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# An Endemic Commensal Leucothoid Discovered in the Tunicate *Cnemidocarpa bicornuta*, from New Zealand (Crustacea, Amphipoda)

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Nova Southeastern University  
Halmos College of Natural Sciences  
and Oceanography

An Endemic Commensal Leucothoid Discovered in the Tunicate *Cnemidocarpa  
bicornuta*, from New Zealand (Crustacea, Amphipoda)

By  
Kaitlyn M. Brucker

Submitted to the Faculty of  
Nova Southeastern University  
Halmos College of Natural Sciences and Oceanography  
In partial fulfillment for the requirements for  
The degree of Master of Science with a specialty in:

Marine Biology and Coastal Zone Management

Nova Southeastern University

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**Thesis of**  
**Kaitlyn M. Brucker**

Submitted in Partial Fulfillment of the Requirements for the Degree of  
**Masters of Science:**  
**Marine Biology and Coastal Zone Management**

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Halmos College of Natural Sciences and Oceanography

March 2016

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## **Abstract**

Precise descriptions and comprehensive taxonomies of species and their ecology are essential in monitoring changes in marine biodiversity at multiple spatial scales. A currently undescribed species of commensal amphipod in the genus *Leucothoe* is reported from New Zealand, collected from the endemic tunicate *Cnemidocarpa bicornuta*. This species differs from others in the genus in having a one-articulate first maxilla palp and an apically produced tuberculate lobe on the inner margin of the outer plate of the maxilliped. Previous taxonomic surveys in New Zealand waters did not document this species, indicating that it may be a recent arrival. This research highlights the importance of biodiversity monitoring and taxonomic surveys to record occurrences of undescribed or recently-arrived taxa.

Key words: new species, amphipod, ascidian, commensalism, invasive species, *Leucothoe*

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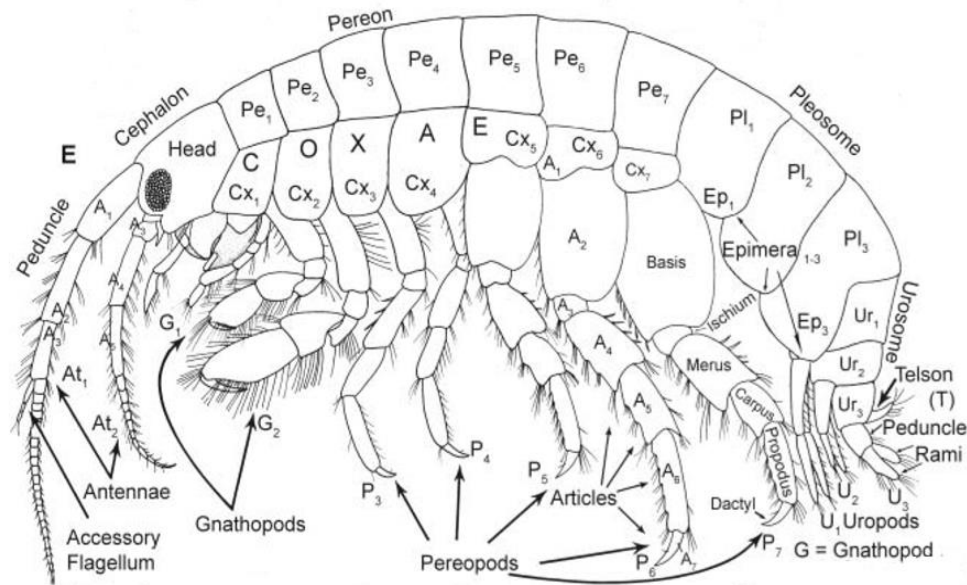
## **1. Introduction**

### **1a. Amphipoda:**

The order Amphipoda includes over 6,000 described species, making it the largest taxon among Pericarida—the brooding crustaceans that also include isopods, mysidaceans, cumaceans, and tanaidaceans (Ruppert, Fox et al. 2004). Amphipods (Figure 1) are small, chiefly laterally compressed crustaceans, usually between 5 and 15 mm long. They may be benthic, pelagic, free-living, or commensal (Barnard 1962, Chapman 1988, De Broyer, Chapelle et al. 2003, Ruppert, Fox et al. 2004, White and Reimer 2012, Martín, Díaz et al. 2013). Although most species are marine, some inhabit terrestrial, semi-terrestrial and freshwater environments (Barnard 1962, Chapman 1988, Ruppert, Fox et al. 2004, Chapman 2007, White and Reimer 2012, Martín, Díaz et al. 2013). Some occur in dense colonies, and a few species are eusocial (White and Reimer 2012). Ecologically, amphipods are important in the conversion of inorganic and organic matter back into biomass as a food source for many species of fish (Ruppert, Fox et al. 2004, Grabowski, Bacela et al. 2007, Martín, Díaz et al. 2013). In a benthic survey of the northern Gulf of Mexico, an ampeliscid amphipod contributed 90% of macrofaunal abundance; densities in some samples reached over 26,000 individuals m<sup>-2</sup> (Soliman and Rowe 2008).

Amphipods have well-developed antennae and compound sessile eyes but no carapace; the head and first thoracic segment fuse to form a cephalothorax. The cephalothorax is followed by the pereon, composed of the last seven thoracic segments, which bear coxae and pereopods. The coxal plates protect the gills in the thoracic region. The first two pairs of pereopods, termed gnathopods, are larger than the rest and are

generally subchelate. The pereon is followed by the abdomen, which is divided into an anterior pleosome and posterior urosome, each composed of three segments.



**Figure 1:** Generalized diagram of an amphipod labeling the major structures. The diagram was modified from Chapman (2007). Abbreviations: A = appendage article, At = antennae, Cx = coxae, E = epimera, G = gnathopod, H = head, LL = lower lip, Md = mandible, Mx = maxilliped, N = gnathopod, P = Pereopod, Pe = Pereonite, Pl = pleonite, T = telson, U = uropod, UL = upper lip, X = maxillae. Subscript numbers indicate place of feature in sequence from anterior to posterior, e.g., Pe<sub>1</sub> = first pereonite, Cx<sub>4</sub> = coxa of fourth pereonite. Subscript numbers associated with 'A' indicate sequence of appendage articles from proximal to distal, e.g., A<sub>3</sub> on At<sub>1</sub> is the third article of the first antenna.

Each pleosome segment bears a pair of pleopods, which are used for swimming and in creating currents through the branchial chamber. The uropods, attached to the urosome, are stiff and used for jumping, digging, kicking and swimming. The most posterior segment is the telson (Ruppert, Fox et al. 2004, Chapman 2007)

Amphipods exhibit direct development; juveniles and adults are morphologically similar. Eggs are brooded by females in the marsupium and during gestation; the eggs are ventilated by the pleopods. Once hatched, juveniles stay in the marsupium for a number

of days depending on the species as well as the environmental conditions (Borowsky 1980, Borowsky 1980, Ruppert, Fox et al. 2004).

### **1b. Gammaridea:**

The vast majority of amphipods, about 5,000 species, belong to suborder Gammaridea (MacNeil, Dick et al. 1997, Ruppert, Fox et al. 2004). They are laterally compressed, with large coxal plates, large abdomen, small compound eyes, and a small cephalothorax (Ruppert, Fox et al. 2004). The gnathopods are modified for a wide range of functions, including feeding, grooming, burrowing and courtship (MacNeil, Dick et al. 1997). Gammarideans can swim, but often remain closely associated with the benthos (Ruppert, Fox et al. 2004). They are often found in dense populations, making them important components of the benthic community (Platvoet, Hou et al. 2008). Many create and inhabit tubes secreted from glands near the fourth and fifth pereopods.

