A NEW SPECIES OF COPEPODA (THAUMATOPSYLLIDAE) SYMBIOTIC WITH A BRITTLE STAR FROM CALIFORNIA, U.S.A., AND DESIGNATION OF A NEW ORDER THAUMATOPSYLLOIDA

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A B S T R A C T

A new species of thaumatopsyllid copepod, *Caribeopsyllus amphiodiae*, is described based on adult specimens reared from nauplii inhabiting the stomach of a burrowing, amphiurid brittle star, *Amphiodia urtica* (Lütken), collected in California, U.S.A. It is the first member of the family to be reported from the eastern Pacific Ocean. *Caribeopsyllus chawayi*, its only known congener, inhabits the Caribbean Sea. All other thaumatopsyllid species occur in the eastern hemisphere. *Caribeopsyllus amphiodiae* is only the second thaumatopsyllid of which both sexes and sexual dimorphism have been described and for which a host species has been identified. *Caribeopsyllus* differs from confamilial genera by the presence of a 1-segmented leg 4 exopod. The new species is distinguished from *C. chawayi* by its general habitus, and by the absence of spiniform processes at the base of each exopodal spine of leg 1 and the first three exopodal spines of leg 2. Analysis of the phylogenetic relationships of Thaumatopsyllidae shows that it is not a member of any of the other previously established orders of Copepoda. Accordingly, a new order, Thaumatopsylloida, is proposed to accommodate the five species of thaumatopsyllids thus far reported and is shown to be a member of a Thaumatopsylloida-Monstrilloida-Siphonostomatoida clade.

Thaumatopsyllus paradoxus Sars, 1913, was the first species of Thaumatopsyllidae to be discovered. Sars (1921: 6) noted that the adult lacks antennae and oral appendages and therefore surmised that the enigmatic juvenile "leads a true parasitic existence within the body of some invertebrate animals." He evidently assumed that it has a life cycle like the monstrilloid copepods to which he allied the species, and which also lack mouthparts as adults. Later studies showed that the metanauplius of *T. paradoxus* lives in the stomach of several ophiuroid species, and revealed that it does not noticeably damage the host and has a life history very different from that of the monstrilloids (Bresciani and Lützen, 1962; Fosshagen, 1970). The symbiotic, benthic metanauplius of *T. paradoxus* was found to develop into a non-feeding, free-swimming copepodite. Advanced larval stages give rise to sexually dimorphic adults that swim near the benthos. At locales in several Norwegian and Swedish fjords up to 50% of the host populations were infested with *T. paradoxus* naupliar stages (Bresciani and Lützen, 1962; Fosshagen, 1970). As the hosts are com-mon and widespread species, the parasite is presumably abundant as well, although, curiously, exceedingly few adults of *T. paradoxus* have ever been gathered from the plankton.

Following the discovery of *T. paradoxus*, three additional thaumatopsyllid species and as many monospecific genera were described, each on the basis of only a few female individuals collected from the plankton. They are *Orientopsyllus investigatoris* Sewell, 1949, based on two female specimens from the Nicobar Islands, Indian Ocean; *Australopsyllus fallax* McKinnon, 1994, based on nine females from southern Australia; and *Caribeopsyllus chawayi* Suárez-Morales and Castellanos, 1998, based on one female from the Caribbean coast of Mexico. Thus, among the known thaumatopsyllids, both sexes have only been described for *T. paradoxus*, and although the family has been characterized as parasitic on ophiuroids (Huys and Boxshall, 1991; Suárez-Morales and Castellanos, 1998), *T. paradoxus* was the only species with known hosts and illustrated larvae.

In the present paper, a new species of Thaumatopsyllidae is described and discussed,

based on the adult female and male individuals reared from nauplii associated with *Amphiodia urtica* (Lütken), a small, burrowing ophiuroid, which inhabits the Pacific Coast from Mexico to Alaska (Hendler, 1996). The information on thaumatopsyllid morphology that we compiled enabled us to appraise the ordinal placement of the family, which has been problematical since its debut in the scientific literature. We provide an analysis of the position of the Thaumatopsyllidae, directing particular attention to its relationship to three orders of Copepoda, the Monstrilloida, Siphonostomatoida, and Cyclopoida, with which it has been suggested to have its closest affinities. Only the systematics of the adult stage of the new species is treated herein; the developmental stages and other aspects of its biology will be dealt with elsewhere.

It should be noted that Wilson (1924) suggested replacing the name *Thaumatopsyllus* with *Thespesiopsyllus*, deeming the former name to be preoccupied. The change was upheld by Davis (1949), Sewell (1949), Bresciani and Lützen (1962), Fosshagen (1970), and Huys and Boxshall (1991). However, Bowman and Abele (1982) argued, based on the International Code of Zoological Nomenclature, that *Thaumatopsyllus* was available for use by Sars, even though it previously had been published as a synonym of the harpacticoid genus *Aegisthus*. Therefore, they reinstated Sars' (1913) generic and familial names. McKinnon (1994) and Suárez-Morales and Castellanos (1998) adopted Bowman and Abele's (1982) opinion, with which we too concur.

Materials and Methods

Individuals of *Amphiodia urtica* were collected at a depth of 60 m, at 33°59′33″N, 118°36′11″W, in Santa Monica Bay, California, which is a sampling station routinely studied by the Environmental Monitoring Division, Bureau of Sanitation of the City of Los Angeles. Sediment collected with a modified Van Veen grab at the site was washed on a 1.0 mm mesh sieve. Ophiuroids retained on the screen were placed in containers of cooled sea water, and held on ice in an insulated chest during transport to the laboratory.

The ophiuroids were examined for the presence of parasites by inducing autotomy of each animal's disk. After the disk detached from the oral frame and arms, it was inverted in a dish of sea water, and the stomach was examined through the oral opening using a stereomicroscope. Copepod nauplii were collected from the stomach using a glass Pasteur pipette and transferred to 90×50 mm Pyrex® (Trademark of Corning, Inc.) crystallizing dishes of sea water, afterwards covered with Saran Wrap[TM] (Dow Chemical Co.) and placed in a 15°C water bath, which approximated the ambient temperature of the natural habitat. They were maintained in a 16:8 h light : dark cycle. The

nauplii were examined daily, and all exuviae were collected. Groups of individuals that successfully transformed into adults (copepodid VI) were pooled in crystallizing dishes and maintained for 1–2 weeks to permit them to mate.

Specimens were preserved in 70% ethanol, and those to be drawn were transferred to lactic acid and cleared for at least 24 h, measured with an ocular micrometer, and dissected on wooden slides (Humes and Gooding, 1964). Illustrations were made with the aid of a drawing tube attached to either a Nikon Optiphot HFX-1 or an Olympus BH-SW compound microscope. Type specimens have all been deposited in the Natural History Museum of Los Angeles County (LACM).

