Invertebrate Biology 126(2): 120–137.

DOI: 10.1111/j.1744-7410.2007.00083.x

Morphology and life cycle of a new loriciferan from the Atlantic coast of Florida with an emended diagnosis and life cycle of Nanaloricidae (Loricifera)

Reinhardt Møbjerg Kristensen,[1,a] Iben Heiner,[1] and Robert P. Higgins[2]

[1] Zoological Museum, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark
[2] 122 Strawbridge Court, Asheville, North Carolina 28803, USA

Abstract. A new interstitial loriciferan, *Nanaloricus gwenae* sp. nov., is described from coarse-sand and shell-hash habitats (*Amphioxus* sand), at 15–17 m depth, 6–7 miles off the coast of Fort Pierce, FL, USA. The new species is very closely related to the type species *Nanaloricus mysticus* found off the coast of Roscoff, France in nearly the same kind of sediment (*Dentalium* sand). All life stages (Higgins larva, postlarva, and adult) in the life cycle of the new species were found through the 10-year investigation, leading to the conclusion that all species of Nanaloricidae have only a sexual reproductive cycle. The adult of the new species can be distinguished by the different body shape, the lorical spikes and sculpture, and the shape of the scalids. The postlarva has stronger longitudinal ridges on the lorical plates and the Higgins larva has toes with smaller mucrones than those of *N. mysticus*. An emended definition of the Nanaloricidae is provided based on the new formula of the number of rows and shape of the scalids on the introvert. The associated meiofauna found in the subtidal sand or shell hash is both abundant and diverse. Tardigrades, gastrotrichs, and kinorhynchs are among the most common associates. *N. gwenae* sp. nov. is represented by only eight specimens collected during a 10-year period of sampling this habitat.

Additional key words: meiofauna, *Nanaloricus gwenae*

In 1983, the phylum Loricifera was erected and *Nanaloricus mysticus* KRISTENSEN 1983 was designated as type species (Kristensen 1983). The type species was described from 79 specimens of both adults and larval stages collected in March 1982 from shelly gravel at 25–30 m depth off the coast of Roscoff, France. In the description of *N. mysticus* from Roscoff, additional specimens were included from the Barlett Expedition, January 31, 1975, near the Azores Islands at 480 m depth and from a shelly substrate 6–7 miles off the coast of Fort Pierce, Florida. These latter specimens were, at the time of the publication of the new phylum, believed to be conspecific with *N. mysticus* (see Kristensen 1983). However, more detailed studies have shown that the specimens from Azores Islands and Florida represent two separate new species. As a result of this discovery, intensive sampling for more specimens was conducted over a 10-year period in the vicinity of Fort Pierce, Florida. Unfortunately, 10 years of intensive sampling of the coarse shell-hash habitat has only resulted in a total of eight specimens, which are reported and described in this article.

Since the discovery of *N. mysticus* and subsequently the phylum Loricifera, several new species have been described. The description in 1986 of a new family of Loricifera, Pliciloricidae, including two new genera with eight new species, was the beginning of intensive loriciferan research (Higgins & Kristensen 1986). Presently, a total of 21 species have been described, arranged in two families and six genera. Several more species are on the verge of publication and several hundred specimens are waiting to be described, e.g., several hundreds of specimens have been collected in the northern part of the Gulf of Mexico (Hubbard et al. 1988) from depths of 298–2959 m. These specimens represent as many as six new species and are currently being described (I. Heiner, unpubl. data).

The family Nanaloricidae currently includes three genera, *Nanaloricus*, *Armorloricus*, and *Phoenicilloricus*, with two, three, and one species, respectively.

[a] Author for correspondence.
E-mail: rmkristensen@snm.ku.dk

After the description of *N. mysticus* in 1983, the next nanaloricid to be described was *Nanaloricus khaitatus* TODARO & KRISTENSEN 1998 from Livorno, Italy. The two species *N. mysticus* and *N. khaitatus* were both collected from shallow waters in the intertidal and subtidal zones and it was assumed that the family consisted of solely interstitial species. This, however, was reevaluated with the description of the second genus and third species of Nanaloricidae, *Phoenicilloricus simplidigitatus* GAD 2004, from a depth of 1813 m near the Kilinailau trench close to Papua New Guinea. In 2004, the third genus *Armorloricus* was described from a locality (Trezen ar Skoden, Roscoff) near the type locality of *N. mysticus*. The genus included two new species, *Armorloricus elegans* KRISTENSEN & GAD 2004 and *Armorloricus davidi* KRISTENSEN & GAD 2004, and two types of Higgins larvae, which could not be linked reliably to the corresponding adult stages (Kristensen & Gad 2004). However, further investigations have shown that the Higgins larva of *Armorloricus* sp. 1 corresponds with *A. elegans*, and the other Higgins larva *Armorloricus* sp. 2 corresponds with *A. davidi* (Heiner 2004; Kristensen & Gad 2004). Shortly after the description of this genus, a new species, *Armorloricus kristenseni* HEINER 2004, was described from the Faroe Bank, located southwest of the Faroe Islands.

In the description of the type species *N. mysticus*, some errors were made. This is especially evident in the interpretation and numbering of the scalids on the introvert in both adults and larvae and of the trichoscalids and their basal plates on the neck in adults. Since then, new information has been gathered from descriptions of the five additional species and the new species *N. gwenae* sp. nov. described herein. Hence, an emended definition of the family Nanaloricidae is necessary and will be presented. Additionally, a reevaluation of the life cycle of Nanaloricidae will be included with new data from an undescribed nanaloricid from the Galapagos Islands (I. Heiner, unpubl. data).