### **1c. Leucothoidae:**

Members of family Leucothoidae occur in all of the world's oceans (Frutos and Sorbe 2013). They are primarily endocommensal inhabitants of sessile, suspension or filter-feeding invertebrates, including sponges, ascidians, bivalves and octocorals (White and Reimer 2012, Thomas and Klebba 2006). The inhalant current generated by the host, or ambient water movements, provide nutrients and oxygen, and remove wastes (White and Reimer 2012). Their association with sessile hosts and life history traits allow species to serve as potential proxies for evolutionary history (Thomas 2015). Leucothoidae currently consists of 176 species in five genera that are separated into two informal clades: anamixids and leucothoids (Thomas 2015). Anamixids are found in tropical to warm temperate regions, display profound sexual dimorphism in

terminal males (White and Reimer 2012), and demonstrate two of the three criteria for eusociality (Michener 1969): 1) generations within anamixid colonies overlap, and 2) different morphologies separate the animals into an organized caste system. However, the third criterion, restriction of reproduction to specified individuals within the colony, has not been confirmed (Michener 1969, White and Reimer 2012). By contrast, members of the leucothoid clade display little sexual dimorphism and are found in tropical to polar waters (White and Reimer 2012).

Within Leucothoidae, *Leucothoe spinicarpa* Abildgaard (1789), was once thought to represent the entire family and was reported from all oceans and seas and at depths ranging from intertidal to 4,000 m (Thomas and Klebba 2006, Krapp-Schickel and De Broyer 2014, Thomas 2015). Additionally, vague illustrations and loss of the holotype complicated understanding of taxonomic relationships among what turned out to be a complex of morphologically similar cryptic species with highly specific host and niche associations. Thomas and Klebba (2006) and Thomas (2015) noted at least 12 different accounts in the Caribbean in which a distinct species was attributed to *L. spinicarpa*.

#### **1d. Genus *Leucothoe*:**

The cosmopolitan genus *Leucothoe* currently includes 132 morphological species (Ishimaru 1985, Serejo 1998, Crowe 2006, White and Reimer 2012, Thomas 2015). Eyes, when present, are usually well developed and contain ocelli of 10 or more facets. Mandibles lack molars, and the palp is three-articulate. Species exhibit little to no sexual dimorphism (White and Reimer 2012). Common hosts include ascidians, bivalves and sponges, but species have been documented in association with algae, corals, and coral rubble, as well as fine sand and mud habitats (Crowe 2006).

### **1e. Amphipod Taxonomy:**

Many of the early taxonomic descriptions of gammaridean amphipods are of species that have subsequently been recognized as Leucothoidae, the earliest being *Leucothoe spinicarpa* (Abildgaard 1789, Thomas and Klebba 2006, Thomas 2015). These amphipods were easily accessible, due at least in part to their shallow coastal marine distribution, and had distinct morphologies. Despite their historic value, the illustrations that accompanied the early descriptions are imprecise and lack the details required for accurate taxonomic assignment (Thomas and Klebba 2006, Minelli 2012, Thomas 2015). In addition, many holotypes have been lost, damaged, or improperly preserved. The combination of these problems makes diagnoses of new leucothoid taxa challenging (Thomas and Klebba 2006, Thomas 2015). However, recent major advances in leucothoid taxonomy have helped resolve such issues. An online taxonomic database now allows comparison of collected specimens with descriptive species illustrations (Thomas and Klebba 2006, Platvoet, Hou et al. 2008, Thomas 2015). New underwater techniques permit collection of amphipods together with their hosts to document associations. These advances have sparked the re-examination of existing collections and new descriptions (Thomas and Klebba 2006, Myers 2013, Krapp-Schickel and De Broyer 2014, Thomas 2015).

Interest in leucothoid research has increased recently due to the role of these amphipods as important environmental proxies. Reish and Barnard (1979) and Thomas (2015), for example, have illustrated the use of amphipods for research focused on global climate change and biodiversity loss due to their vulnerability to toxins and pollutants.

Amphipods lack a planktonic dispersal stage, which has led to high levels of endemism (Lopes, Marques et al. 1993, Thomas 1993, Richards, Thomas et al. 2007, Richards, Stanhope et al. 2012, Thomas 2015), producing a framework of biodiversity that can be compared biogeographically, making them useful proxies in monitoring ecosystem change (Myers 1993, Thomas 1993, Thomas 2015). However, leucothoid amphipods may disperse through other means, such as rafting on seaweed, algae, floating debris, and transport in ballast water, all of which have facilitated broad dispersal of tube-dwelling and fouling community taxa. Co-introduction of amphipod species via their hosts has also been a concern (Myers 1993, Muir 1997, Carlton 2009, Thomas 2015), e.g., Muir (1997) considered the spread of *Paraluciothoe flindersi* (now = *L. eltoni*) across the Hawaiian Islands to be the result of a co-introduction with its host sponge *Mycale* sp.

**1f. Host specificity:**

Leucothoid amphipods are common commensals of a variety of organisms including bivalves, sponges, ascidians, and brachiopods (Thomas 1997, Thiel 1999, Thiel 2000, Crowe 2006, Thomas and Klebba 2007). These organisms create microhabitats that provide residential species with shelter and food sources (Thiel 1999). The relationships between host species and their associates differ among major phyla. For example, amphipod associates living ascidians have access to inhalant water before it is filtered by the host, whereas sponges filter water before it reaches the amphipods in the spongocoel (Thiel 1999). Bivalves are comparable to ascidians in that amphipod associates inhabit the mantle and have direct access to nutrients but differ in that associates of bivalves need to compensate for excessive mucus. This is done with modified hooked setae (Thomas

and Klebba 2007, Vader and Tandberg 2013). Since hosts such as bivalves, ascidians, and sponges are relatively long-lived stable species, compared to leucothoid amphipods, they can provide suitable environments for copulation and rearing offspring (Thiel 1999, Thiel 2000, Thomas and Klebba 2007). Host size can have profound effects on commensal population size, e.g., larger hosts are better ecological targets, with increased internal space that provides higher current flow with more food availability, and have existed for longer periods increasing colonization time (Gage 1966, Dalby Jr 1996, Thomas and Klebba 2007). Smaller host organisms, such as ascidians and bivalves, usually only harbor a few amphipods that are usually small family units consisting of parents and offspring (Thiel 1999, Thiel 2000, Vader and Tandberg 2013). Further, *Leucothoe spinicarpa* offspring may inherit their ascidian hosts from their parents, a finding that correlates with advanced social behavior, which Thiel (1999, 2000) suggested evolved from residing in the host's stable environment and having to defend it from other competitors.

Host canal morphology is also a factor in host preference. Henkel and Pawlik (2005) documented a positive correlation between number of commensals and internal surface area in sponges (Thomas and Klebba 2007). Although most commensal amphipod species may occupy a variety of hosts, some are highly host specific (Duffy and Hay 1991, Gestoso, Olabarria et al. 2014). Thiel (1999) noted that individuals of *L. spinicarpa* in ascidians stayed within the first 5 mm of the inhalant opening with their antenna oriented outward. He also noted that juvenile and smaller adult individuals of *L. spinicarpa* have been found in less favorable hosts, suggesting that intraspecific competition for limited host organisms may exist.