The terminology used for anatomical features follows that employed by Huys and Boxshall (1991), in the most rigorous analysis of copepod taxonomic characters to date. The 54 characters used in their cladistic analysis of the copepod orders were adopted for the present study of the phylogenetic relationships of the Thaumatopsyllidae. The character matrix used herein (Table 2), is adapted from Huys and Boxshall (1991: 394, Table 13), with the addition of Thaumatopsyllidae. The use of ordered, multistate characters in the matrix implies a linear sequence between character states, wherein the most derived state (= 2) is presumed to have evolved from the ancestral state (= 0) via the intermediate state (= 1). The symbol "?" signifies that a character is absent, or could not be accurately scored.

The cladistic analysis was performed with the computer program PAUP*, Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0b6 (Swofford, 2000). Based on the results of Huys and Boxshall (1991), our analysis used the order Platycopioida as the outgroup, thereby polarizing the data and rooting the cladogram with that taxon. The unordered analysis resulted in four very similar, equally parsimonious phylograms, which are cladograms that convey the degree of anagenetic change using varying branch lengths.

Results
Caribeopsyllus amphiodiae, new species
Figs. 1–4.

Material Examined.—39 (27 ♀♀ and 12 ♂♂) free-living adults reared from nauplii found in stomachs of *Amphiodia urtica* (Lütken) collected from February to July 1992 in Santa Monica Bay, California. Holotype ♀ (LACM CC 1992-196.1), allotype ♂ (LACM CC 1992-196.2), and 20 paratypes (10 ♀♀ and 10 ♂♂) (LACM CC 1992-196.3) deposited in the Natural History Museum of Los Angeles County, Los Angeles, California.

Female.—Total body length 1.64 (1.51–1.89) mm and width 0.51 (0.49–0.55) mm based on 10 specimens. Prosome oval and urosome slender (Fig. 1A). Cephalothorax of recently preserved specimen with large red pigmented area in anteriormost region, bearing 2 dorsolateral conspicillae (large refractile lenses; shown in Fig. 1B, but not in Fig. 1A) and 1 median ventral conspicilla. Rostrum broad, rounded. Urosome distinctly 4-segmented, long and
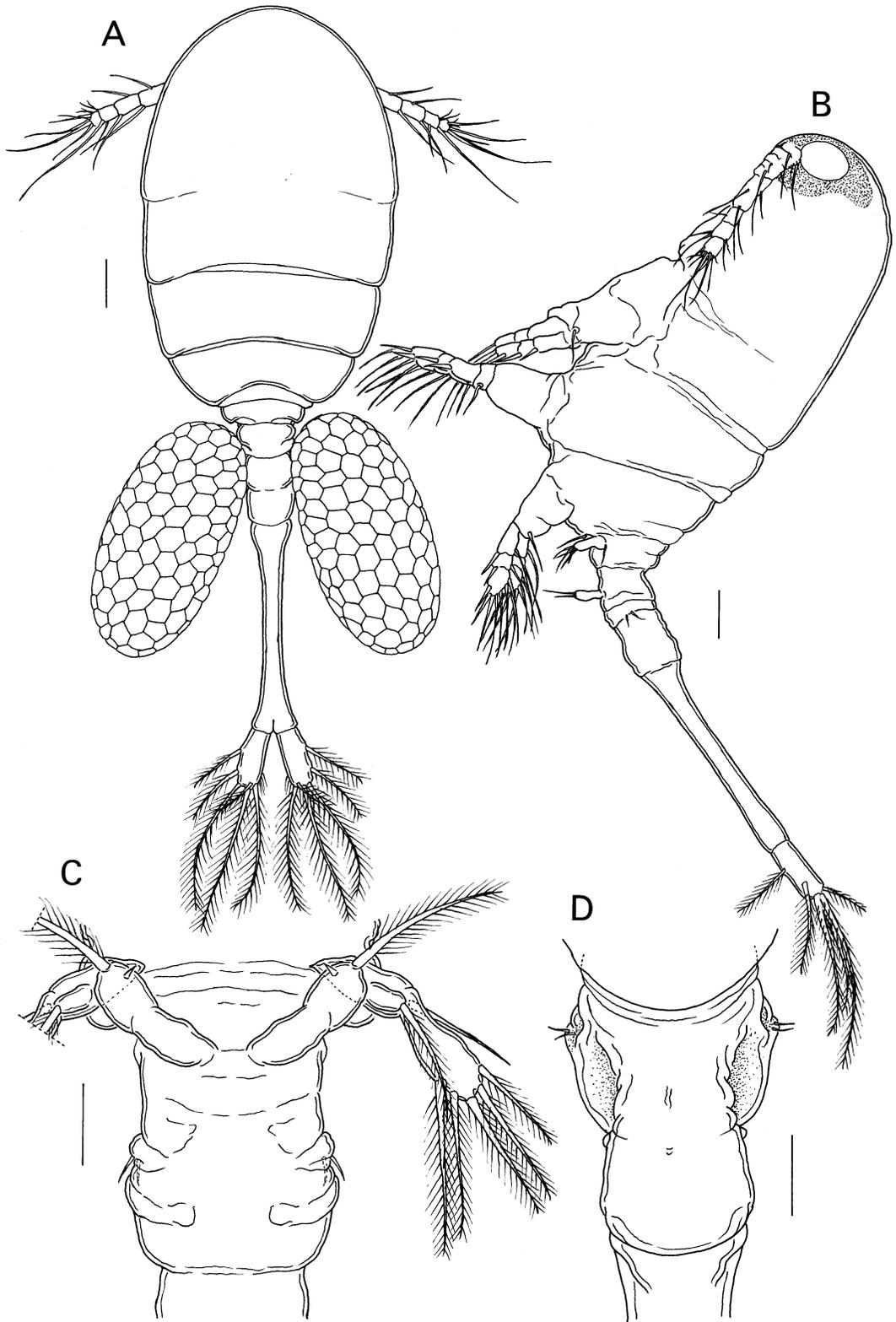
slender. Fourth pediger (Fig. 1A) much smaller than third pediger, 3.15 times broader than long. Fifth pediger (Fig. 1C) narrower than fourth pediger, about 1.64 times broader than long. Genital double somite (Fig. 1C, D) broader in anterior region, about 1.5 times broader than long and carrying 2 small setae representing leg 6 in area of egg sac attachment. Multiseriate egg sacs with eggs measuring 0.05 mm in diameter. Anal somite (Fig. 1A) long and slender, at least 4.8 times longer than broad, with concave lateral borders. Caudal ramus (Fig. 2B) 2.85 times longer than wide, bearing 6 pinnate setae and inner distal rows of setules. Digestive tract and anus appear to be absent; however, vestigial mouth and transverse cuticular structure, possibly labrum, present.