Methods

Collection of specimens

Specimens were obtained from samples of coarse quartz particles mixed with shell hash taken at five sites 15–17 m deep, along the Atlantic coast of Florida near Fort Pierce (Fig. 1), 9.66 km (6 miles) and 11.27 km (7 miles) east of Fort Pierce Inlet (27°30.0′N, 80°12.1′W). As noted in two earlier publications (Kristensen & Higgins 1989; Thomas et al. 1995), this habitat offers ample interstitial space

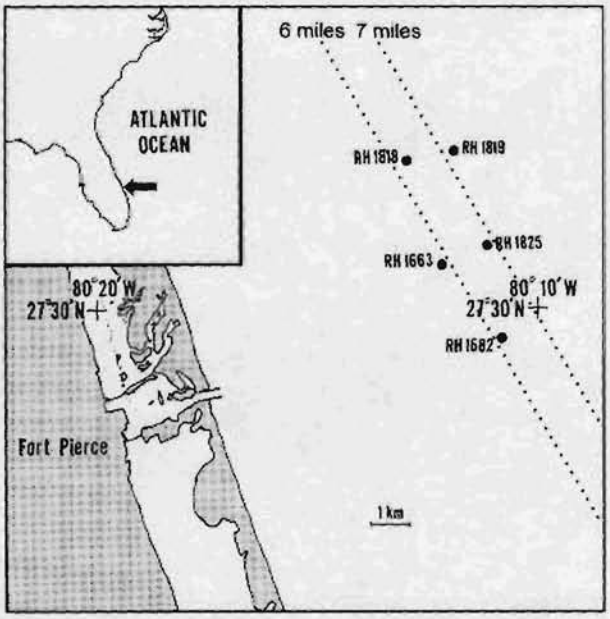

Fig. 1. Map of Fort Pierce, FL, USA, with the five different stations marked with RH numbers.

for the existence of a highly diverse mesopsammal assemblage of invertebrates. The five stations refer to R.P. Higgins' collection numbers (RH) and are noted in Fig. 1. All specimens have an RMK number (R.M. Kristensen's specimen number) and an official type species number from the Zoological Museum of Copenhagen (ZMUC).

RH 1818: October 4, 1983, from coarse quartz sand, 17 m depth, 27°33.1′N, 80°13.3′W. One adult female (holotype), Figs. 2 and 4D (RMK 831004.2) (ZMUC LOR 454).

RH 1819: October 4, 1983, from coarse sand with shell hash, 17 m depth, 27°33.3′N, 80°11.9′W. One postlarva (paratype), Figs. 3 and 4B (RMK 831004.3) (ZMUC LOR 455).

RH 1825: October 4, 1983, from medium sand mixed with shell hash, 17 m depth, 27°31.2′N, 80°11.1′W. One Higgins larva, ventral aspect, Figs. 4A and 5 (RMK 831004.9) (ZMUC LOR 456) and one exuvium of postlarva, Fig. 4C (RMK 831004.8) (ZMUC LOR 457).

RH 1663: January 31, 1983, from shell hash, 15 m depth, 27°30.9′N, 80°12.1′W. One Higgins larva (RMK 830131.10) (ZMUC LOR 458).

RH 1682: April 5, 1983, from coarse quartz sand mixed with shell hash, 17 m depth, 27°29.4′N, 80°13.3′W. One Higgins larva, lateral aspect, Fig. 7 (RMK 830405.1) (ZMUC 459), one last larval stage Higgins larva, dorsal aspect, Fig. 6 (RMK 830405.2)