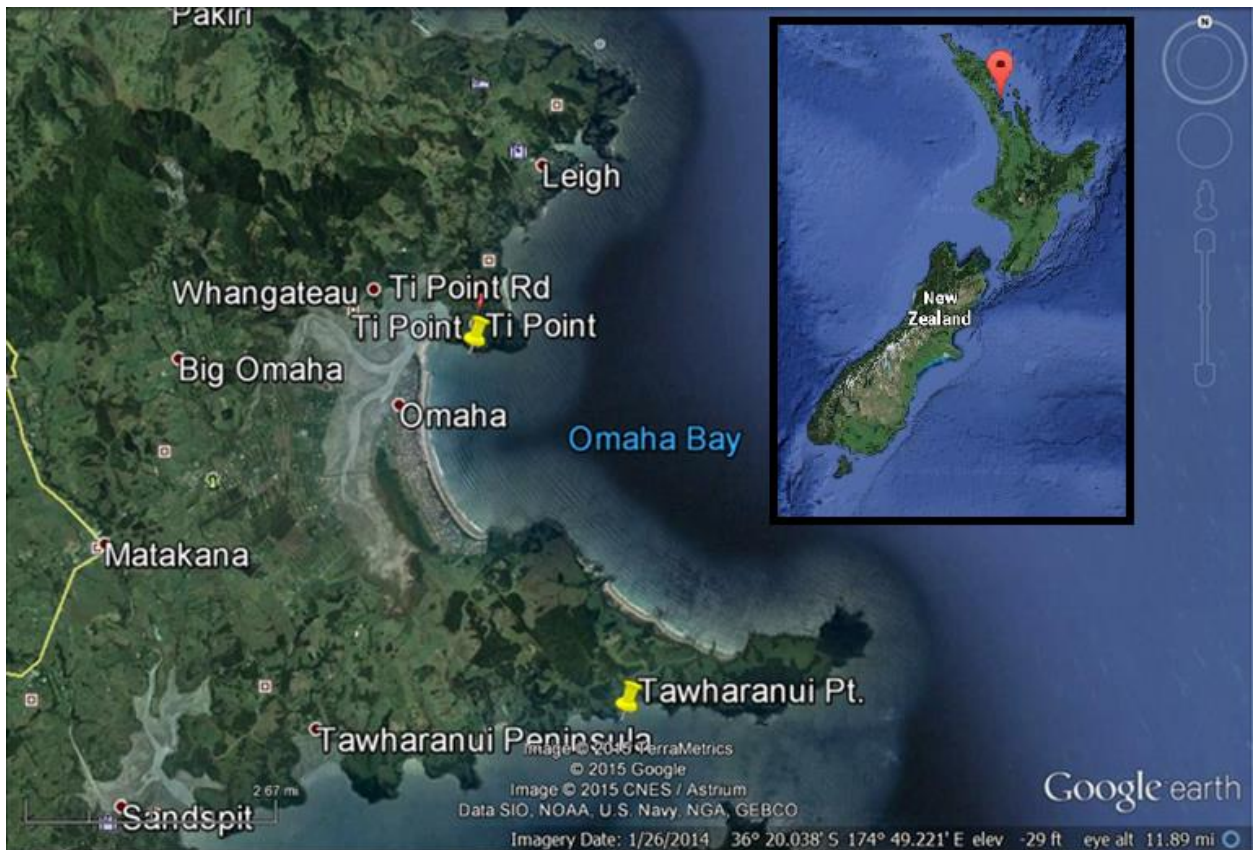


Degree of host specificity may change with locality. In the Florida Keys, Crowe (2002) only found *Leucothoe ashleyae* in *Callyspongia vaginalis* hosts with low populations, whereas *Leucothoe kensleyi* was associated with 14 different host species. In contrast, in Belize, *L. ashleyae* occurs in association with nine different sponge species, while the presence of *L. kensleyi* in host organisms is uncommon (Crowe and Thomas 2002, Thomas and Klebba 2007). Vader (2013) noted that most amphipod associates of bivalves inhabit closely related hosts.

## **2. Materials and Methods:**

### **2a. Collection:**

Amphipods and their hosts were collected together by SCUBA in 2013 and 2014, following techniques in Thomas (2015), at two sites around Omaha Bay on North Island, New Zealand: Ti Point and Tawharanui Point (Figure 2). Individual host ascidians, *Cnemidocarpa bicornuta*, a New Zealand endemic, were removed from the substrate using a dive knife and immediately placed into plastic Ziploc bags and brought to the surface. At the surface, ascidians were bisected with a knife, and amphipods were extracted from the atrium. Samples were fixed in either a 2% buffered formalin, or a 70% ethanol solution. Debris was removed from amphipods with small sable hair brushes before being transferred into glycerin for dissection, illustration, and analysis.



**Figure 2:** Sites at Ti Point, Omaha Bay, and Tawharanui Pt., Jones Bay.

*Study Site:*

Figure 2. Sample collection sites around Omaha Bay, North Island, New Zealand.

**2b. Morphological Analysis:**

Amphipods were examined and dissected using a stereomicroscope. All appendages were analyzed. Appendages were removed using fine-tipped forceps and mounted onto a glass microscope slide with a drop of glycerin and a coverslip. Alternatively, gnathopod 1 (right and left), gnathopod 2 (right and left), and the maxilliped were removed and cleared of muscle tissue via dissolution in a Bioquip clearing agent (lactic acid, phenol, and glacial acetic acid) and incubation at 40 °C for 24 h. All appendages were analyzed

for novel and/or distinguishing features. Appendages used for primary taxonomic comparison were gnathopod 1, maxilliped, and the first antenna, because these appendages had distinctive morphological features for comparison. Comparisons were completed using the Leucothoe Taxonomic Database ([http://cnso.nova.edu/jthomas/Current%20Leucothoidae\\_16Sep14.pdf](http://cnso.nova.edu/jthomas/Current%20Leucothoidae_16Sep14.pdf)).

### **2c. Illustration and Description:**

Digital illustrations were prepared using methodology described by Coleman (2003). Pencil illustrations were drawn using a camera lucida attached to an Olympus BH-S compound microscope with Nomarski optics and then scanned into Adobe Illustrator<sup>®</sup> and digitally traced using a Wacom<sup>®</sup> drawing pad. Lines were traced using the arc tool and then fitted to the pencil drawing using anchor points. Line thickness in Illustrator was based on the feature being illustrated: setae 0.25, spines 0.50, and appendages 1.00. Descriptive terminology was adapted from Thomas (2015) and (Bousfield 1973).

### **2d. Abbreviations:**

Appendage and body segment abbreviations are as in Figure 1. Additional abbreviations include: **A** = antennae, **C** = coxae, **E** = epimera, **H** = head, **LL** = lower lip, **Md** = mandible, **Mx** = maxilliped, **N** = gnathopod, **P** = Pereopod, **T** = telson, **U** = uropod, **UL** = upper lip, **X** = maxillae. Each illustration has an associated descriptive abbreviation. A capital letter to the right of a body part or appendage (e.g., P4 = fourth pereopod) refers to either **L** = left, **R** = right. Lower case letters represent the following: **l** = lateral, **m** = medial, **x** = magnified. LW = length to width ratio. The symbols ♂ and ♀ represent male and female sexes, respectively. Material is deposited at Tamaki Paenga

Hira—Auckland War Memorial Museum Natural History Centre, Auckland, New Zealand (AWMNHC).