Antennule (Fig. 2A) 6-segmented, with armature formula 6, 2, 4, 2, 4, and 7 plus one aesthetasc; setae sparsely pinnate or naked. Antenna and oral appendages absent.

Legs 1–4 (Fig. 2C, D; Fig. 3A, B) biramous, with 3-segmented rami, except for leg 4 with 1-segmented exopod and endopod. 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. 2D) and first to third exopodal segments of leg 3 (Fig. 3A) 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. 2C, D) with spiniform processes next to outermost setae; spiniform process next to outermost seta on terminal segment of leg 3 endopod (Fig. 3A). Intercoxal plate well developed in legs 1 (Fig. 2C), 2 (not shown in Fig. 2D), and 3 (Fig. 3A), but missing in leg 4 (Figs. 1C, 3B). Leg 5 (Fig. 1C) with 2 members of pair situated closely on ventromedial surface of somite; each member elongate and broad at base (Fig. 3C), about 1.8 times longer than broad, bearing 1 small naked and 1 large pinnate terminal setae.

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Fig. 1. *Caribeopsyllus amphiodiae*, new genus, new species. Ovigerous paratype female. A, habitus of ethanol-preserved specimen, dorsal; B, habitus of fresh specimen, lateral; C, pedigers 4 and 5, and anterior part of genital double somite, ventral; D, genital double somite, dorsal. Scale bars: 0.1 mm in A, B; 0.05 mm in C, D.

Male.—Body (Fig. 4A) differs most conspicuously from female in having less elongate anal somite and markedly narrower cephalosome, laterally constricted near antennules. Total 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. Cephalothorax (Fig. 4A, B) with large red pigmented area in anteriormost region and bearing 2 dorsal and 1 median ventral conspicillae (Fig. 4B) as in female. Rostrum broad, rounded. Urosome distinctly 4-segmented. Fifth pediger (Fig. 4C) quadrangular, about 1.5 to 2.0 times broader than long. Genital somite (sixth pediger) (Fig. 4C) 1.6 times broader than long, widest at midregion. First abdominal somite (Fig. 4C) short, about 1.3 times broader than long. Anal somite (Fig. 4C) elongate, almost 3 times longer than broad, with concave lateral margins as in female. Digestive tract and anus appear to be absent. Vestigial mouth and transverse cuticular structure, possibly a labrum, present.

Antennule (Fig. 4D) geniculate, 13-segmented, with armature formula as follows: 3, 1, 1, 1, 0, 0, 1, 4, 2, 2, 3, 1, and 11; some setae sparsely pinnate. Segments XV–XVI fused, lacking sheath; geniculation occurring between fused ancestral segments of XIX–XX and XXI–XXIII; terminal (thirteenth) segment representing fused ancestral somites of XXIV–XXVII. All other cephalic appendages absent.

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

Etymology.—The naupliar stages live in the stomach of *Amphiodia urtica*, and the species is named after the host genus.

Remarks.—The new species shares a number of morphological features with *Caribeopsyllus chawayi* Suárez-Morales, 1998, a copepod that occurs on the Caribbean coast of the Yucatan Peninsula, Mexico. Both species have a partially fused cephalothorax and first pedigerous segment, 4-segmented urosome, 6-segmented antennule, and similar segmentation and armature of legs 1–5 (Table 1). For these reasons, the new
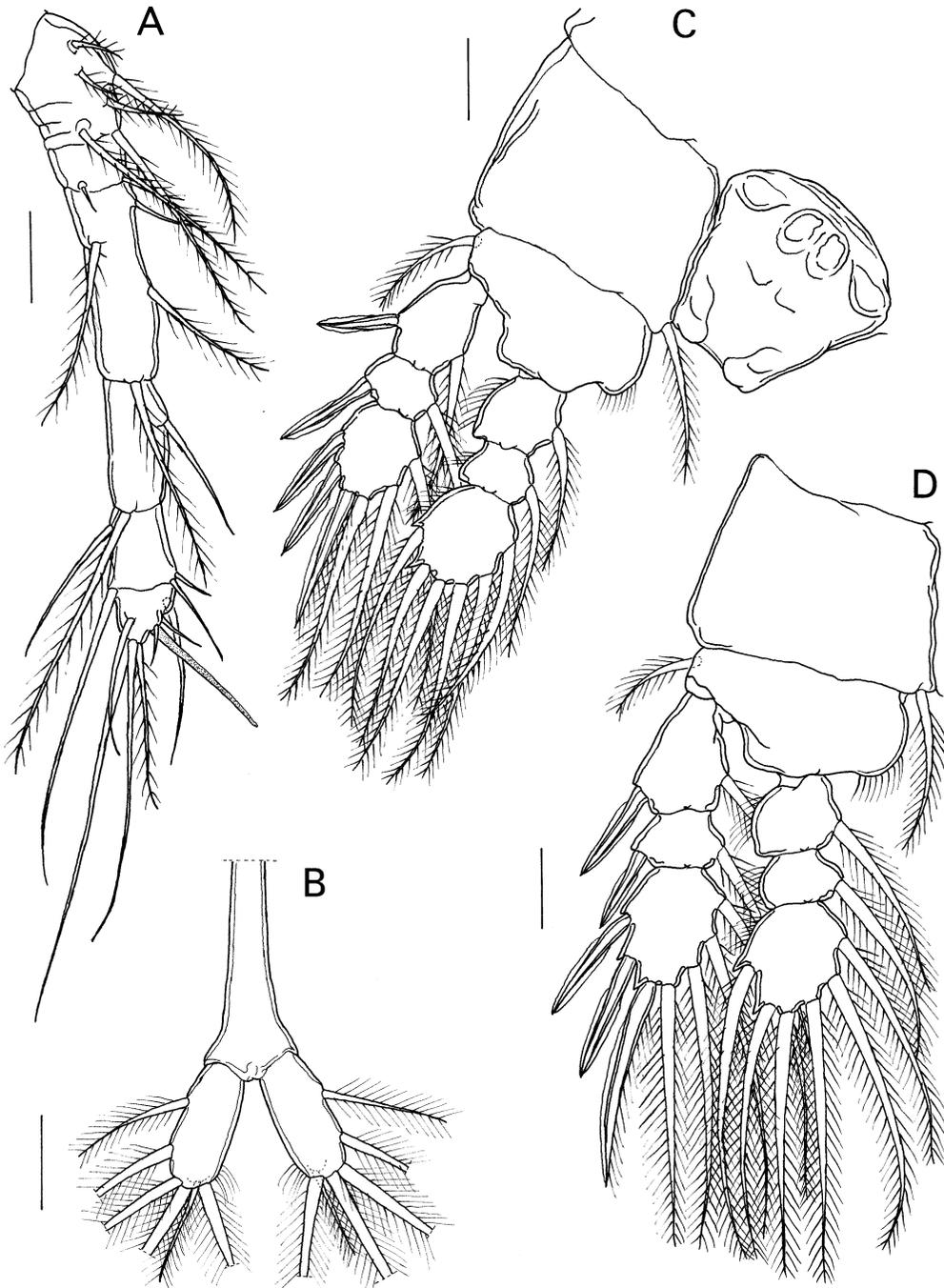
Fig. 2. *Caribeopsyllus amphiodiae*, new genus, new species. Ovigerous paratype female. A, antennule; B, posterior part of urosome; C, leg 1 and intercoxal plate; D, leg 2. Scale bars: 0.05 mm in A, C, D; 0.1 mm in B.