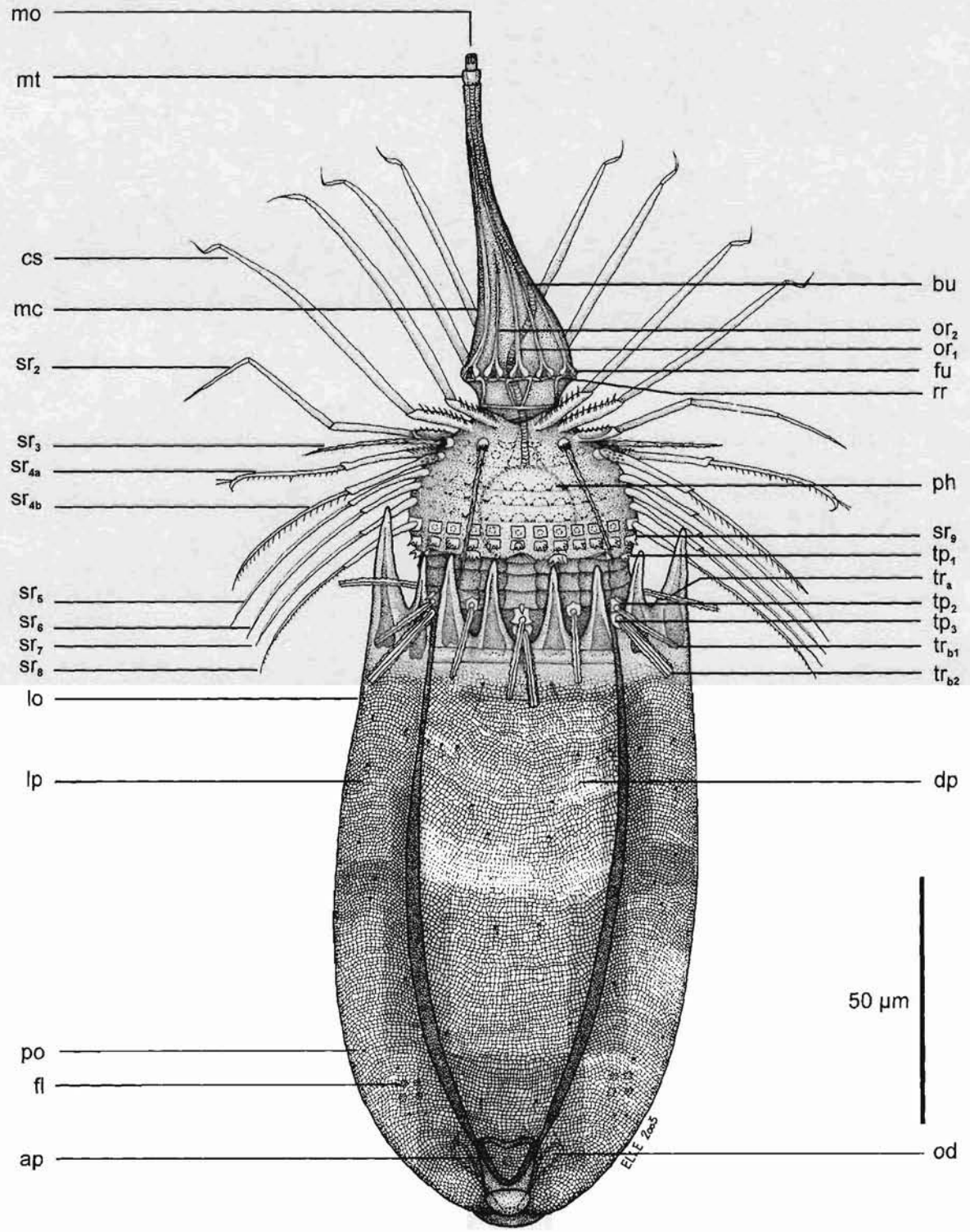

Fig. 2. Drawing of *Nanaloricus gwenae* sp. nov. holotypic adult female (ZMUC LOR 454), dorsal view.

(ZMUC LOR 460), and one exuvium of a Higgins larva (RMK 830405.3) (ZMUC LOR 461).

A Higgins anchor dredge was used to obtain shell-hash sediment. Meiofauna and associated lighter-weight fractions were separated from the shell hash by placing ~1 L of sediment in 5 L of freshwater, stirring for <15 s, and decanting through a 62-μm-mesh sieve. Material retained in each successive sieve was transferred immediately into a container of filtered seawater until the entire sediment sample was processed. This material was again decanted through the sieve. Some was transferred to small dishes for live sorting, and the remainder was fixed in 4–6% buffered (sodium borate) formalin to which a tincture of "Rose Bengal" was added to stain all organisms in order to facilitate sorting.

Observation of fixed specimens

Specimens were transferred to a small dish with 1 part glycerin to 20 parts 70% ethanol, which was allowed to evaporate to glycerin only. Glycerin-impregnated specimens were mounted in this same medium on glass microslides or on Cobb aluminum slide frame mounts. The microslide preparations were sealed with Murrayite. In 2005, the microslides were refilled with glycerin and resealed with Glyceel® (J.W. Bates, USA). Illustrations were made using a camera lucida. Photographs were taken through a Nikon Microphot-Fx microscope (Japan) equipped with interference contrast optics. Specimens of the new species, *Nanaloricus gwenae* sp. nov., have been deposited in the ZMUC, Denmark.

Abbreviations used in figures and text:

af	anal field
an	anus
ap	anal plate
bu	buccal tube
cp	closing plate of thorax
cs	clavoscalid of row 1
dp	dorsal lorica plate
eg	egg
fl	flower-shaped flosculum
fu	oral furca
la_1	anterolateral seta
la_2	anteromedial seta
la_3	anteroventral seta
lo	lorica
lp	laterodorsal lorica plates
mc	mouth cone
mo	mouth opening
mt	mouth tube
mu	mucrones
od	ovary duct
or_1	primary oral ridge
or_2	secondary oral ridge
ph	pharynx
po	pore on the lorica plates
rr	rectangular ridge on mouth cone
se_1	posterodorsal seta
se_2	posterolateral seta
se_3	posteroterminal seta
sr_{2-9}	scalid row 2–9
sr_2	leg-shaped scalid of second row in adult
sr_3	feather-like scalid of third row in adult
th	thorax
to	toe
tp_{1-3}	trichoscalid basal plates 1–3
tr_a	single trichoscalid
tr_{b1}	primary appendage of double trichoscalid
tr_{b2}	secondary appendage of double trichoscalid
tu	tubes

Results

Systematic account

Phylum Loricifera Kristensen 1983

Definition. Adults are 115–425 μm long and are bilaterally symmetrical marine metazoans. The following features are autapomorphic characters of the adult loriciferans: (1) the body is divided into five regions: mouth cone, head (introvert), neck, thorax, and abdomen; (2) the internal buccal armature is arranged in a strict tri- or hexagonal pattern; (3) there are nine rows of sensory or locomotory appendages (scalids), each with muscles attached to the introvert; (4) the first row of scalids consists of eight multiciliary club-shaped or blunt-tipped clavoscalids; (5) the second to ninth rows of scalids consist of spinoscalids of various shapes and numbers; (6) the neck consists of three rows of basal plates and one row of trichoscalids; (7) trichoscalids are flattened and serrated; there are either 15 single or seven double alternating with eight single trichoscalids; (8) the thorax is accordion-like, without appendages; (9) the abdomen is covered with a more or less cuticularized lorica with six to ten plates or 22–40 plicae; (10) the mouth cone is not eversible, but protrusible; and (11) the mouth cone and head can be retracted into the loricated abdomen.