### 3. RESULTS

#### Systematics

Order Amphipoda Latreille, 1816

Suborder Gammaridea Latreille, 1802

Family Leucothoidae Dana, 1852

Genus *Leucothoe* Leach, 1814

**Type-species.** *Gammarus spinicarpus* Abildgaard (1789). Coxa 2 at least as long as broad, rounded ventrally and anteriorly, coxa 1 not concealed; mandibular palp 3-articulate; outer plate of maxilliped reaching less than halfway along palp article 1 (Barnard and Barnard 1969).

**Diagnosis.** Accessory flagellum vestigial, 1- or 2-articulate, or lacking, very small; mandible lacking molar; outer plates of maxilliped very small, probably never larger than inner plates; telson entire; gnathopod1 carpocheate. See Anamixidae, Sebidae, Amphilochidae, Cressidae, Thaumatelsonidae.

#### *Leucothoe* n. sp.

**Type Locality.** Omaha Bay, Tawharanui Pt, New Zealand, 36°22.7375 S; 174°49.134 E, station JDT-NZH, 27May2013, 7.0 m, rocky ledge with kelp.

**Holotype.** [AWMNHC]-XXXX, male A, 8.6 mm, 27-May-2013, James Thomas, collector.

**Paratype.** [AWMNHC]-YYYY, female C, 8.8 mm, 27-May-2013, James Thomas, collector.



**Figure 3:** *Leucothoe* sp. n. under microscope.

**Diagnosis.** Male holotype “A”. Antenna 1 and 2 short, less than 0.22x body length; maxilliped outer plate 0.60x length of article 1, with 2 apicomedial teeth, inner margin sinuous; maxilla 1 palp 1 articulate; gnathopod 1, article 7 reduced, article 5 with row of 13 short submarginal setae; gnathopod 2, article 6 with dense linear rows of mediofacial setae 0.73x of propodus, second oblique mediofacial row extending from midproximal margin to 0.39X of posterior margin, palm with 4 major 2 minor projections, with 2 setae each, except second most distal projection which has 1 setae, 2 apical setae at base of dactyl; dactyl smooth, curved, 0.61x of propodus.

**Description of male holotype, cat. no. XXXX (specimen A)** Figures 3 and 4. Ratios of antenna 1 and 2, 0.22 and 0.15x body length; antenna 2 0.68x length of antenna 1; flagellum 9-segmented on antenna 1, 6-segmented on antenna 2.

**Coxae.** Coxa 1 bilobed, coxae 1-4 with LW 1.00:1.57:1.38:1.66; coxa 4 posteriorly excavated and widest along mid-posterior margin; Coxa 5 bilobed, coxae 6-7 reduced, ovate.

**Upper lip.** (Figure 4). Rounded, lacking ornamentation.

**Mandibles.** (Figure 4). Molars lacking; Left mandible with small incisor equal in width and length, moderately dentate. Palp 3-articulate, articles 1-3 length ratios 1.00:2.65:1.5; article 2 with 4 apicodistal anterior and 2 apical setae; lacinia mobilis large, as broad as incisor; row with 13 spines, 3 distal spines enlarged, flattened and bladelike. Right mandible, palp articles 2 and 3 with anterior distal and apical setae. Raker row with 15 spines, the 4 most distal flattened and bladelike.

**Maxillae.** (Figure 4). Maxilla 1, palp 1-articulate with slight constriction at approximately 2.9 mark, apex constricted with subapical cleft, distal apex with 2 shovel-like setae, subapical cleft with 2 thick setae; outer plate with 7 thick apical setae in 2 rows with 4 medial and 3 lateral and 4 fine brush subdistal setae; inner plate, small, ovate, with one apical seta. Maxilla 2, inner plate with 7 submarginal setae at distal end, 2 of which are pectinate, inner margin with 4 facial setae, line of brush setae on outer margin and between most proximal 2 setae on inner margin; outer plate with 2 apical setae, 1 of which is pectinate, and 1 submarginal pectinate seta, brush setae on inner and outer margins.



**Figure 4:** *Leucothoe* sp. n. male holotype "A", 8.6 mm. Hd, Head; A1, antenna 1; A2, antenna 2; X1, maxilla; X2, maxilla 2; MdL, Left mandible, MdR, right mandible; Mx, Maxilliped; UL, Upper lip.

**Maxilliped.** (Figure 4). Palp articles 1-3 of approximately equal size and each as wide as long, article 4 curved, ~1.5x length of article 3; palp article 1 with 5 apicodistal setae on

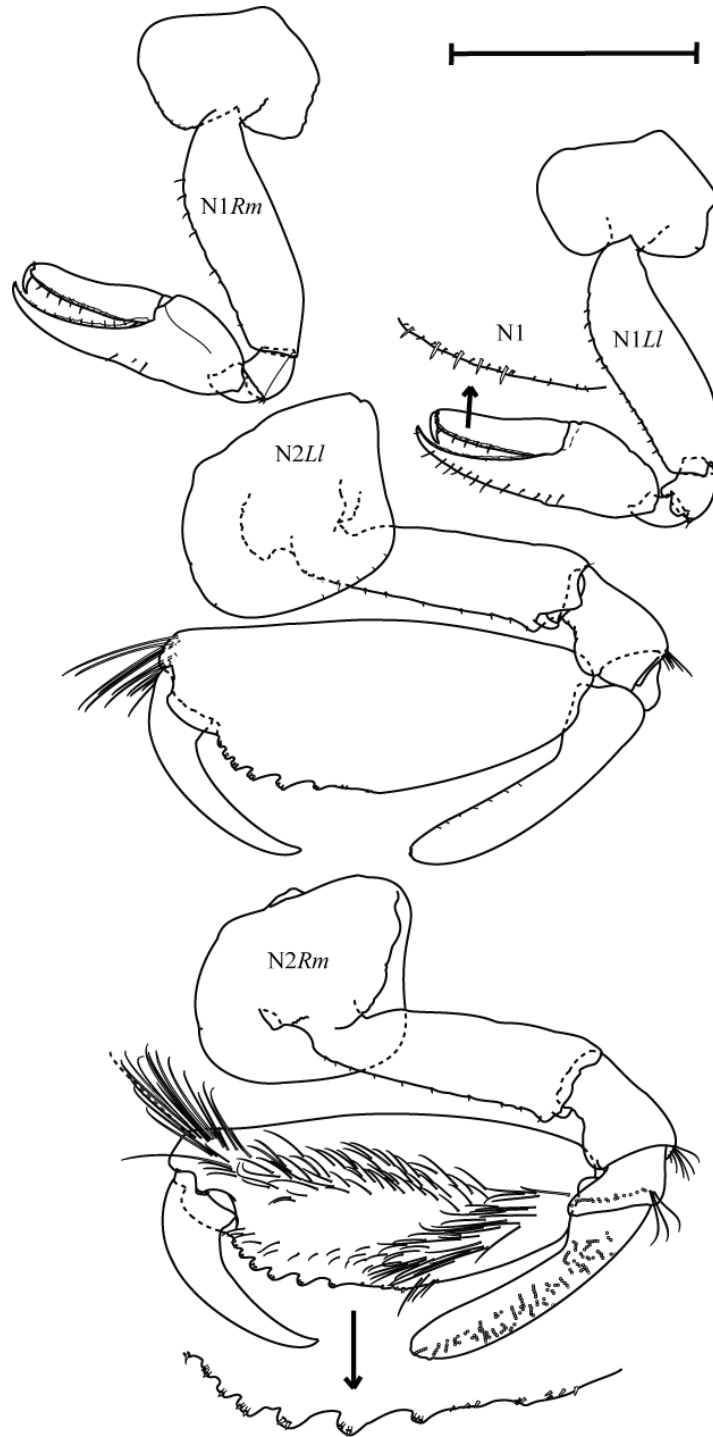
inner margin, 1 dorsal facial seta at mid-inner margin, outer margin with 1 dorsal apical seta; article 2 with 14 setae along inner margin, 4 with submarginal dorsal attachment; article 3 with 8 distal apical setae on inner margin and 1 apical seta on outer margin; article 4 with dense row of brush setae along inner margin: inner plate with 2 shovel-like apicomedial setae, medial spine larger and thicker than lateral spine; outer plate extending to 0.6 length of article 1, with 2 apicomedial teeth, inner margin sinuous;

