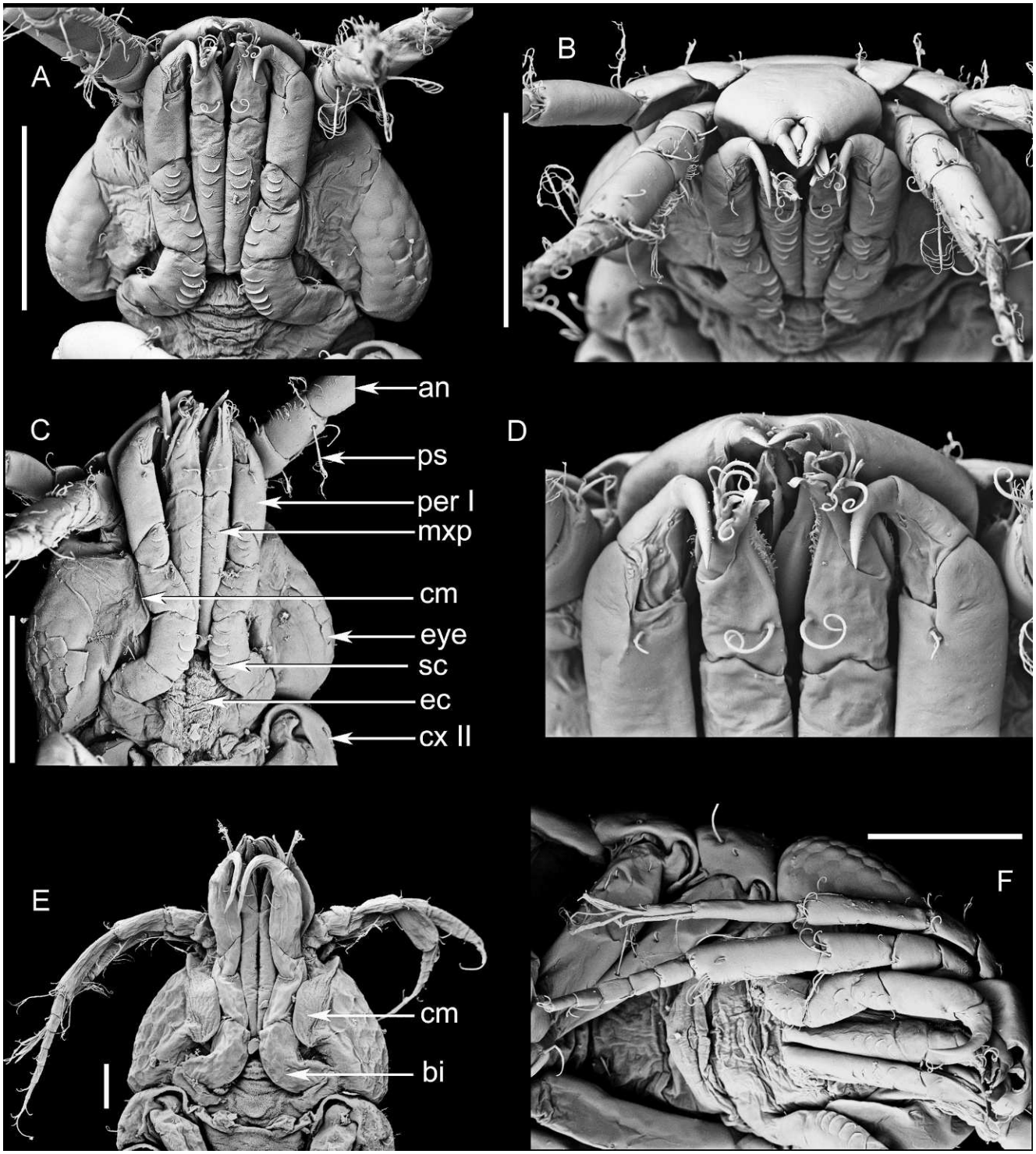

Fig. 3. Head in ventral views. *Gnathia aureamaculosa*. A-B, D (enlargement of A), AM P.81394 (Z2); C, AM P.81395 (Z2); F, AM P.81396 (Z3). E, an undescribed species from Lizard Island, AM P.81399 (Z3). Labels: an = antenna; ps = penicillate seta; per I = pereiopod I, propodus indicated; bi = basis-ischium; mxp = maxilliped, basis indicated; sc = posterior facing cuticular scales, found on thoracic limbs; cm = marginal carina; ec = membranous cuticle of pereionite 1; cx II = coxa of pereiopod II. Scale bars 100 µm.

with this somite are entirely coxal. Monod (1926: Fig. 71) shows that the ''pullus'' or zuphea of *P. formica* has an oval tergal plate on pereionite 5, so this might be a useful feature for identification in other taxa. The tergite of pereionite 6, which is not divided in any way, is nearly as long as pereionite 4 but curves posteriorly. Again, Monod (1926) shows that the tergite 6 might be partially separated from the coxae by articular or expansion cuticle. The tergite of pereionite 7 appears as an oval insert between pereionite 6 and pleonite 1, in which it is embedded.