Higgins larvae are 48–780 μm long, with the same body regions as the adult. Autapomorphic characters of the larval loriciferans are as follows: (1) the mouth cone is sometimes with a midventral seta and/or six to 12 oral stylets; internal armature (when present) is in a strict tri- or hexagonal pattern; (2) there is an eversible introvert with eight clavoscalids and up to

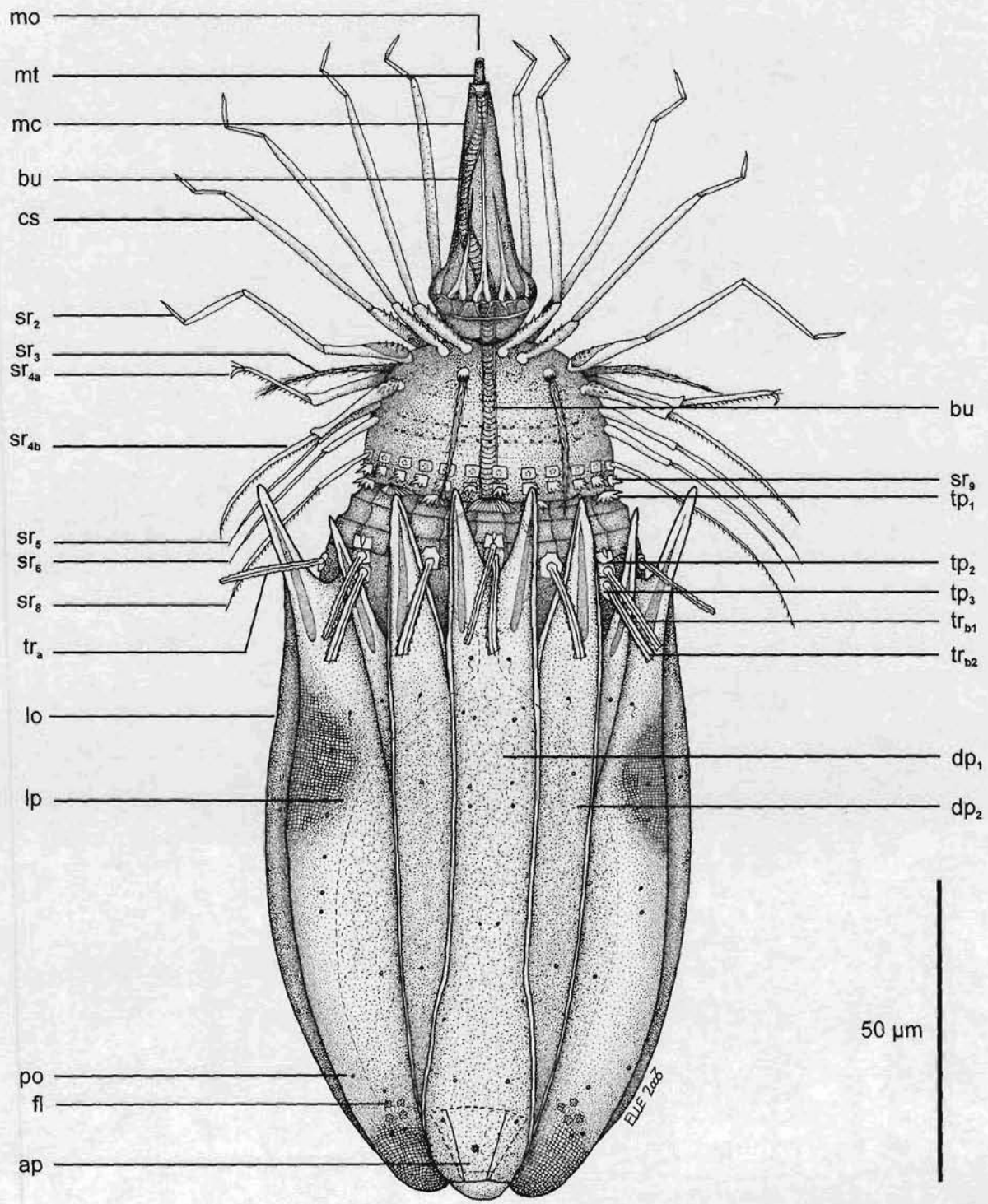

Fig. 3. Drawing of *Nanaloricus gwenae* sp. nov. paratypic postlarva (ZMUC LOR 455), dorsal view.

seven rows of spinoscalids; (3) the neck is used as a closing apparatus; (4) the accordion-shaped thorax has plates formed from transverse and longitudinal folds; (5) the lorica is longitudinally folded; (6) two or three locomotory setae are present ventrally between the thorax and the abdomen; (7) toes are located caudally; (8) two or three sensory setae are present on the posterior part of the lorica; and (9) there are several larval instars growing by a series of molts.