**Gnathopod 1.** (Figure 5). Coxa bilobed, LW 1.16; article 2 LW 2.96, linear, anterior margin undulate, with 13 short marginal setae and 1 posterodistal seta; article 5 LW 2.83, expanded at insertion of article 4, tapering distally, with sharp apex, lateral posterior margin with row of 13 short submarginal setae, medial posterior margin with 3 short mediodorsal setae, anterior margin tuberculate with 8 marginal setae and 6 short distal setae; propodus 0.93x length of carpus, LW 3.60, lateral posterior margin with 5 stout submarginal setae and 14 small submarginal setae; dactyl 0.26x length of propodus, curved, closing posterior to apex of carpus.

**Gnathopod 2.** (Figure 5). Coxa subquadrangle, expanded, LW 1.12; article 2 linear, LW 3.18, anterior margin lined with 10 short submarginal setae; article 3 posterodistal margin expanded dorsally, with tuft of 6 setae; article 5 LW 1.72, medial margin with single oblique row of 23 submarginal setae; carpus curved and apically rounded, 0.43x length of propodus, medial margin with multiple dense rows of facial setae numbering 50+; article 6 LW 2.44, 0.73x length of propodus, medial margin with dense linear rows of mediodorsal setae, second oblique mediodorsal row extending from midproximal margin to 0.39x of posterior margin, palm with 4 major and 2 minor projections, each



with 2 setae except second most distal projection, which has 1 seta, 2 apical setae at base of dactyl; dactyl smooth, curved, 0.61x length of propodus.



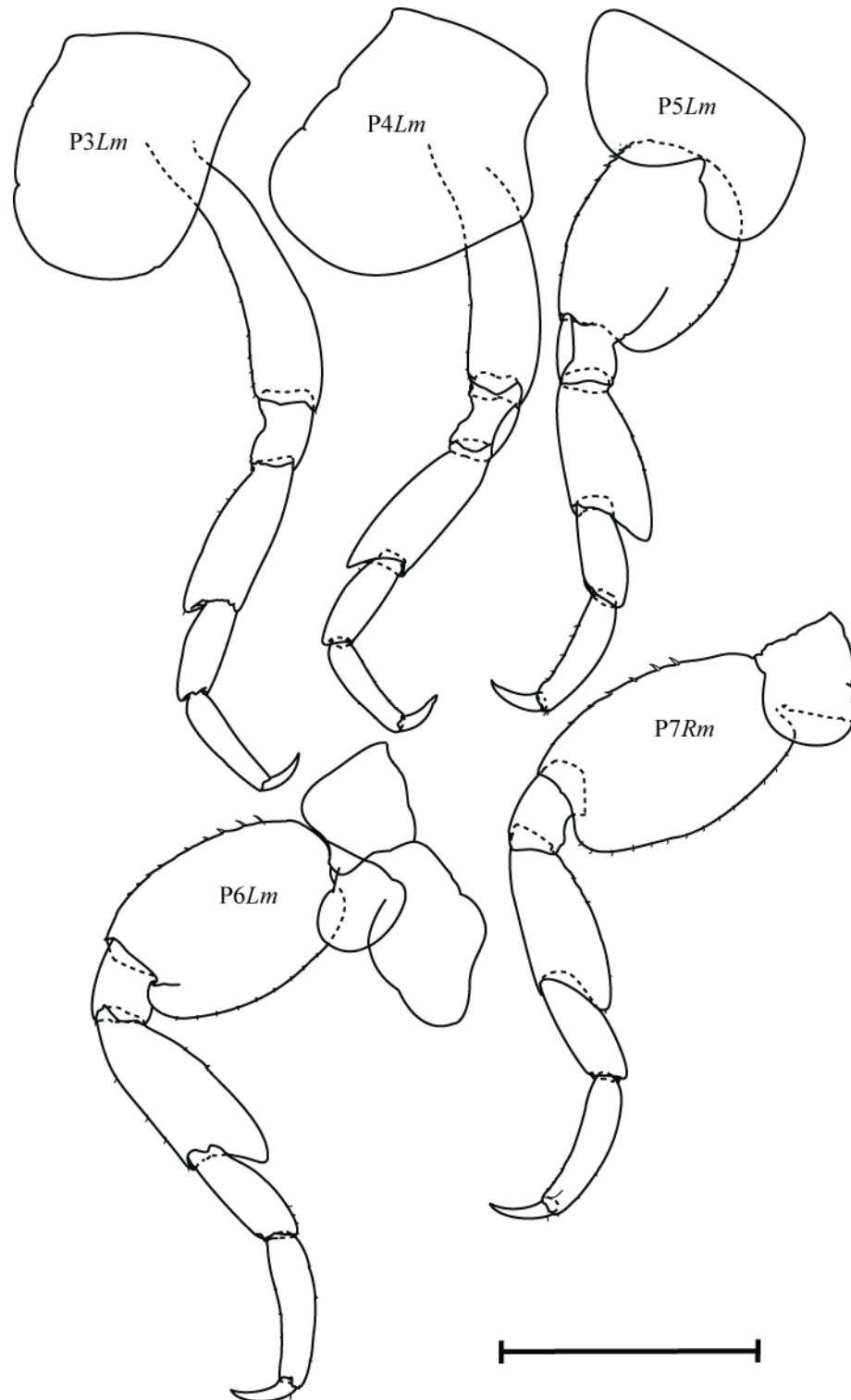
**Figure 5:** *Leucothoe* sp. n. male holotype "A", 8.6 mm. N1Rm, gnathopod 1 right medial; N1LI, gnathopod 1 left lateral; N2LI, gnathopod 2 Left lateral; N2Rm, gnathopod 2 right medial.

**Pereopods 3-4.** (Figure 6). Pereopod 3, coxa lobate, LW 1.32; basis linear, LW 3.96, slightly expanded distally, anterior margin with 8 small brush setae; merus anteriorly excavate. Pereopod 4, coxa excavate posteriorly, LW 1.16; basis linear, LW 3.26, with 7 small brush setae along anterior margin; merus anteriorly excavate.