species has been placed in the genus *Caribeopsyllus*.

These two species may be distinguished from each other by the general habitus of the females: the cephalothorax of *C. chawayi* has a distinctly

narrowed anterior end, giving the cephalothorax a slightly triangular profile. In addition, at the juncture of the cephalothorax and the first pedigerous segment there is a small pointed process projecting laterally on each side. The new
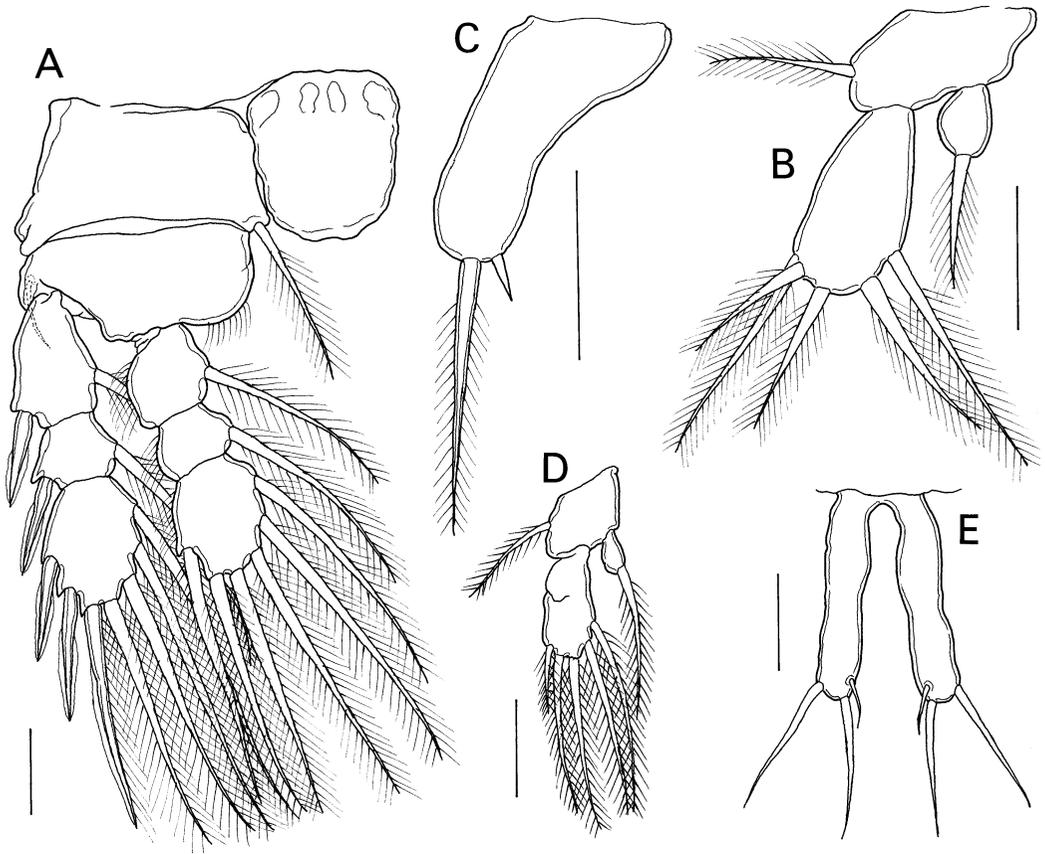
Fig. 3. *Caribeopsyllus amphiodiae*, new genus, new species. Ovigerous paratype female. A, leg 3 and intercoxal plate; B, leg 4; C, leg 5. Male: D, leg 4; E, leg 5. Scale bars: 0.05 mm in all drawings.

species *C. amphiodiae* has a broadly rounded anterior end with no pointed lateral projections. *Caribeopsyllus chawayi* also has distinct spiniform processes present on the outer edge of each exopodal spine of legs 1 and 2, which are absent on leg 1 and the first three exopodal spines of leg 2 in the new species.

Caribeopsyllus amphiodiae is the first thaumatopsyllid found to have two dorsal and one ventral conspicillae. Paired ocelli were observed in *C. chawayi* and possibly also in *T. paradoxus* (Fosshagen, 1970; Suárez-Morales and Castellanos, 1998). The metanauplius of the latter species has paired conspicillae, judging from the "well-separated eye spots, at their sides flanked by an oblong silvery spot" described by Bresciani and Lützen (1962: 388). Whether the eye structures of *C. amphiodiae* are exceptional or whether a triad of conspicillae has been overlooked in other thaumatopsyllids remain to be determined.

Cladistic Analysis

Evaluation of one million trees sampled equiprobably (randomly) from the set of all possible trees resulted in a slightly skewed distribution of tree lengths, with a mean of 112.42. The degree of skewness or $g_1 = -0.78$ indicates the presence of significant hierarchical structure in the data set (Table 2), confirmed by the relatively high retention index (RI), a measure of branch support.