Order Nanaloricida KRISTENSEN 1983

Definition. Same as the phylum.

Family Nanaloricidae KRISTENSEN 1983

Emended definition. *Adults* are 210–425 μm long. The length of males and females is the same. The definition of body parts (mouth cone, introvert, neck, thorax, and loricated abdomen), as described in Higgins & Kristensen (1986), is emended.

The protrusible mouth cone, centered anterior to the introvert, consists of three sections. The first section (closest to the introvert) has eight cone retractor muscles, each attaching distally to a large cuticular apodeme, the furca. The so-called eight oral stylets (Kristensen 1983) are not stylets but cuticular oral ridges. The cuticle of the second section is strongly sclerotized and the section forms a hexagonal part terminally. The third section of the mouth cone continues as a mouth tube that can be withdrawn telescopically inside the mouth cone. The length of the mouth tube varies greatly. The mouth tube is an extruded portion of the cuticularized buccal canal. Inside the mouth cone and the head, the buccal canal continues as a long, flexible, annulated buccal tube. The buccal tube enters the pharyngeal bulb mesially and forms a large apophysis complex. The triradiated myoepithelial pharynx has three rows of placoids, each with five distinct macroplacoids.

The introvert has nine rows of scalids. In the first row, the clavoscalids, sexual dimorphism is present. Females have eight two-segmented clavoscalids. Males have two ventral clavoscalids similar to those of the females and three pairs of dorsal clavoscalids divided into primary, secondary, and tertiary clavoscalids. Tertiary branches of the dorsal pairs are enlarged. Males always have a total of 20 clavoscalids. The second to ninth rows consist of spinoscalids. The second row consists of nine leg-shaped scalids and the third row consists of seven feather-like scalids. The fourth row consists of 16 smaller leg-shaped spinoscalids, either only of one type or of two different types. The fifth to seventh rows consist of 30 simple uniform scalids in each row. The eighth row consists of 30 trichoscalid-like scalids and the ninth row consists of 30 beak-like (*Armorloricus*) or teeth-like (*Nanaloricus*) scalids situated on basal plates.

The neck consists of three rows of trichoscalid basal plates with 15 plates in each row and 15 trichoscalids, seven double and eight single. This makes a total of 22 appendages on the neck. Males have the two ventral trichoscalids modified into hook-shaped claspers. They are probably used to hold the female during copulation, although this has never been observed. The thorax has no appendages and most of it is usually hidden by the abdominal lorica.

The lorica consists of six longitudinal plates with anteriorly oriented hollow spikes. The number of lorical spikes varies, because the midventral one is sometimes reduced; however, in most species there are 15 lorical spikes. No tubuli or adhesive tubes are present, but several unicellular glands (pores) open directly on the surface of the lorica. These pores are arranged in a species-specific pattern over the lorica. The lorica is either smooth as in *Armorloricus* or has honeycomb sculpture as in *Nanaloricus*. Nine flosculi are present posteriorly on the lorica: four on each dorsolateral plate and one on the anal plate. Internally, there is a pair of saccate gonads, ovaries or testis, which opens terminally through the anus. Protonephridia are located inside the gonads.

The *postlarva* is 189–300 μm long and resembles the adult female, except for a few distinct characters such as a shorter mouth cone, a missing seventh row of the spinoscalids, a dorsal lorica plate that is sometimes split into three, and a lack of gonads. The postlarva lives in the sediment as the adults.

Higgins larvae are 80–206 μm long. The mouth cone is without an external or internal armature. The introvert has seven rows of scalids. The first row consists of eight clavoscalids. The number of spinoscalids in the second row varies from zero in *Armorloricus* to seven in some species of *Nanaloricus* and to ten in *Phoeniciloricus*. The third, fourth, and fifth rows consist of 15 scalids of various appearances in each row. The sixth and seventh rows consist of 13 scalids in each row. These scalids are more tooth-like and most of them are positioned on basal plates. The middorsal scalid in the last rows is modified into a club-shaped or leaf-like scalid.

The neck is indistinct and the thorax consists of five folds or plates; the first two ventral plates are enlarged and form a closing apparatus for the retractable head. The lorica has several longitudinal folds and a distinct honeycomb sculpture. There are three pairs of locomotory setae located ventrally on the anterior edge of the lorica. Posteriorly there are two caudal leaf-shaped toes, each with mucrones located on the basal part.