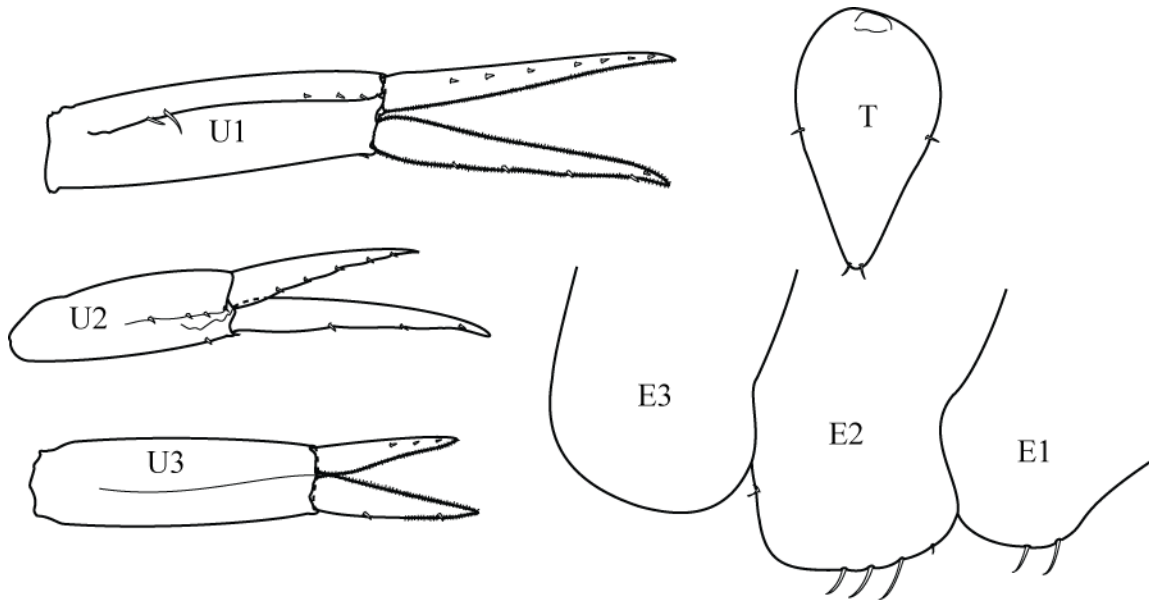
**Pereopods 5-7.** (Figure 6). Coxa 5 bilobed; coxae 6-7 reduced, ovate. Pereopods 5-7 with bases broadly expanded, LW 1.21: 1.32: 1.38; anterior margins serrate, with 7, 10, and 11 spines, respectively; posterior margins smooth, with marginal setae; article 3 with posterior distal lobe 0.28x: 0.36x: 0.38x of article 4.

**Epimera 1-3.** (Figure 7). E1 distal margin curved with 2 setae. E2 distal margin subtruncate with 3 ventral setae. E3 distal margin convex, smooth, lacking setae.

**Uropods.** (Figure 7). Uropods 1-3 LW 1.00: 0.77: 0.71; peduncle 1-3 LW 1.00: 0.66: 0.84. Uropod 1 peduncle 1.13x of inner ramus, with 6 medial spines and 1 spine on inner margin; rami subequal in length, outer ramus slightly shorter; outer ramus outer margin with 7 marginal spines, inner margin serrate; inner ramus with 4 spines on inner margin and 1 distal medial spine, margin serrate along entire length. Uropod 2 peduncle 0.87x length of inner ramus, 4 medial spines and 2 marginal spines on inner margin; outer ramus 0.76x length of inner ramus; outer ramus outer margin smooth, inner margin smooth with 5 submarginal spines; inner ramus, outer margin smooth, inner margin with 3 marginal setae. Uropod 3, peduncle 1.67x length of inner ramus, smooth; outer ramus 1.19x inner ramus, outer margin with 3 marginal spines, inner margin with double row of serrations along entire length; inner ramus outer margin serrate beyond 0.53 of inner margin, inner margin serrate.



**Figure 6:** *Leucothoe* sp. n. male holotype "A", 8.6 mm. P3Lm, pereopod 3 left medial; P4Lm, pereopod 4 left medial; P5Lm, pereopod 5 left medial; P6Lm, pereopod 6 Left medial; P7Rm, pereopod 7 right medial.



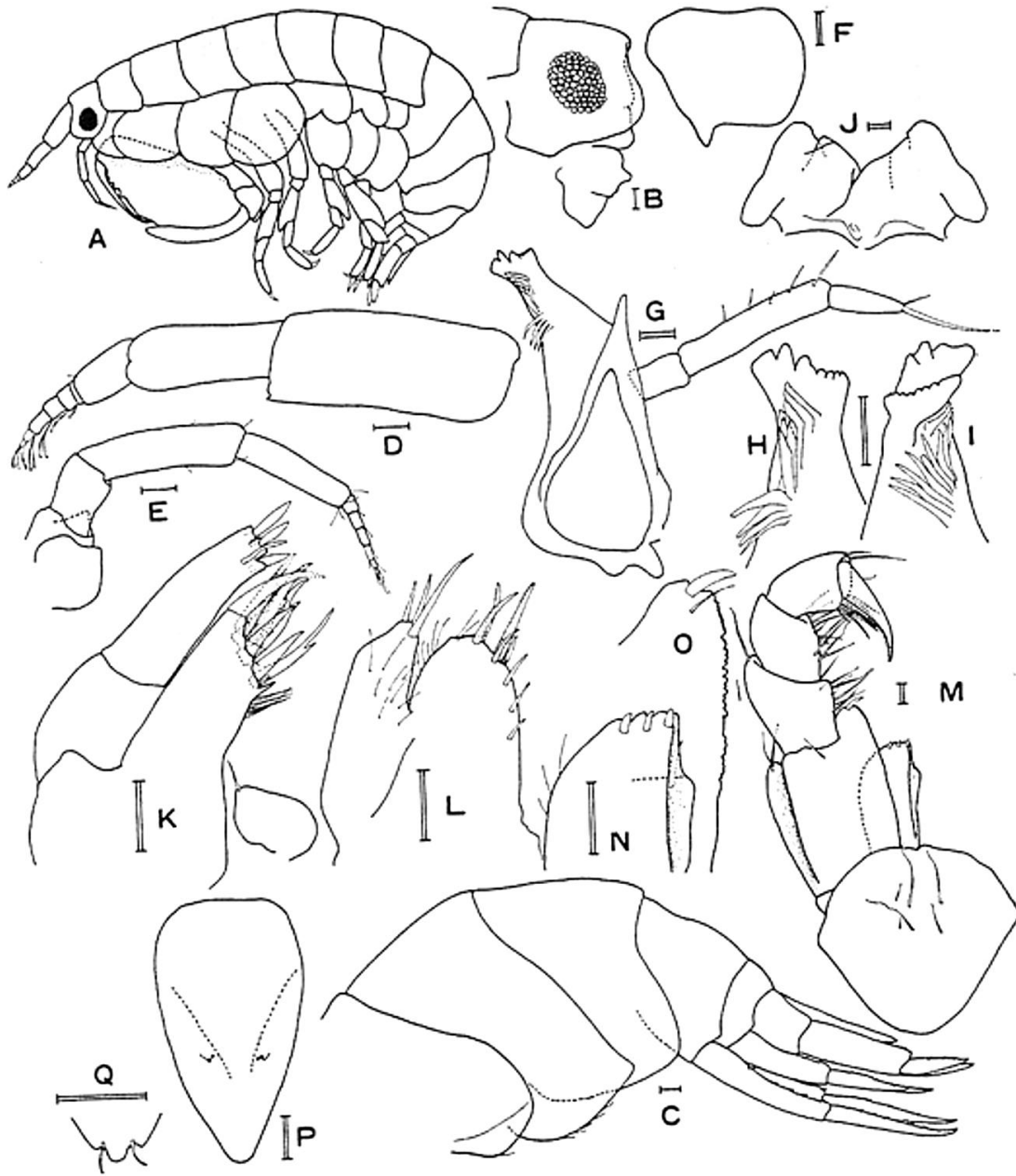
**Figure 7:** *Leucothoe* sp. n. male holotype "A", 8.6 mm. U1, uropod 1; U2, uropod 2; U3, uropod 3, T, telson; E3, epimera 3; E2, epimera 2; E1, epimera 1.