The cladistic analysis of the copepod orders herein was performed on unordered characters, as ordered analyses have the unfavorable property of predetermining topologies or results. The analysis resulted in four similar and equally parsimonious phylograms with a tree length of 78 steps (Fig. 5). The phylograms possess a consistency index of 0.81 and a retention index of 0.75 (maximum value of perfect fit = 1.0). The difference among the four trees is due
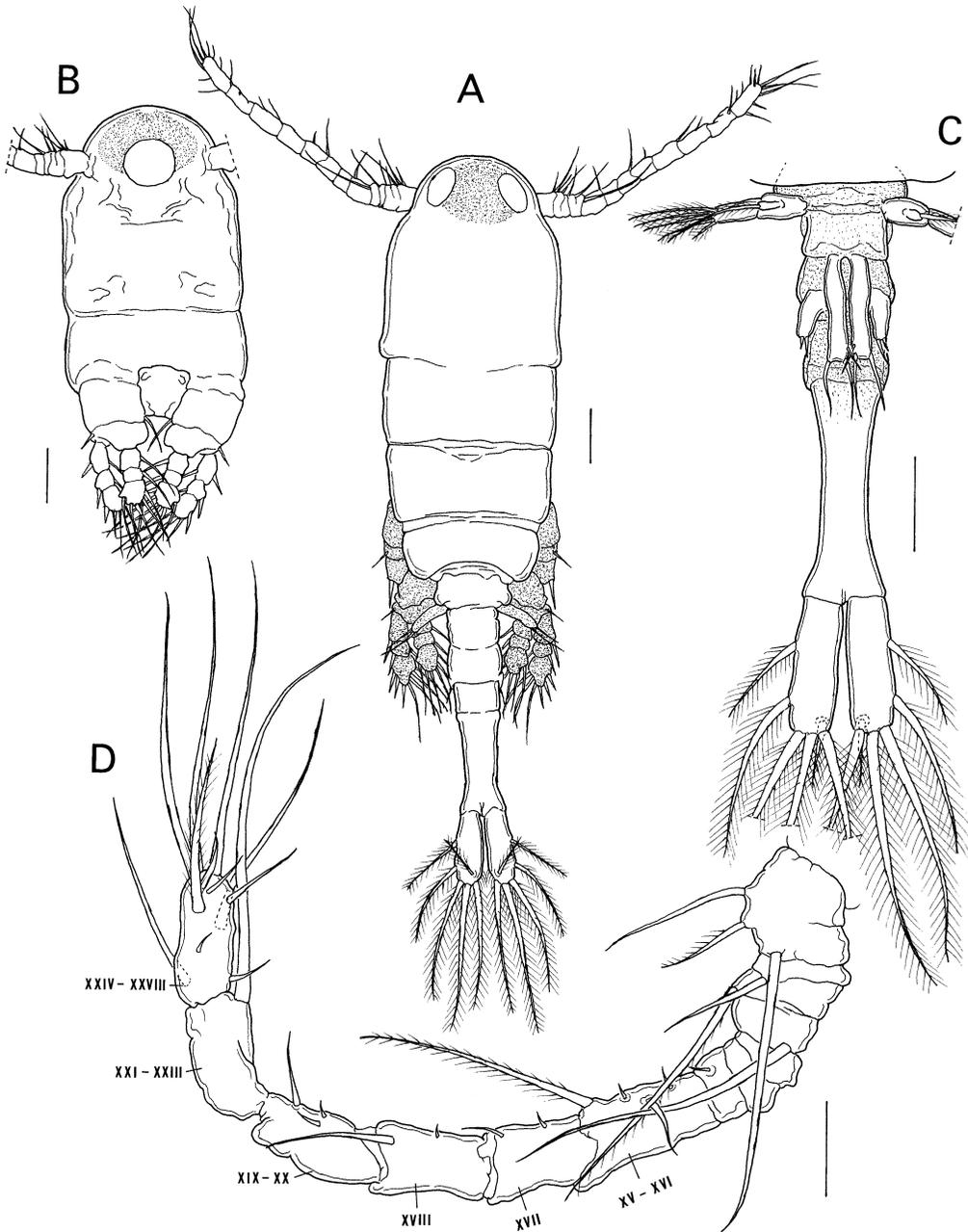
Fig. 4. *Caribeopsyllus amphiodiae*, new genus, new species. Paratype male. A, habitus of fresh specimen, dorsal; B, cephalothorax of fresh specimen, ventral; C, fourth pediger and urosome, ventral; D, antennule (Roman numerals indicate ancestral segments). Scale bars: 0.1 mm in A, B, C; 0.05 mm in D.

to the inconsistent positions of the Mormon-illoida and Harpacticoida on the phylograms, occurring either as the sister taxa on a clade or individually in separate clades. Huys and Boxshall (1991) noted similar variations in their cladograms.

Of utmost significance is the stability of the relationships within the most derived clade in all four cladograms. In every case, the Poecilo-stomatoida was the sister taxon to the clade composed of the Thaumatopsyllidae and its sister taxa the Monstrilloida and Siphonosto-

Table 1. Comparison of the features of females of the five known species of Thaumatopsyllidae. Data and armature formulae of the legs for *Thaumatopsyllus* are based on information from Sars (1921), for *Orientopsyllus* from Sewell (1949), for *Australopsyllus* from McKinnon (1994), for *Caribeopsyllus chawayi* from Suárez-Morales and Castellanos (1998, personal communication), and for *Caribeopsyllus amphiodiae* from the present paper. Armature formulae for legs are presented as outer margin first, with Roman numerals for the number of spines, and Arabic numerals for setae. Question mark (?) follows characters that were not clearly described or illustrated, and information that was based on damaged material. Asterisk (*) denotes cases where formulae were corrected for missing, probably broken, setae.

Character/taxon	*Thaumatopsyllus paradoxus*	*Orientopsyllus investigatoris*	*Australopsyllus fallax*	*Caribeopsyllus chawayi*	*Caribeopsyllus amphiodiae*
Dorsal suture between pediger 1 and cephalosome	Absent	Present	Present	Absent	Absent
Somites of urosome	5	3	4?	4	4
Antennule segments	15	6	8	6	6
Setae (+ aesthetascs) on terminal antennule segment	6 + 1	4 + 0	8 + 0	7 + 1	7 + 1
Furcal setae (primary elements)	7	6	6	6	6
Leg 4	Biramous	Uniramous	Uniramous	Biramous	Biramous
Leg 4 exopod	2-segmented	3-segmented?	2-segmented	1-segmented	1-segmented
Leg 4 endopod	1-segmented	absent	absent	1-segmented	1-segmented
Elements of terminal exopodal segment of leg 4	3	4	5	5	5
Leg 5	3-segmented	1-segmented	2-segmented	1-segmented	1-segmented
Leg 1					
Coxa	0-1	0-1	0-1	0-1	0-1
Basis	1-0	1-0	1-0	1-0*	1-0
Exopodite	I-1; I-1; II,1,3	I-1; I-1; II,1,3	I-1; I-1; II,I,3	I-1; I-1; I,6	I-1; I-1; II,I,3
Endopodite	0-1; 0-1; 6	0-1; 0-1; 6	0-1; 0-1; 6	0-1; 0-1; 6	0-1; 0-1; 6
Leg 2					
Coxa	0-1	?	0-1	0-1	0-1
Basis	1-0	?	1-0	1-0*	1-0
Exopodite	I-1; I-1; II,I,4	?	I-1; I-1; II,5	I-1; I-1; II,I,4	I-1; I-1; II,I,4
Endopodite	0-1; 0-1; 6	?	0-1; 0-1; 6	0-1; 0-1; 6	0-1; 0-1; 6
Leg 3					
Coxa	0-1	0-1	0-1	0-1	0-1
Basis	1-0	1-0	1-0	1-0	1-0
Exopodite	I-1; I-1; II,1,4	I-1; I-1; II,1,4	I-1; I-1; II,I,4	I-1; I-1; II,I,4	I-1; I-1; II,I,4
Endopodite	0-1; 0-1; 6	0-1; 0-2; 6	0-1; 0-1; 6	0-1; 0-1; 6	0-1; 0-1; 6
Leg 4 (e)					
Protopod	1-0	0-0	1-0	0-0	1-0
Exopod	0-0; 3	1-0; 4	0-0; 5	0-0; 5	5
Endopod	0,1,0			0,1,0	0,1,0
Leg 5 (e)					
Protopod	0-0				
Exopod	0-0; 1	2	0-0; 2	2	2