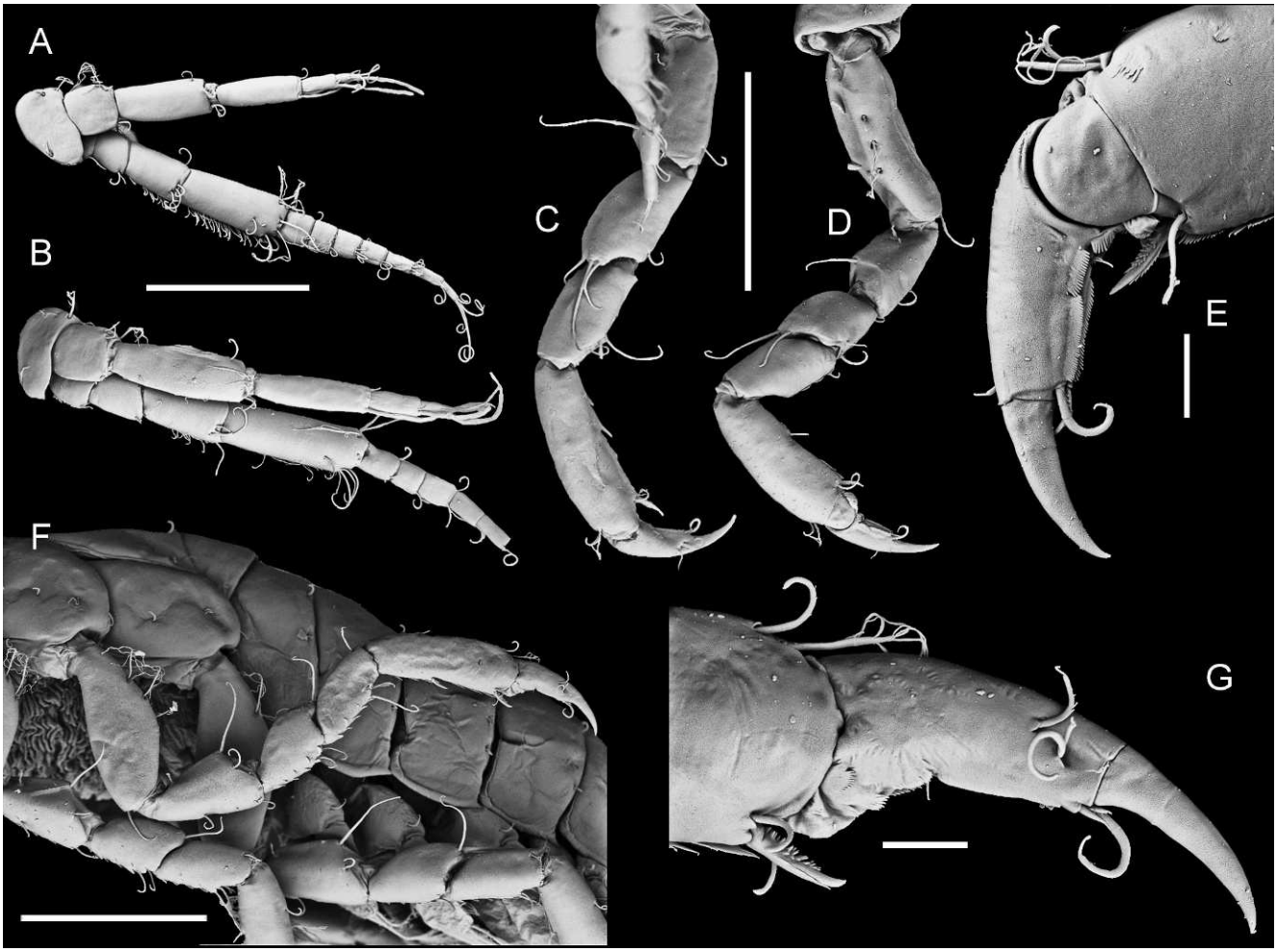

Fig. 4. Antennula, antenna, and pereiopods, *Gnathia aureamaculosa*. A, D-G, AM P.81394 (Z2). B-C, AM P.81396 (Z3). A-B, antennula and antenna. C-D, pereiopod III. E, dactylus, pereiopod II. F, pereiopod V on posterior body. G, dactylus, pereiopod V. Scale bars: A-B, C-D, F, 100 μm. E,G, 10 μm.

In ventral view, pereionites 1, 5 through 7 do not have well-defined sternites, but consist of the expansion cuticle. A detailed image (Fig. 5A) of pereionite 5 shows that the expansion cuticle has many transverse folds, but also has axial folds at a much finer scale. Together, these microfolds allow the gnathiid's impressive ability to engorge blood and increase in length and width.

Pleonites (Fig. 1).—In dorsal view, pleonite 1 has a strongly concave margin with pereionite 7, but has a relatively linear posterior margin. The remaining pleonites are approximately subequal in length and with approximately sublinear margins. Pleonite 5 is no longer dorsally than the more anterior pleonites. The pleurae (the ventrally projecting part of the pleonite tergites) have smoothly concave ventral margins, which are thickened and rounded in cross section, with a small angular posterior spine.