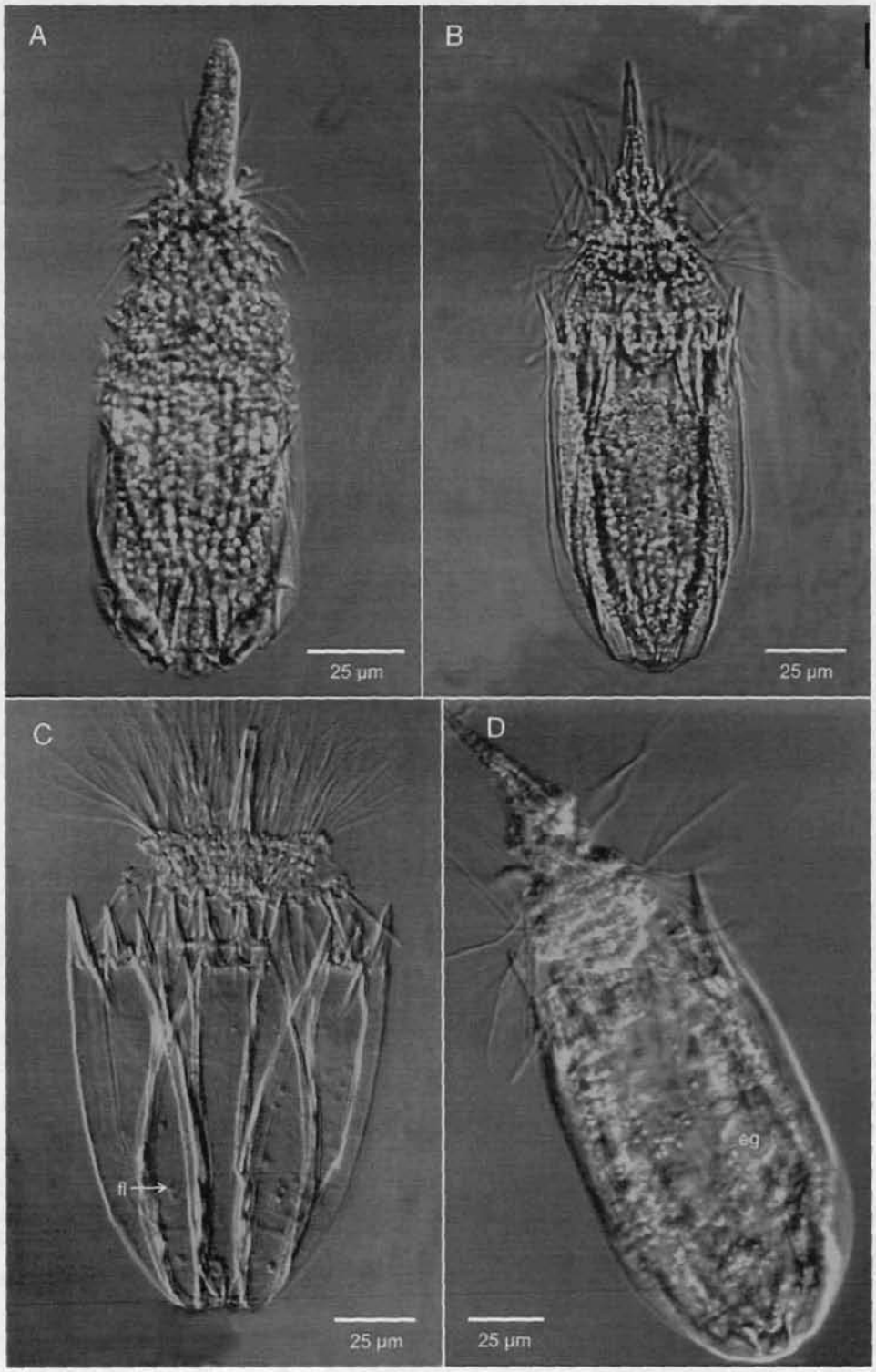

Fig. 4. Photos of *Nanaloricus gwenae* sp. nov. **A.** Paratypic Higgins larva, ventral view (ZMUC LOR 456). **B.** Paratypic postlarva (ZMUC LOR 455). **C.** Postlarval exuvium (ZMUC LOR 457). **D.** Holotypic female (ZMUC LOR 454).

The terminal part of the toe is spine shaped with a terminal pore for an adhesive gland. There are three pairs of sensory setae: the first two have a large base (cirrophore) and are located on the two lateral anal plates; the last pair of setae are short, without bases, and are located on each side of the anus, which is situated dorsally on an anal plate. Three flosculi are present on the posterior dorsal plates.

Genus *Nanaloricus* KRISTENSEN 1983

Definition. Same as the family.

Type species. *Nanaloricus mysticus* KRISTENSEN 1983.

Etymology. The generic name is derived from the Greek *nana* (dwarf), plus *lorica* (belt or girdle).

Nanaloricus gwenae sp. nov.
(Figs. 2–9)

Diagnosis. The adult female is 239 μm long (including the 75-μm mouth cone), with a total of 190 scalids in nine rows on the introvert. The first row has eight slender clavoscalids with small papillae at the base; the second row consists of nine large leg-shaped spinoscalids alternating asymmetrically with the third row, which consists of seven feather-shaped spinoscalids; the fourth row consists of eight smaller leg-shaped spinoscalids alternating with eight shorter claw-tipped spinoscalids; the eighth row has 30 trichoscalid-like scalids; and the ninth row has 30 tooth-like scalids. The neck has seven double and eight single trichoscalids; trichoscalids are relatively short (15–16 μm). The first row of trichoscalid basal plates is positioned just below the introvert. The lorica has six strongly sclerotized plates, the honeycomb sculpture barely visible with light microscopy. There are four flosculi on each laterodorsal plate and one flosculum on the anal plate. The anal cone is indistinct or reduced. Adult male unknown.

The postlarva is 189 μm long, with eight rows of scalids; the seventh row of 30 spinoscalids present in the adult is absent. The lorica has six poorly sclerotized plates, with 15 longitudinal double ridges aligned with each lorical spike; the spikes are longer in the postlarva than in the adult. The anal cone is located ventrally. The body cavity has no gonads, but is filled with coelomocytes.