matoida. The gain of characters 43 (the loss of the intercoxal sclerite between the fifth legs of the female) and 50 (the loss of the intercoxal sclerite between the fifth legs of the male), along with the derived state of character 30 (reduced number of setae on the basis of the maxilliped) separate the Thaumatopsyllidae-Monstrilloida-Siphonostomatoida clade from the basal elements of the tree. The derived gain of character 1 (first pedigerous somite fused to cephalosome forming a cephalothorax) separates the Monstrilloida-Siphonostomatoida clade from Thaumatopsyllidae. This feature, in conjunction with the autapomorphic presence of character 38 (the absence of the first exopodal segment and associated spine of leg 5) in the Thaumatopsyllidae, confirms that the species of Thaumatopsyllidae comprise a new and unique order within the Copepoda. Moreover, in combination with the morphological apomorphy of the adult, there are other characteristics that set the Thaumatopsyllidae apart, including a parasitic feeding nauplius with hooked appendages, which develops to a copepodid I stage before emerging from the host.

Table 2. Character matrix adopted from Huys and Boxshall (1991: 394; Table 13) with the elimination of the character states for a hypothetical ancestor and the addition of character states for the Family Thaumatopsyllidae.

Taxon	Character											
	1	5	1 0	1 5	2 0	2 5	3 0	3 5	4 0	4 5	5 0	5 4
Platycopioida	00000000000100000110???0??011000000000?000000100000010
Calanoida	00110010110010000000100000000011111111000000001000000
Misophrioida	01110010120100111101100010000100011111110101010001011
Harpacticoida	01111111120101011101000100011111111111111011101101010
Monstrilloida	1111111112111?0??????????????????11111111111111111111111?
Mormonilloida	01111?11121110101102100 0???10111?111?2222??????????????
Gelyelloida	01111?11121110111110211011111111??11??????????????????
Cyclopoida	011110111211101??0102110111111111111101110100?1001001
Siphonostomatoida	111111111121111 0??1112???1???1211111111101111111111111111
Poecilostomatoida	011111112211110??1112???1???111111111111111110111?1101111
Thaumatopsyllidae	011111111121110??????????????11111112111?1111?1111111

Comparison of the New Species

In 1949, when Sewell described the second species of thaumatopsyllid discovered, *O. investigatoris*, he assigned it to a new genus based on five distinguishing features. The inventory of characters used to discriminate among thaumatopsyllids grew as additional species were described (see McKinnon, 1994; Suárez-Morales and Castellanos, 1998). An updated tabulation is provided in this report (Table 1). The character matrix comprises a mosaic with a unique combination of attributes characterizing each species and reveals that each species has at least one autapomorphy and a mixture of other features shared with its confamilials.

The genus *Caribeopsyllus* is distinguished from other thaumatopsyllids by the 1-segmented exopod on leg 4 (2-segmented in all other confamilial genera) and a unique combination of other characters. The new species is most similar to *C. chawayi*, if the features most informative of generic identity, including the identical numbers of urosomal somites, antennule segments, segments of legs 4 and 5, and the absence of a dorsal suture, are taken into account. The similarity in the morphology of the adult females of the two species amply justifies placement of the new species in the genus *Caribeopsyllus*. Although the antennule of the new species has the same number of segments as *O. investigatoris* and *C. chawayi*, it shows greater evidence of fusion in the basal segment with more complex folding and more (= 6) setae than any of the other thaumatopsyllid species. In that respect, *C. chawayi*, with 4 setae on the basal segment of the antennule, is more

similar to *C. amphiodiae* than any other thaumatopsyllid species.

The males of *O. investigatoris*, *A. fallax*, and *C. chawayi* are unknown. The male of *T. paradoxus* was first described by Fosshagen (1970), and in 1991, when Huys and Boxshall published their monograph on the evolution of Copepoda, they included in it a partial re-description of both sexes of *T. paradoxus*. There were some discrepancies between their and Fosshagen's descriptions, and we have used the more detailed information provided by Huys and Boxshall (1991) for comparison with the present new species.

The male of *T. paradoxus* has one more abdominal somite than *C. amphiodiae*. Males of the two species also differ in the structure and/or armature of 6 of the 12 antennular segments. Leg 4 in *T. paradoxus* is uniramous (instead of biramous) and tipped with 2 (instead of 5) unequal elements. Leg 5 is 2- (instead of 1-) segmented and armed with 4 (instead of 3) unequal elements in the distal region, and the appendages are separated instead of fused, or nearly so, as in *C. amphiodiae*. Also, the anal segment of the male *T. paradoxus* is shorter and broader than that of *C. amphiodiae*.