Pleotelson (Fig. 1E, H-I; 6G).—The terminal somite, as in the more anterior dorsal surfaces, is smooth without any spines or ridges. The distal tip is indented between a pair of short triangular spines. Although the general triangular form of the pleotelson is typical for the family, the length to width ratio is consistent within this species: the median ratio from 3 specimens is 0.99 (Z2 $n = 2$, Z3 $n = 1$). Each specimen also had consistent positions for pairs of setae: the lateral margin, adjacent to the uropods, centrally (elongate and straight), posterior margin in the convex part, and the distal tip between two small spines. These setal positions are retained in most instances in the adult male (image not shown), although the setal sizes may be different.

The pleotelson has landmarks, which are present in other species, that should be noted. When viewed in dorsal view, the pleotelson is nearly as wide as pleonite 5, with the lateral angle positioned nearly to the lateral margin. The tapering margin of the pleotelson has a weak concavity where the uropodal protopod extends under the pleotelson, and then a convex inflection just posterior to the uropods, followed by a convexity before the margin terminates at two small posterior spines. This last shape causes the pleotelson to appear posteriorly attenuated.

Antennula and Antenna (Figs. 1-4).—The bean-shaped antennular first article is dorsally flat with two posterolateral and one anteromedial penicillate seta. The second article, which is approximately as long as wide, is also flattened on its dorsal surface and has two penicillate setae

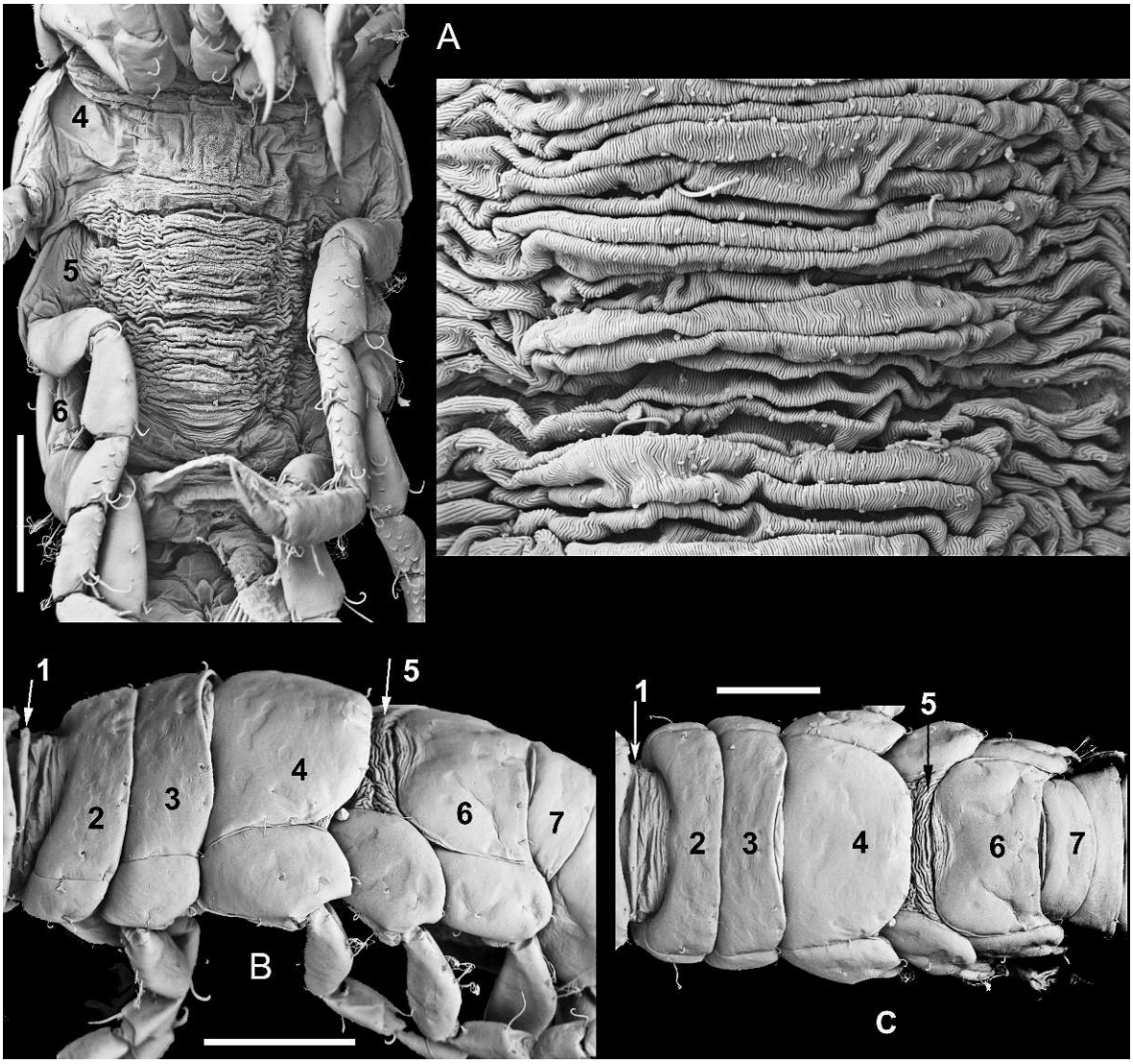

Fig. 5. Pereionites, showing expansion cuticle on segment 5, *Gnathia aureamaculosa*, AM P.81394 (Z2). A, ventral view (left), with enlargement of pereionite 5 (right); numbers indicate pereional somites. B, dorsolateral, showing coxae in plan view. C, dorsal. Scale bars 100 µm.

posteriorly on distal margin. Article 4 is extremely short, which is probably an accentuated plesiomorphic state in the isopods; this article is distinctly shorter than articles 3 or 5 as in most other orders (Wägele, 1983). The 3 articles distal to the fourth article decrease in width distally and have an aesthetasc each. Presumedly the "peduncle," a deprecated term (Wilson, 2009), corresponds to the first three articles.