Higgins larvae are 128–189 μm long, with a long two-parted mouth cone. The introvert has seven scalid rows: the first row consists of eight clavoscalids with small papillae, the second row has four spinoscalids in young larvae and six (two extra) in older larvae, three midventral pairs of hook-shaped spinoscalids (s4a, s4a′, s5a, s5a′, s5b, s5b′), and a single middorsal clavoid sensory organ (s7g), and the seventh row has teeth-like scalids. The locomotory spines of the lorica are located midventrally and consist of three pairs, each pair with a common base. Two tubes leading into spiraled multicellular glands are located laterally on the abdomen. Three pairs of sensory setae (se_{1-3}) are present posteriorly on the caudal plates. The dorsocaudal setae (se_3) near the anus are short (4 μm) and barely visible with light microscopy. The honeycomb sculpture of the lorica is more distinct than in the adult.

Etymology. The species is named in honor of Gwen Higgins, wife of Robert P. Higgins.

Description of the adult. The holotypic adult female is 239 μm long, including the mouth cone, and 74 μm wide (Figs. 2, 4D). The mouth cone is long (75 μm), flexible, and with three distinct sections. The first section consists of eight large sclerotized furcae (fu) and eight oral ridges (or_1 and or_2). Below the furcae are eight cuticularized rectangular ridges (rr). The shorter secondary oral ridges (or_2) are only in the first section whereas the primary oral ridges (or_1) continue into the second section. Therefore, there are only four oral ridges in the second section. The primary oral ridges are extremely long (50 μm), nearly twice as long as the secondary ones (26 μm). The third section consists of a short (6 μm) mouth tube (mt), which is subsequently divided into two parts. There are no external oral stylets. Internally there is a long annulated buccal tube (bu), which originates adjacent to the mouth tube and terminates anterior to the pharynx (ph).

The introvert consists of nine rows of scalids. The first row (cs) has eight slender clavoscalids divided into three segments. The first segment has many small papillae at the base, the second segment is very long and narrow, and the third segment is short and hook shaped. The second row (sr_2) consists of nine large leg-shaped spinoscalids. The large leg-shaped scalids are divided into four segments. The first segment is short with several papillae. The second, third, and fourth segments are of nearly equal length and the fourth ends in a sharp spine. The second row alternates asymmetrically with the third row, because there are no third row scalids below the large leg-shaped scalids in the midventral and almost middorsal positions. The third row (sr_3) consists of seven feather-shaped spinoscalids. The feather-shaped scalids are short (30 μm) with numerous short thin hairs. The fourth row (sr_4) consists of 16 spinoscalids, eight smaller leg-shaped spinoscalids alternating with eight claw-tipped spinoscalids. The claw-tipped scalids (sr_{4a}) are two-segmented. The first segment, half the total length of the scalid, is thick, with several stiff hairs near the base, and has a pronounced knee.