The naupliar stages of *O. investigatoris* and *A. fallax* are also unknown. However, because the naupliar stages of both *T. paradoxus* and *C. amphiodiae* develop in the stomachs of ophiuroids, we suspect the naupliar stages of the former species do as well. The details of larval development, as well as the life cycle of *C. amphiodiae*, will be described elsewhere. Nonetheless, to underscore the differences between *T. paradoxus* and *C. amphiodiae*, we point out that the smallest larvae of *T. paradoxus* re-
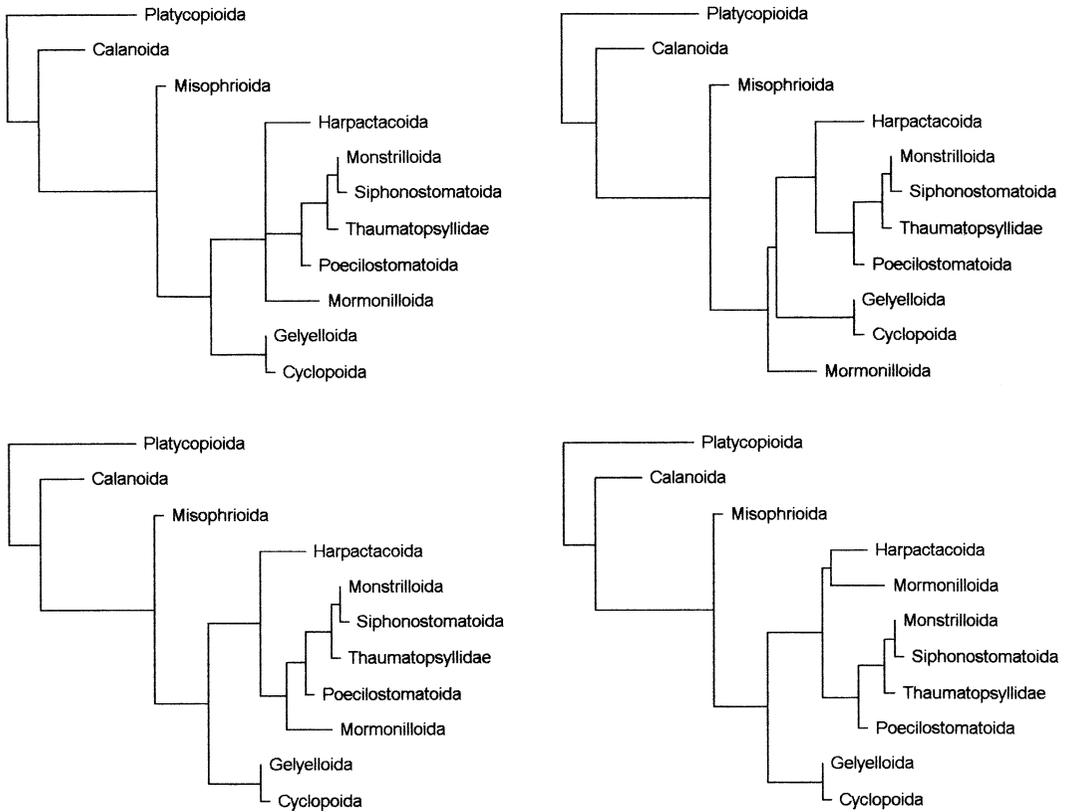
Fig. 5. The four, very similar, equally parsimonious phylograms, all showing the Thaumatopsyllidae as sister taxon to the Siphonostomatoida and Monstrilloida.

covered from the host were metanauplii with four pairs of appendages (Bresciani and Lützen, 1962; Dahms *et al.*, 1999), but the smallest *C. amphiodiae* residing in *A. urtica* were nauplii with three pairs of appendages.

Sexual Dimorphism

Huys and Boxshall (1991) described sexual dimorphism between the adult male and female individuals of *T. paradoxus* involving the antennule segments and fourth–sixth legs. There are also anatomical differences in the same structures between female individuals of *T. paradoxus* and *C. amphiodiae* (see Table 1), raising a question of whether sexual dimorphism is similarly manifested in the two species. A comparison between them indicates that dimorphism is more extreme in the morphology of the antennules in *C. amphiodiae*, and in the legs of *T. paradoxus*. The dimorphic differences are presumably associated with specializations for mating peculiar to each species, such as the manner in which the male grasps the female and

transfers spermatophores, but mating behavior of thaumatopsyllids is still unknown.

The males of *T. paradoxus* and *C. amphiodiae* are smaller than the females, and have a markedly more slender cephalothorax and a shorter anal somite. Furthermore, the sixth legs of the female are lateral, but ventral in the male of both species. The males of both species differ from the females in having a geniculation between the antennule segments that are homologous to the ancestral XIX–XX and XXI–XXIII. However, the antennule of *T. paradoxus* is markedly less sexually dimorphic than that of *C. amphiodiae*. In the former species, the antennule of the female is 15-segmented, and it is 14-segmented in the male. In the latter species, the antennule of the female is only 6-segmented, but it is 13-segmented in the male. The female *T. paradoxus* has the fifth leg with one more segment, but three fewer armature elements than in the male. In *C. amphiodiae*, the female has one less armature element than in the male, and although leg 5 consists of only one segment in both sexes, the legs of the males are

fused basally. The fourth leg is biramous in female *T. paradoxus* and is uniramous in the male, but except for a difference in the relative size of the appendage, there is no dimorphism in the fourth leg of *C. amphiodiae*.

Systematic Position of the Thaumatopsyllidae

When Sars (1913) described *T. paradoxus*, he created a new family to accommodate it, Thaumatopsyllidae, and placed it in the "Division Monstrilloida" exclusively on the basis of the absence of antennae and mouthparts. He furthermore placed the family within "Monstrilloida cyclopimorpha," to distinguish it from the other section of the division "Monstrilloida genuina," from which it differs in many respects.

The placement of Thaumatopsyllidae in the Order Monstrilloida has been repeatedly questioned since Sars' (1913) treatment. Davis (1949: 245) suggested the monstrilloids to be "most closely allied to the Cyclopoida." Sewell (1949: 129) proposed that the family "approximates...to the Families Artotrogidae and Cancerillidae of the Cyclopoida." Fosshagen (1970: 38), however, considered "placing *T. paradoxus* in the Cyclopoida rather than in the Monstrilloida." The so-called Cyclopoida in the last two works refers to the Sarsian section "Cyclopoida siphonostoma," which is now treated as the Order Siphonostomatoida (see Kabata, 1979). Nevertheless, Huys and Boxshall (1991) transferred "Thespesiopsyllidae" to the Order Cyclopoida, a group that was previously known as "Cyclopoida gnathostoma," to distinguish it from the "Cyclopoida siphonostoma." Ferrari (1992) pointed out an inconsistency in Huys' and Boxshall's treatment of tagmosis, and on that basis questioned their transfer of *T. paradoxus* to the Cyclopoida. Ho (1994), in a cladistic analysis of the parasitic cyclopoid copepods, explicitly rejected the transfer of the "thespesiopsyllids" based on their unusual life history (i.e., parasitic naupliar stage) and the absence of antennae and oral appendages. In a phylogenetic assessment of the Calanoida, Harpacticoida, and Poecilostomatoida, based on 28S rRNA, Braga *et al.* (1999) found that the latter group was basal. Their tree was markedly inconsistent with morphology-based phylogenies, and the Thaumatopsyllidae was not included in their analysis. Thus, the attribution of the Thaumatopsyllidae to an existing order has long been problematical and has remained unresolved.