The antennal basal article is not expressed as in many other isopods, so that the antenna has only 5 podomeres. The basal first article is shorter than the others, and is hidden under the basal article of the antennula; it is only visible under pereiopod I (Fig. 3A, C). Articles 3-5 have a ventromedially-directed fringe of setae and penicillate setae on the ventral part of their distal margins (Fig. 3A-B). Articles 4 and 5 have several groups of simple setae on the distoventral margin. The antennal flagellum (Fig. 4A-B) has 7 articles, each with a dorsomedial group of 2-4 curled setae, except for the terminal article which has 5-6 curled setae of differing lengths.

Mouthparts (Figs. 2, 3).—The dorsal surface of the clypeus is smooth, devoid of setae or cuticular ornamentation. It has a posterior indentation to accommodate the basal article of the antennula, and tapers anteriorly to the projecting mandibles. The clypeus has paired thin membranous flaps that surround the mandibles; these possibly are the remnants of the labrum.

Ferreira et al. (2009) found that the P3 mandible had 9 distomedial teeth, although only 5-6 are visible on a Z2 specimen (Fig. 2D), while in an enlargement of another specimen (Fig. 2B) one can see the full complement of 9. A Z3 specimen (AM P.81103) has 9 teeth as well, and another Z3 specimen (AM P.81396; not shown) has a tiny additional tooth distally, or 10 teeth total. A central lobe of the paragnaths projects between the mandibles (which obscures some mandibular distomedial teeth in Fig. 2D). The maxillulae and maxillipeds are visible below the mandibles in the SEM specimens. As indicated in Ferreira et al. (2009), the maxillulae have 5 teeth on the distomedial