**Telson.** (Figure 7). Entire, LW 1.79, apical margin rounded, with 2 apical and 2 mid-lateral marginal setae.

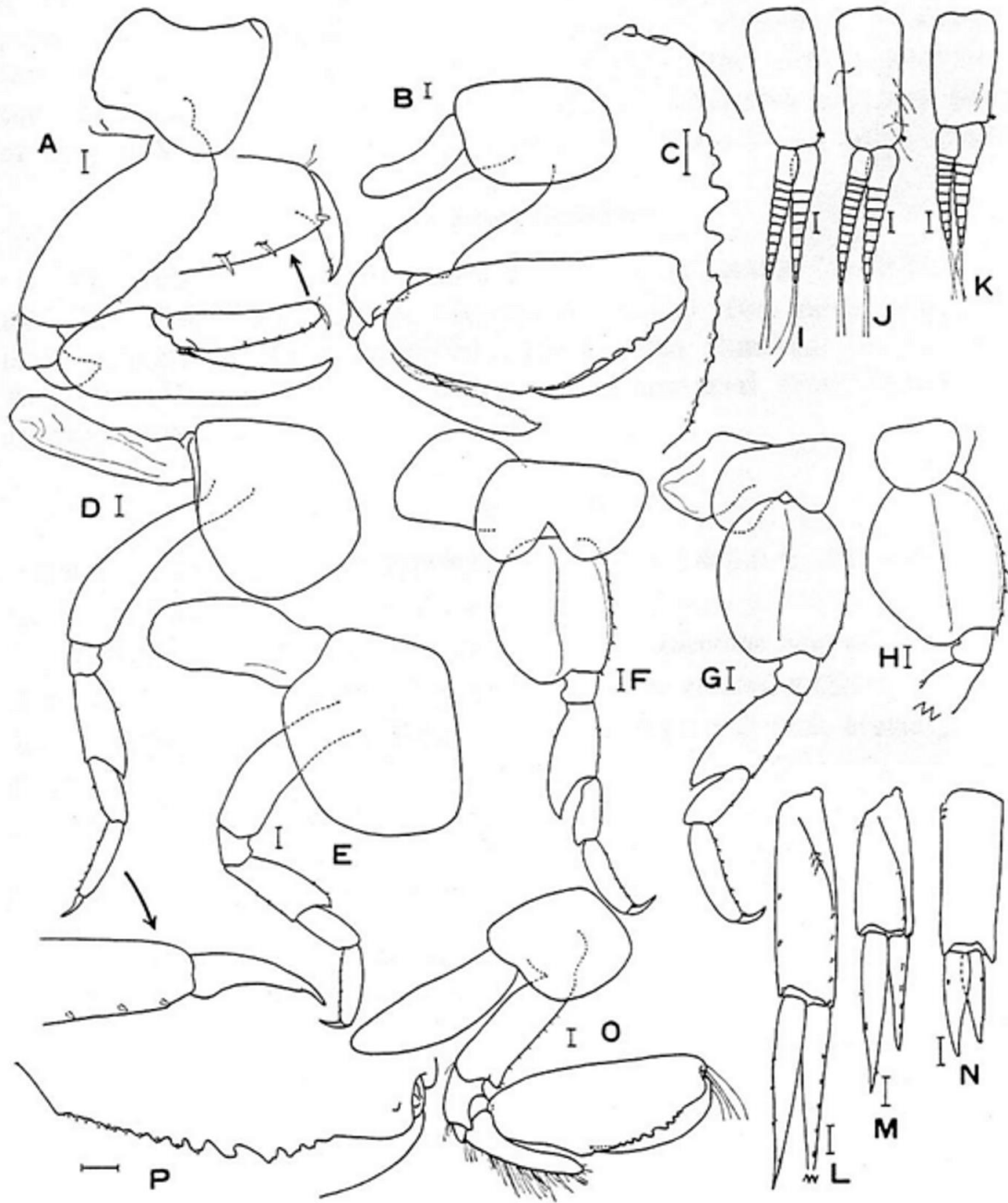
**Description of female paratype cat. no. XXXX (specimen C).** 8.8mm; similar to male except article 1 of gnathopod 1 more robust, LW 0.38.

**Relationships.** *Leucothoe* n. sp. Brucker and Thomas, 2016, is morphologically similar to *Leucothoe nagatai* Ishimaru, 1985 (figures 8 and 9), endemic to Japan. Both have short robust antenna 1 and antenna 2, modified blade-like spines in the raker rows of the mandibles, sinuous inner margin of the large outer plate on the maxilliped, carpochele gnathopod 1, and preference for ascidians as hosts. Both species also fit in Group IVa of Ledoyer's eight leucothoidean species groups (Ledoyer 1978, Ishimaru 1985). These groups are based on morphological characteristics, do not include any ecological or phylogenetic data, and do not represent a key for the genus *Leucothoe*. Group Iva is based

on several shared characteristics, including a shortened article 7 on gnathopod 1, a rounded corner on epimeron 3, and a dentate palm on gnathopod 2 (Ledoyer 1978, Ishimaru 1985). *Leucothoe nagatai* differs from *Leucothoe* n. sp. in having no accessory flagellum on article 3 of A1, with overall fewer accessory flagella on A1 and A2; a 2-articulate palp on maxilla 1; a smaller outer plate on the maxilliped, only 0.50x length of palp article 1; bilobed coxa 6, and unarmed projections on article 6 of gnathopod 2. In contrast, *Leucothoe* n. sp. has a 1-articulate palp on maxilla 1; the outer plate is 0.60x the length of palp article 1; coxa 6 is reduced and ovate, and the projections of the palm of gnathopod 2 are armed.



**Figure 8:** *Leucothoe nagatai* Ishimaru, 1985. Male, 9.3 mm (holotype). A, habitus; B, head; C, pleon; D, antenna 1 (R, inn); E, antenna 2 (R, out); F, labrum; G-H, mandible (R, inn); I, mandible (L, inn); J, labium; K-L, maxillae 1-2 (R, vt); M-O, maxilliped (vt); P, telson (ds). Male, 9.3 mm. Q, apex of telson (ds). Bar scales 0.1 mm, double bar scales 0.05 mm. at, anterior view; inn, inner view; out, outer view; vt, ventral view; ds, dorsal view; R, right part; L, left part.



**Figure 9:** *Leucothoe nagatai* Ishimaru, 1985. Male, 9.3 mm (holotype). A-B, gnathopods 1-2 (R, out); C, palm of gnathopod 2; D-H, pereopods 3-7 (R, out); I-K, Pleopods 1-3 (R, at); L-N, uropods 1-3 (R, ds). Female, 9.6 mm (allotype). O, gnathopod 2 (R, out); P, palm of gnathopod 2. Bar scales 0.1 mm. Abbreviations as in Figure 8.