The phylograms in Fig. 5 supports Sars' original hypothesis that Thaumatopsyllidae is allied with the Monstrilloida, and also indicate that Sewell (1949) and Fosshagen (1970) were correct to suggest its affinity to the Siphonostomatoida. The trees, however, refute Huys' and Boxshall's (1991) transfer of the family to the Order Cyclopoida. An examination of the 54 characters in Table 2 reveals that thaumatopsyllids differ from the Order Cyclopoida in the following eight characters:

Character 6: *Female antennule with segments IX through XII fused to form quadruple segment.*— These four antennular segments are free (= 0) in Cyclopoida but fused (= 1) in Thaumatopsyllidae (see Fig. 2A). The derived state of this character is a synapomorphy grouping the harpacticoid-siphonostomatoid complex, which is the clade in each tree containing the Harpacticoida, Mormonilloida, Poecilostomatoida, Thaumatopsyllidae, Monstrilloida, and Siphonostomatoida.

Character 15: *Male antennule with sheath on segment XV, enclosing segment XVI.*—The sheath is present (= 1) in Cyclopoida, but, as shown in Fig. 4D, segment XV in thaumatopsyllids is completely fused to segment XVI and lacks such a sheath (= 0). This character is a synapomorphy grouping the Cyclopoida and Gelyelloida.

Character 38: *First exopodal segment of swimming leg 5 with 2 spines on outer margin.*— Huys and Boxshall (1991) considered the possession of 2 spines on this segment to be plesiomorphic (= 0) and 1 spine, apomorphic (= 1). Because there is no such spine on leg 5 of thaumatopsyllids (see Fig. 3C), the condition is a further transformation from the state of "1" and was accordingly coded with "2." The derived state (= 1) of this character is a synapomorphy grouping all the orders above the Platycopioida. The more derived state (= 2) appears as an autapomorphy on the clade containing only the Thaumatopsyllidae.

Character 39: *Number of setae on inner margin of second endopodal segment of first swimming leg.*—The ancestral state of this character was considered to be 2 setae (= 0), which is what one sees in the Cyclopoida. However, as illustrated in Fig. 2C, the segment in thaumatopsyllids shows a derived state (= 1) in

carrying only one seta. The derived state of this character is a synapomorphy grouping the harpacticoid-siphonostomatoid complex.

Character 43: *Intercoxal sclerite between fifth legs of female lost.*—This sclerite is present (= 0) in the Cyclopoida, but lost (= 1) in the Thaumatopsyllidae (see Fig. 1C). The derived state of this character is a synapomorphy grouping the (Thaumatopsyllidae (Monstrilloida, Siphonostomatoida)) clade.

Character 45: *Coxa and basis of female fifth legs fused.*—These two proximal segments are free (= 0) in Cyclopoida, but completely fused to the rest of the leg (= 1) in Thaumatopsyllidae (see Figs. 1C, 3C). The derived state of this character is a synapomorphy grouping the harpacticoid-siphonostomatoid complex.

Character 46: *Inner coxal seta absent from female fifth legs.*—This seta is believed to be present (= 0) in the ancestral form and in Cyclopoida. However, as shown in Fig. 3C, it is absent (= 1) in Thaumatopsyllidae. The derived state of this character is a synapomorphy grouping the harpacticoid-siphonostomatoid complex.

Character 52: *Coxa and basis of male fifth legs fused.*—Accounts of this character are as for character 45. The derived state of this character is a synapomorphy grouping the (Poecilostomatoida (Thaumatopsyllidae (Monstrilloida, Siphonostomatoida))) clade.

The harpacticoid-siphonostomatoid complex is posited to be monophyletic based on the parsimony analysis conducted herein. Indeed, the shared derived characters (namely, characters 6, 39, 45, and 46) that unite this group clearly separate the Thaumatopsyllidae from the Cyclopoida. Hence, Huys' and Boxshall's (1991) placement of the Thaumatopsyllidae within the Cyclopoida is not corroborated by this analysis.

In addition, the Thaumatopsyllidae is in monophyly with the Monstrilloida and Siphonostomatoida. Thaumatopsyllidae differs from Monstrilloida in characters 1 and 38, and from Siphonostomatoida in characters 1 and 39. Character 1 denotes the fusion, or lack thereof, of pediger 1 with the cephalosome along a dorsal suture line demarcating the cephalosome from the first pediger (Huys and Boxshall, 1991).

These two entities are fused (= 1) in all representatives of both Monstrilloida and Siphonostomatoida, but they are not fused (= 0) in the thaumatopsyllid genera *Australopsyllus* and *Orientopsyllus*. Their fusion in *Thaumatopsyllus* and *Caribeopsyllus* is considered to have independently evolved after the separation of the lineage of the Thaumatopsyllidae from the Monstrilloida-Siphonostomatoida clade. Accordingly, a new order, Thaumatopsylloida, is herein proposed to accommodate the Family Thaumatopsyllidae, and it is defined as follows:

Thaumatopsylloida, new order

Diagnosis.—Copepoda. Adult: Body cyclopiform, unmodified. Urosome 3- to 5-segmented. Anal somite distinctly elongated. Caudal ramus with 6 or 7 setae. Rostrum broad and rounded. Antennule with 6–15 segments. Antenna and oral appendages absent. Vestigial labrum and mouth may be present.

Legs 1–3 biramous, with 3-segmented rami. Leg 4 biramous or uniramous; exopod 1- to 2-segmented and endopod 1-segmented or absent. Leg 5 uniramous; 1- to 3-segmented.

Naupliar stage: With 3 appendages, presumably antennule, antenna, and mandible; all with plumose setae. Nauplius parasitic, with recurved spines on 3 appendages used for attachment to host.

Adults and copepodids free-living, planktonic; nauplius stage lives in the stomach of Ophiuroidea.

Type Family.—Thaumatopsyllidae Sars, 1913.

Finally, a caveat is appropriate regarding the hypotheses of copepod relationships presented herein, in that they are based on a limited data set derived almost exclusively from previous work and restricted to adult morphology. Several additional, remarkable similarities between the Thaumatopsylloida and Monstrilloida are apparent upon further inspection. First is the loss of the antenna and the reduced or lost mouth in the adults. Second is a protelean life cycle, which involves a crawling, parasitic nauplius, and the self-expulsion of the copepodid from its benthic invertebrate host. This combination of characteristics that they share suggests a greater affinity between the two orders than that presented in the preceding discussion. Indeed, after the addition of two characters to the data set herein, namely the crawling, parasitic nauplius, and loss of the

antennae, a cladistic analysis of the copepod orders results in four equally parsimonious trees (not shown herein). They differ from the trees produced in the first analysis (Fig. 5) in only one respect, by positing the monophyly of the Monstrilloida and Thaumatopsylloida, with the Siphonostomatoida as the sister taxon to this most apical clade. We anticipate that with additions of morphological and ontogenetic data, arising from further study, this hypothesis will be corroborated.

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