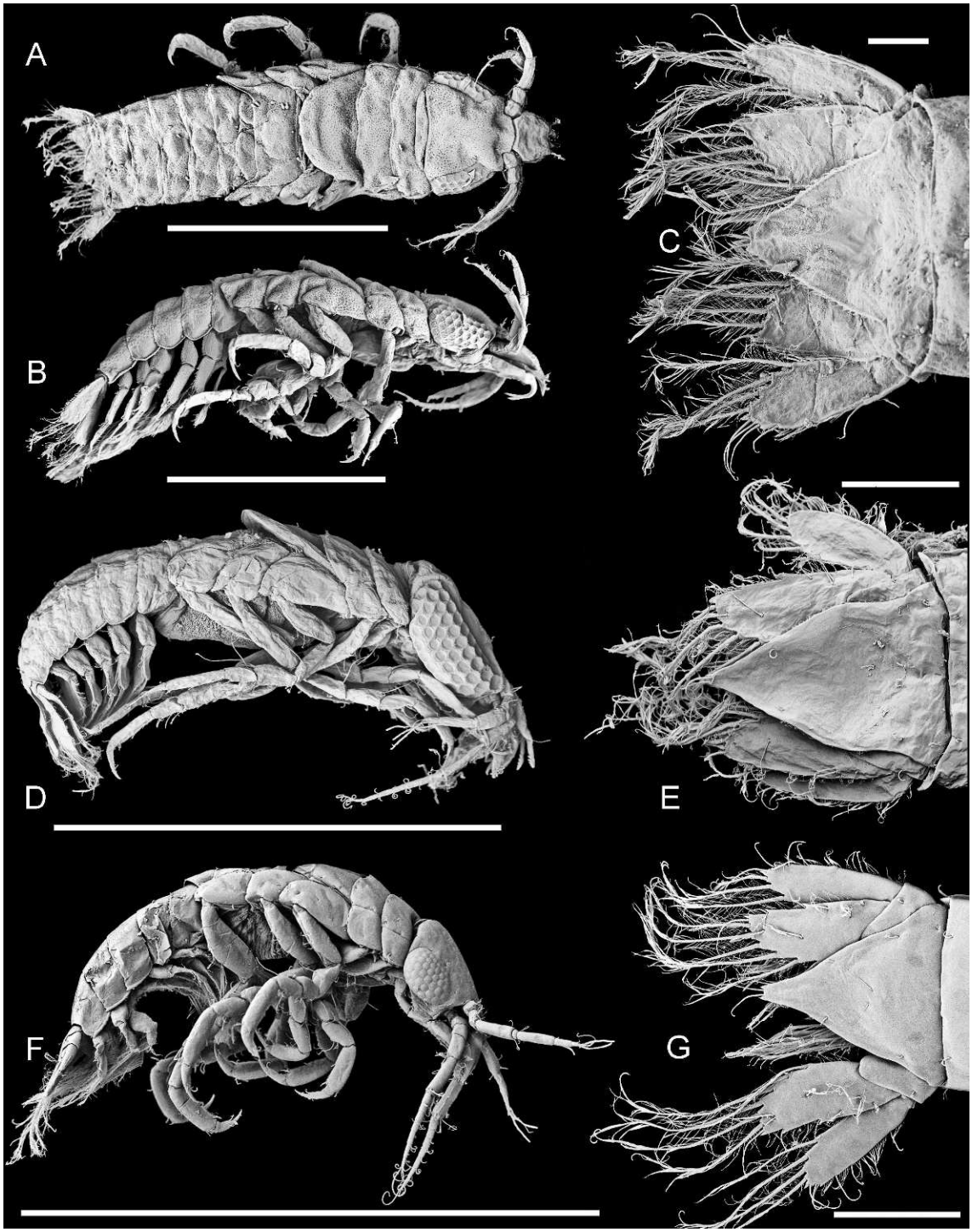

Fig. 6. Zuphea instar 3 of three species of *Gnathia* from Lizard Island. A-C, undescribed species, AM P.81399. D-E, *G. falcipenis*, AM P.81398. F-G, *G. aureamaculosa*, AM P.81103. A, dorsal view. B, D, F, lateral views. C, E, G, pleotelson and uropods, dorsal view. Scale bars: A-B, D, F, 1 mm; C, E, G, 100 μm.

margins. These mouthparts exhibited some curling from the drying process that made them visible; they might be obscured by the mandibles in a fresh specimen.

In ventral view (Fig. 3), the maxilliped is an elongate limb with a distally inserted palp; the endite was not observed in situ on these specimens. The palp consisted of 4 articles (Ferreira et al. (2009) reported only 3), although the articulations of the basal article and the distal article are only partially expressed. The basal article is probably homologous with the first article of a plesiomorphic 5-articled maxilliped. The basal two articles each have elongate medial projections that are distally pointed and have medial paired fringes of submicroscopic setules. Setae occur on the distal articles 2 and 3, including a single pair

of large curled setae on the ventromedial side of the second article. The distal article of the maxilliped (Fig. 3C-D) is attenuated with a thicker basal part having a few curved setae. The narrow curved terminal part seems to form an opposing claw against the thin distal projection of third article. This terminal article probably is made of 2 articles with an unexpressed articulation at the base of the curved claw-like section, bringing the total article count in line with other isopods. The maxillipedal basis has 4 posteriorly-facing hemicircular scales, similar to those seen on the more posterior thoracic limbs. A fifth basal scale, that was more weakly defined, was seen on some specimens. The number of these scales did not vary between Z2 and Z3 stages. Additionally, the length of the maxilliped did not vary much with body length (ranging 0.14-0.17 mm, $n = 3$). This is in accord with other non-gnathiid taxa where the head is proportionally larger in juvenile stages (Wilson, 1982).

Pereiopods (Figs. 1, 3-4, 6).—Monod (1926:147) reported that coxae I, and coxae III-V of the Paragnathia *formica* praniza were ''entièrement soudé'' [entirely fused], although our SEM images of *G. aureamaculosa* shows that only coxa I and VII were not seen, the former being not expressed on the ventral surface of the sternite and the latter owing to the absence of the last pereiopod. The other coxae were well developed and formed the lateral margin of the dorsal surface.

Pereiopod I (Fig. 3), often referred to in the literature as ''gnathopod,'' is closely adpressed to the ventral surface of the head and maxillipeds. The body articulations for the coxae are not expressed, and appear only as stiffer areas adjacent to the highly flexible sternal cuticle. The basis is unadorned with scales, but has a penicillate seta on its anterior margin. The ischium has 5 hemicircular scales. Four scales can be found on the merus, although one is weakly expressed and sits laterally to a larger distal scale. The triangular (in lateral view) carpus has 3 hemicircular scales. The propodus is strongly built and lacks scales or an articulation with the articular plate. The dactylus has a smooth sharp, strongly curved claw that is typical for most gnathiids. The junction between the dactylus and the claw is visible, and the ventral margin of the dactylar cuticle appears somewhat flexible. All segments except for the basis and dactylus have a distomedial seta.

Pereiopods II-VI are all approximately similar (pers II, III, V shown in Fig. 4), each having a smooth cuticle except for posteriorly-directed ventromedial scales on the ischium to propodus. The dorsal side of each basis has a group of around 3 penicillate setae. The dactyli have several proximal cuticular ridges and scales (Fig. 4G) with an adjacent denticulate seta on the propodus; these are likely to be visible only under high magnifications.

Pleopods (Fig. 1B).—The pleopods are similar, comprising protopods and two distally setose rami (endopod and exopod). The protopods have medial projections furnished with two distally setulate setae and a distal group of fine setae on the medial margin. Each exopod broadens distally to an angular inflection with the shallowly curving distal margin; this margin has 8-9 elongate plumose setae.