The close morphological relationship between *Leucothoe* n. sp. and *L. nagatai* requires examination of two other similar species: *Paraleucothoe novaehollandiae* (Haswell, 1879), and its junior synonym *L. brevidigitata* (Miers, 1884), established by White (2013), and *L. alata* (Barnard, 1959). *Leucothoe alata* differs from *Leucothoe* n. sp. in having a small outer palp on the maxilliped with article 3 on the palp wider than article 4, a 2-articulate palp on maxilla 1, lacinia mobilis much smaller than incisor, mediofacial setae absent near base of propodus on gnathopod 2, and telson lacking mid-lateral setae. The characteristics distinguishing *P. novaehollandiae* from *Leucothoe* n. sp. include: the distal tapering palm structure of gnathopod 2, lacinia mobilis on both right and left mandibles, and telson lacking ornamentation. In contrast, *Leucothoe* n. sp. the palm of gnathopod 2 does not taper distally, lacinia mobilis is located only on the left mandible, and the telson has 2 apical and 2 mid-lateral marginal setae.



**Figure 10:** *Cnemidocarpa bicornuta* photo by James D. Thomas



*Leucothoe* n. sp. and *L. nagatai* resemble the genus *Paraleucothoe* in having an extended outer plate on the maxilliped. However, in *Paraleucothoe* species, the outer plate extends beyond article 1 of the palp. In addition, terminal males of *Paraleucothoe* species have a greatly enlarged “shoe shaped” gnathopod 1.



**Figure 11:** *Corella eumyota* photo by James D. Thomas

**Ecology.** Primarily found as male and female pairs with juveniles in the atrium of the subtidal, solitary ascidian *Cnemidocarpa bicornuta* (Sluiter, 1900) (Figure 10). On New Zealand, *C. bicornuta* occurs on North Island from Ninety Mile Beach in the north to Wellington in the south on the west coast, and has also been reported from East Cape on the east coast. On South Island it ranges from Canterbury Bight (northernmost) to

Dunedin (southernmost) in the Pacific and Milford Sound (northernmost) to Long Sound (southernmost) in the Tasman Sea. It has also been documented off two islands off the southernmost tip of New Zealand: Ruapuke Island (west coast) and Stewart Island (north coast and Paterson Inlet on the east coast). It has also been observed at Lord Howe Island (Australia) in the Tasman Sea (Secretariat: 2013 ). Occasionally found in association with *Corella eumyota* Traustedt (1882) (Figure 11) and *Asterocarpa coerulea* Quoy and Gaimard (1834) (Figure 12) (J.Thomas personal observations).

**Distribution.** Omaha Bay and Jones Bay, New Zealand, 2-10 m.



**Figure 12:** *Asterocarpa coerulea* photo by James D. Thomas

#### **4. Discussion:**

The documentation of *Leucothoe* n. sp. is important, because it is associated with an endemic ascidian and is one of only four leucothoid species documented as native to New Zealand. The others are *L. macquariae* Krapp-Shickel and DeBoyer, 2014, *L. trailli*

Thompson, 1882, and *L. tridens* Stebbing, 1888. Of these species, only one is documented with an ascidian host: *L. macquariae* (Thomas 2014). *Leucothoe* n. sp. is a resident in the host *Cnemidocarpa bicornuta*, a subtidal, solitary tunicate studied for its production of purines (Lindsay, Battershill et al. 1998). Although considered endemic to New Zealand, *C. bicornuta* has also been observed at Lord Howe Island, which is in Australian waters (Secretariat: 2013 ).

*Cnemidocarpa bicornuta* is in suborder Stolidobranchia, which is characterized by a heavily pleated pharyngeal lining with transverse and longitudinal vessels and gill slits varying in number with tunicate size (Ruppert, Fox et al. 2004, del Mundo 2009). Stolidobranch families include Molgulidae, Pyuridae, and Styelidae (del Mundo 2009), of which the latter two are most common in New Zealand (Morton and Miller 1973, del Mundo 2009). *Cnemidocarpa* is a styelid. Morphologically, styelids have branchial baskets with 4 simple branchial tentacles and endocarps, semi-transparent dermal projections, which cover the inner surface of the papillae in the peribranchial cavity. Pyurids have 6 or more tentacles, and only a few species have endocarps, which are smaller than those of styelids. *Cnemidocarpa* differs from other styelids in having ovaries covered by a protective sheath and encasing the tubular male gonads. In other styelid genera, the male and female organs are separated (VanName 1945, del Mundo 2009).

Until detailed dissections and analyses were completed, *Leucothoe* n. sp. was initially mistaken for the recent invader species *L. nagatai*, in New Zealand (J. Thomas, unpublished data). Native to Japan, *L. nagatai* is a known invasive in San Diego Bay, California (Sorensen, Swope et al. 2013). Invasive species are non-indigenous organisms that establish populations in new areas, causing displacement of native species and

changes in community structure (Molnar, Gamboa et al. 2008, Jiménez-Valverde, Peterson et al. 2011). The establishment and success of marine invasive species is a growing threat to biodiversity across the globe (Cebrian, Linares et al. 2012), because they may alter habitat by manipulating and outcompeting native organisms for available resources (Gray 1997, Molnar, Gamboa et al. 2008, Cebrian, Linares et al. 2012). Invasive amphipod species have caused drastic changes in the macroinvertebrate framework in European rivers, e.g., Rhine, Vistula, Moselle, and Oder. Invasive amphipod species including *Gammarus tigrinus* Sexton, 1939, *Chaetogammarus ischnus* (Stebbing 1899), *Obesogammarus crassus* (G. O. Sars 1894), *Dikerogammarus haemobaphes* (Eichwald 1841), and *Dikerogammarus villosus* (Sowinsky 1894) have successfully outcompeted many of the native amphipod species such as *Gammarus duebeni* Lilljeborg, 1852, *G. roeselii* Gervais, 1835, and *G. fossarum* Koch, 1836, for resources. The invaders have also colonized areas that natives could not due to pollution and have replaced native species as prey items for percid and gobiid fishes (Dick, Platvoet et al. 2002, Grabowski, Bacela et al. 2007). Dick and Platvoet (2000) and Dick et al. (2002) found that the introduction of *D. villosus* to the Dutch Lower River Rhine coincided with overall declines of local macroinvertebrates. They also observed direct competition between *D. villosus* and the native *G. duebeni*, in which *D. villosus* preyed upon *G. deubeni* and replaced them. The invasion of *D. villosus* illustrates the potential impact of *L. nagatai* on *Leucothoe* n. sp. The two species share a common host preference, and have similar gross morphologies in the short antennae, color, and relative shape of gnathopods 1 and 2. However, as with other members of the *L. spinicarpa* complex, examination of fine morphological details of appendages reveal consistent

differences among species. Limitations in host resources may cause competition between the two species. Additionally, the endocommensal relationship of *L. nagatai* with ascidians and its similar appearance to *Leucothoe* n. sp. will make eradication of the invader a difficult process.

## **5. Conclusion:**

Biological monitoring of coastal ecosystems, as well as reliable taxonomic diagnoses, are important aspects of addressing changes in marine biodiversity. Carrying out consistent biological surveys to assess the health of local marine habitats contributes to management of the spread and impact of invasive species. This study highlights the importance of morphological taxonomic data. As many studies now rely upon molecular genetic data to compare and identify different species, it is important to remember that morphological characters are important in field identification and are more cost effective as well.

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