Uropods (Fig. 1E, H, I; 6G).—The length of the uropods, measured from the angular anterior margin of the protopod to the distal tip of the endopod (spines included), when compared to the length of the pleotelson, shows an allometric shift. The Z2 uropod length is 95% the length of the pleotelson, whereas the Z3 uropod is 108%. Conversely, the longest plumose setae on the uropod margins are longer in the Z2 (115% of the endopod length), compared to the Z3 (approximately subequal to endopod length).

The uropods also have features that are constant between both instars. The basal segment of the uropods (protopod) is approximately triangular, although the dorsomedial margin has a strong convex inflection. The dorsolateral surface of the protopod has a small simple seta.

The exopod has a convex lateral margin and a curved spine at the distal tip, and a smaller spine adjacent to several setae subdistally. The distolateral margin of the exopod has 5 elongate simple setae and a fringe of smaller setae emerging ventrally to the margin. The medial margin of the exopod has 4 elongate plumose setae medial to the terminal spine.

The lateral margin of the endopod is weakly convex – almost straight – and has 3 sharp spines associated with 3 elongate setae. The margin also has a ventrally positioned fringe of fine setae. The endopod medial margin has elongate 6 plumose setae that decrease in length medially. The exopod dorsal surface has 5 positions with penicillate setae that are present in both instars; the distal position and the second from the proximal group have 2 setae, and the remaining positions each have only a single seta.

Discussion

Colour Patterns

Monod (1926) reviewed published observations of colour patterns, and commented ''La plupart des auteurs qui ont étudié les Gnathiidae sur le vivant ont signalé l'extraordinaire variabilité et la beauté de leur coloris''[Most authors who studied the living Gnathiidae reported extraordinary variability and the beauty of their colour.]. Although most authors prior to Monod had found distinctive patterns, often multiple species and stages were included in a single collection or category. Monod himself (1926) listed numerous different colour patterns observed from juveniles collected from benthic or host samples, but did not assign them to species. Because colour may provide a simple method for sorting live animals in field samples (Smit and Davies, 2004), we briefly review species-specific colour patterns in live juveniles described in recent publications. These colours are independent of the different shades of the internal organs that depend on whether the juveniles had fed or not. *Gnathia aureamaculosa* P3 have a sexually differential pigmentation: P3 females have greenish-yellow spots over pereion, while P3 males have white blotches and light brown and yellow spots on pereion (Ferreira et al., 2009). *Gnathia falcipenis* has paired red spots on head, and mottled red or orange patterns on body. *Gnathia grandilaris* has a black pereion with distinctive white markings and the dorso-lateral surface of the pleon has light brown

pigmentation patterns with brownish-yellow pigmentation on lateral surfaces of uropodal exopod. *Gnathia maculosa* Ota and Hirose, 2009a has a dark red or black pereion with white speckles or dapples. *Gnathia nublia* Ota and Hirose, 2009b has a pereion dorsal surface marked with a black and white cloud-like pattern and the ventral pereion is marked with a distinct white line, with distinct brown spots on eyes and pleonites with a brown pattern on their lateral margins. *Gnathia limicola* Ota, Tanaka and Hirose, 2007 has a pereion that is yellow to dark red (orange in alcohol), and other parts are light brown. *Gnathia pilosus* Hadfield et al., 2008 zupheae have a dark brown pereion with randomly distributed speckles, with a light brown pleon and head. *Gnathia trimaculata* as described in Coetzee et al. (2009, fig. 11) exhibits a yellow-greenish colour, with 3 black lines extending dorsally across pereionites 5-6 and 3 pairs of distinctive black spots within yellow circles on lateral sides of pereionites 4, 5 and 6, whereas the dorsal surface between the eyes has a light brown pigmentation. Given distinctive colour differences between juveniles of these species, obtaining colour data or photographs from freshly captured specimens could add considerably to our ability to identify gnathiid juveniles in the field.

Body Length

Gnathiid juveniles have an extraordinary ability to substantially increase their body volume during feeding using the expansion cuticle of pereionite 5 (Fig. 5). In this study, the Z2 specimens prepared for SEM had mean lengths of 0.95 mm ($n = 3$) and Z3 were 1.24 mm ($n = 2$), which are in accord with much more extensive measurements (measured from the head to pleonite 5, omitting the pleotelson) of Grutter (2003): Z2 and Z3 specimens had lengths of 0.84 mm ($n = 210$) and 1.16 mm ($n = 23$), respectively. These mean lengths, adjusted to include the pleotelson length (approximately 33% of total length in this species) are Z2 = 1.12 mm and Z3 = 1.54 mm. Our measurements are smaller than those observed by Grutter (2003) owing to shrinkage during critical point drying. Preserved zupheal specimens sometimes were partially expanded at pereionite 5, resulting in an increased length. Total body length measurements, while generally indicative of size, should be used with caution. Different preservation methods (formalin vs. ethanol) used on samples of the same species might yield differing lengths on zupheae of the same juvenile instar. Our results suggest that measurements from single somites, such as the head or pleotelson might provide more consistent results (Grutter, 1997a; Smit and Davies, 2004). Grutter (2003) was able to obtain a statistically significant regression using logarithms of body length and gut volume of pranizae where the three instars clustered and were thus identifiable. The body length was confirmed using the head width of known stages (Grutter, 1997a). The praniza stages were expanded to nearly their full length, so the variance was much less. Grutter (2003) found that the recently fed P2 and P3 stages had mean lengths of 1.38 mm and 2.17 mm ($n = 1087, 260$, respectively). Compared to the unfed juvenile figures above, this is an impressive increase in body length of 23% and 41%. Similarly, Grutter (2003) reported that on average, P3 juveniles had between 9 to 20 times larger gut volumes than earlier instars. The last instar has the largest increase, possibly because this is the last meal before moulting to the non-feeding adult.

Head

Monod (1926) commented that the form of the cephalon is nearly the same in all known pranizae at the time. Most descriptions of the head describe shapes that are common for most species, such as the presence of sensory pits (Fig. 2C), or that the eyes are bulbous (but compare eyes in Fig. 6), which would be generally true for shallow water species. Some deep-water gnathiids are blind or have substantially reduced eyes (Cohen and Poore, 1994). The external anatomical features in each species, however, differ considerably in size and composition. The most prominently different features are the clypeus and the eyes.

The clypeus, which has been labelled erroneously ''labrum'' in some publications, has potentially useful variation compared to the head length. Praniza of various species have reported labrum:head length ratios ranging from 0.25 (*G. trimaculata*, Coetzee et al., 2009; *G. aureamaculosa,* Ferreira et al., 2009) to around 2.5 (*G. grandilaris*, Coetzee et al., 2008). These reported lengths appear to be inaccurate because the published images don't show these differences in sizes. For *G. aureamaculosa*, the clypeus to head length ratio is 0.98-1.01 ($n = 3$), where each measurement is taken in approximately plan view (surface perpendicular to the viewing direction). Because the clypeus curves ventrally from the head dorsal surface, two different positions are needed to obtain a ratio of the two measurements. If both measurements are taken when the head is in one position, the clypeus may appear to be foreshortened; e.g., in Fig. 1A, the ratio is as little as 0.19. As a consequence, the clypeus should be measured as nearly in plan view as is possible.

The eyes show useful variation between species (Fig. 6), the size of which can be directly related to the habits of each species. Nagel (2009) found that *G. falcipenis* has visual adaptations for foraging at low light levels, which is related to its nocturnal habits, whereas *G. aureamaculosa* is diurnal. The three species surveyed here have approximately the same number of ocelli (45-48), but of different sizes. The eyes of the undescribed species and *G. falcipenis* (Fig. 6A-B, D) extend nearly the entire lateral length of the head, whereas the eye of *G. aureamaculosa* has a distinctly free anterolateral margin of the head. The lateral shape of the eye is evenly oval in the latter species while the former two species have linear dorsal margins. The dorsal shape of the eye also shows easily distinguished differences: the undescribed species with a distinct anterior angle (Fig. 6A), *G. falcipenis* somewhat linear (not shown) and *G. aureamaculosa* smoothly curving (Fig. 2A, E). These simple differences should be visible in a dissecting microscope.

Monod illustrated a ridge on the head ventral surface of the adult male of *P. formica* that he termed the ''carène marginale'' or marginal carina. This structure can be seen in the zuphea of *G. aureamaculosa* (Fig. 3C *cm*), where it forms a lateral support for the first pereiopod. Although Monod (1926) provided a detailed description of the

internal structures of the head, he didn't mention the external ridge. This carina differs in size between species. In *G. aureamaculosa*, the marginal carina is nearly indistinguishable, and might not be seen in light microscopes. Although this feature is not described in most species, we did observe that this ridge distinctly extends over the basis of the undescribed species (Fig. 3E).

The mandibles have 9 or 10 distomedial teeth in Z2 and Z3 stages, so this feature, which might be visible in a compound microscope, could be useful for morphometrics or identification. The other head limbs, paragnaths, maxillulae and the maxilliped are likely to be difficult to employ on a regular basis for identification owing to the small size and requirement of dissection. The maxillipeds do differ between the taxa; compare for example Fig. 3C and 3E), where *G. aureamaculosa* has well defined hemicircular scales, *G.* sp. does not. The variable quality of illustrations of these limbs in the literature, wherein some coarse illustrations of the maxilliped are difficult to interpret, will require that they are reillustrated with more detail for some of the described species.

Pleotelson and Uropods

The terminal segment of the body and associated limbs are possible morphological links between the juveniles and adults (Smit and Davies, 2004). Between the two zuphea stages these features were nearly identical, except for allometric changes in the length of the uropods and length of the marginal plumose setae. Comparison with the other two species (Fig. 6C, E, G) shows many points of difference, so the pleotelson and uropods should figure importantly in developing a taxonomic system for the zupheas. The uropods have distinctly different configurations of the rami and lengths, and the pleotelsons are distinctly different once the landmarks identified for *G. aureamaculosa* are compared with the other species. The setation of the two teleost parasites is decidedly different from that of the undescribed species, which may have an elasmobranch host (Coetzee, 2006).

Morphometrics

The pleotelson length to width ratio for *G. aureamaculosa* Z2 and Z3 stages is nearly identical (median l/w = 0.99, range 0.95-1.03; Fig. 1E, H-I, 6G), but it differs substantially from the other two species: 0.72 for *G.* sp. (Fig. 6C) and 1.3 for *G. falcipenis* (Fig. 6E). In making these measurements, achieving a consistent plan view is essential. Assuming that ontogenetic consistency applies to these other two species, this simple ratio, in combination with other measurements, may assist distinguishing species. Other potentially useful ratios include: head length/width, eye length/head length, labrum length/width, antennula length and antenna length compared to each other and to the head length, uropodal exopod and endopod lengths compared to each other and to pleotelson length. These measurements, combined with data on setation, spination, and shape of particular parts, should provide effective species identification without resorting to time-consuming molecular or culture techniques. Data from these ratios should be useful regardless of whether the juvenile has fed or not, i.e., whether zuphea or praniza, because they do not involve the central expandable region of the body. If ontogenetic consistency proves to be the rule for most species, then gnathiid species should be identifiable regardless of which instar is being observed.

Abbreviated Developmental Sequence

Gnathiidae are unique among isopods because they have only 3 postmarsupial instars before adulthood. Most other isopods have 3 free-living manca stages and several juvenile stages before reaching adulthood (Asellota: Elizalde and Sorbe, 1993; Hessler, 1970; Wilson, 1981; Phreatoicidea: Wilson and Ho, 1996). Although gnathiid juveniles lack pereiopod VII, as in a manca stage, this limb is suppressed in all gnathiid instars, including the adults. The heterochronic absence of pereiopod VII is well known in other isopods (Kavanagh et al., 2006; Poore, 1984; Wilson, 1976, 1989). Unlike early manca instars of other isopods, the free-living juvenile gnathiids have a fully developed pereionite 7 (Fig. 5B-C). Monod (1926) made detailed observations on the embryonic and marsupial development of *P. formica*, and identified three different stages. Stage I in Monod's system is equivalent to the embryonic development of other isopods (cf. Wolff, 2009; Milatovic et al., 2010). Monod's stage II appears to be fully developed marsupial embryos, equivalent to the last marsupial instar or first free living manca stage in other other isopods (Wolff, 2009; Milatovic et al., 2010). Monod's stage III embryos are fully developed, with setae and functional mouthparts. The latter part of stage II and all of stage III appear to be equivalent to the missing manca instars. The 3 free-living juvenile stages of the Gnathiidae are therefore equivalent to instars 4-6 in other isopods.

The term ''larva'' is often applied to free-living gnathiid juveniles (Giannetto et al., 2003; Grutter and Poulin, 1998; Marino et al., 2004) and indeed Monod (1926) used this term extensively, although he recognised primary (marsupial) and secondary (free-living parasitic) larvae. In Monod's view (ibid, p. 238), the term ''larva'' could be used in a broad meaning (sensu lato) despite that these crustaceans lack the holometabolous insect cocoon or chrysalis resting stage. He also regarded the gnathiid transformation as ''true hypermetamorphosis,'' similar to some insects and monstrilloid copepods. We have not used ''larva'' here to promote clarity on the developmental sequence of instars in gnathiids, although we find no reason to deprecate this term in general usage.

Phylogenetic Significance

The morphology of the zupheae that we have described here clarifies ongoing discussions about the relationships of gnathiids to other isopods. Although recent studies (Cohen and Poore, 1994; Brandt and Poore, 2003) have continued to assert a close relationship between *Protognathia bathypelagica* (Schultz, 1977) and Gnathiidae, these taxa do not share apomorphic features originally claimed by Wägele and Brandt (1988; see Wilson, 1996). *Protognathia* is a typical cymothoidean and could be more accurately placed among the Cirolanidae as originally done by Schultz

(1977). Several fundamental features distinguish gnathiids from cymothoideans. Although the body tagmosis of Gnathiidae agrees with the other members of Cymothoida (3 limbs anteriorly directed, remaining limbs directed posteriorly), the anterior pereiopods II-III are walking legs that are morphologically similar to the posterior limbs (Fig. 1), and are clearly not hook-like, raptorial limbs as seen in most other Cymothoida. The gnathiids lack the cymothoidean frontal lamina, and have an anteriorly projecting clypeus, which is unlike the cymothoidean posteroventrally projecting form. The antennulae and antennae emerge anterolaterally from the head, whereas in the typical cymothoid pattern these limbs emerge anteriorly and then extend laterally from a geniculate articulation on the basal podomeres. Altogether, these features suggest a lack of relationship between the gnathiids and cymothoideans. These observations are reflected in a total evidence phylogeny using 18S rDNA and morphological data (Wilson, 2009) that casts doubt on the unity of Cymothoida as currently defined (Wägele, 1989; Brandt and Poore, 2003). That analysis, however, did find a close relationship between Gnathiidae and Cymothoidae, although the data set included only a single gnathiid, the well-studied *P. formica*. Returning to the use of the suborder name Gnathiidea may be a useful option for the classification of this family, but more evidence is needed, particularly gathering more sequences from diverse taxa in the family, and expanding the morphological matrix to include the data illustrated in this work.

Clearly, much remains to be discovered regarding the diversity and evolution of Gnathiidae. Our hope is that further taxonomic works on this group will provide detailed data on the zuphea juveniles as well as the adults.

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