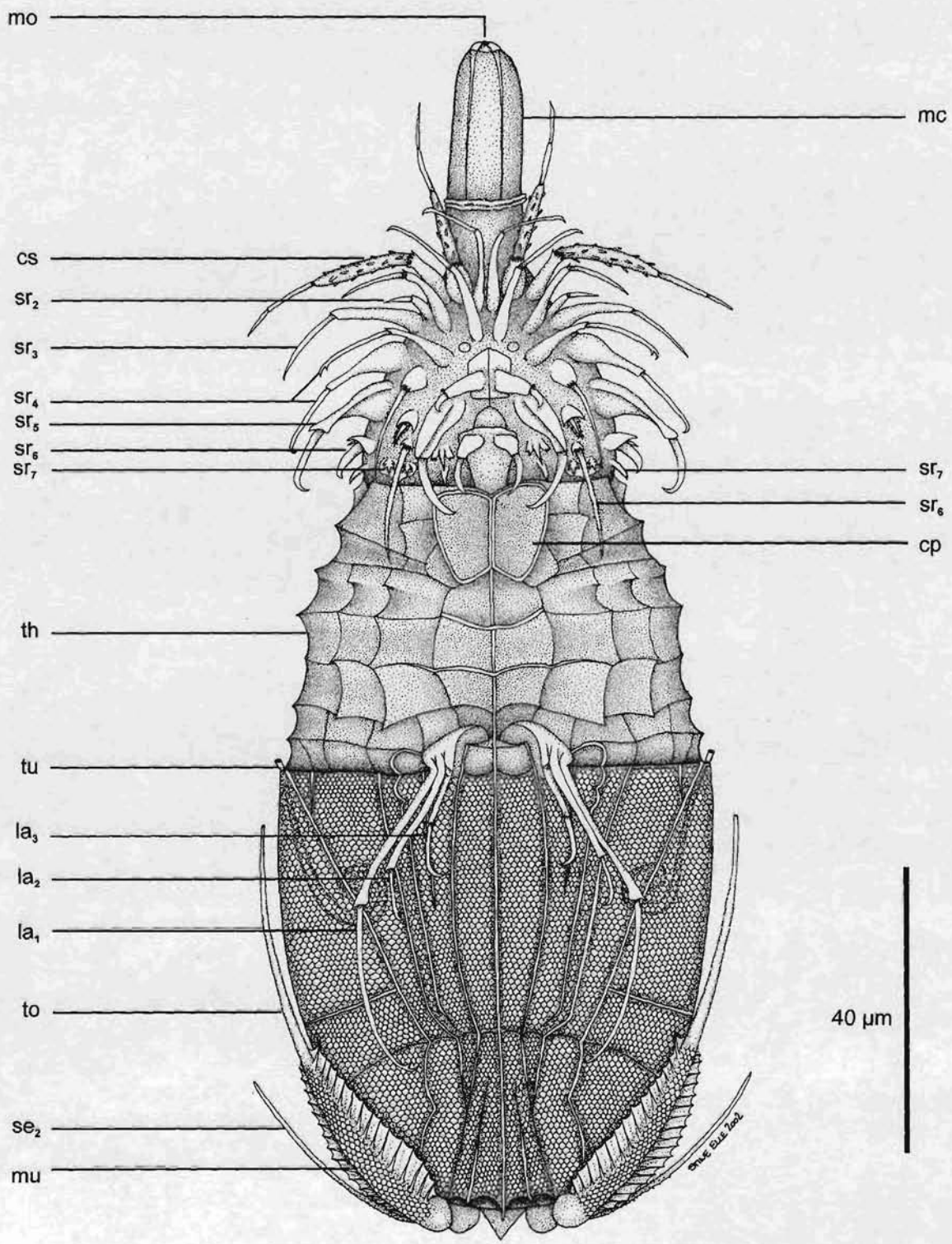

Fig. 5. Drawing of *Nanaloricus gwenae* sp. nov. paratypic Higgins larva (ZMUC LOR 456), ventral view.

The second segment is thin, with several hairs pointing posteriorly, and has a claw-shaped tip with two stiff hairs. The smaller leg-shaped scalids (sr_{4b}) have three segments. The first segment is a bulbous base and the second is thick with a few papillae near the base. The third segment is feather shaped with numerous thin small hairs. The fifth through seventh rows (sr_{5-7}) consist of 30 simple uniform two-segmented spinoscalids in each row. The eighth row (sr_8) consists of 30 trichoscalid-like scalids. These scalids are two-segmented and the second segment has serrated margins. The ninth row (sr_9) consists of 30 teeth-like scalids positioned on rectangular basal plates and each scalid has three teeth.

The neck has three rows of trichoscalid basal plates (tp_{1-3}) with 15 basal plates in each row and 15 trichoscalids (tr): seven of these are double and eight are single. The first basal plate row (tp_1) is positioned just below the ninth row of the introvert. The first basal plate row consists of eight tooth-like basal plates, with four teeth on each plate alternating with seven striated triangular plates. The second and third rows are positioned further down on the neck. The second basal plate row (tp_2) consists of only seven basal plates. These seven plates are rectangular with a short spine anteriorly and support the seven primary appendages of the double trichoscalids (tr_{b1}). The third basal plate row consists of eight hexagonal plates alternating with seven triangular plates. The eight hexagonal plates support the eight single trichoscalids (tr_a) and the seven triangular plates support the seven secondary appendages of the double trichoscalids (tr_{b2}). All trichoscalids are short (15–16 μm) and with serrated margins. The secondary appendages of the double trichoscalids (tr_{b2}) are slightly wider (2 μm) than the other appendages (1 μm). The thorax has no appendages and is almost hidden by the lorical spikes.

The lorica (lo) consists of six strongly sclerotized plates with a honeycomb sculpture, which is difficult to see using light microscopy. Numerous pores (po) are distributed on the lorica plates in a specific pattern. The lorica plates have a total of 15 spikes; the most lateral spikes of the laterodorsal plates (lp) are longer (20 μm) than the remaining spikes (15 μm). The midventral spike on the ventral lorica plate is only one-fourth (4 μm) the length of the other spikes. There are four flosculi (fl) arranged in a rectangular pattern on each of the laterodorsal plates (lp). Additionally, there is a single flosculum located on the small triangular anal plate (ap). Internally there are two ovaries (not drawn in Fig. 2), one with a single egg (e.g., Fig. 4D). The ovaries end in two ovary ducts.

Description of the postlarva. The postlarva (Figs. 3, 4B,C) is 189 μm long, 70 μm wide, and resembles the holotypic female, except for a few details, e.g., there are only eight rows of scalids (cs, sr_{2-6}, sr_{8-9}), because the seventh row of the introvert is absent. The lorica plates are less sclerotized than in the adult. There are three dorsal plates, one middorsal (dp_1) and two dorsal (dp_2), instead of the single dorsal plate in the adult. The double ridges on the three dorsal plates are aligned with each lorical spike and the spikes are nearly twice as long as in the adult. The postlarva has undeveloped reproductive organs and the body cavity is filled with coelomocytes.

Description of the Higgins larva. The Higgins larvae are 128–167 μm long and 46–66 μm wide (Figs. 4A, 5–9). Of the three illustrated specimens (paratypes), the medium instar larva (Fig. 5) is 167 μm long and 60 μm wide, the last instar larva (Fig. 6) is 189 μm long and 66 μm wide, and the early instar larva (Fig. 7) is 128 μm long and 46 μm wide. All other measurements are only from the medium instar larva (Fig. 5). Additionally, all scalid abbreviations correspond to the abbreviations in the introvert in Figs. 8 and 9.

The mouth cone (mc) is cone shaped and divided into two parts, where the last part has six longitudinal lines. There is no external or internal armature. The mouth opening (mo) is positioned anterior to the mouth cone and in the shape of a six-petal flower.

The introvert consists of seven rows of scalids:

- The first row (cs) has eight clavoscalids, which are divided into four segments. The first segment is bulbous with several thick hairs between the first and second segments. The ventrolateral pair (c1b–b′) is longer (33 μm) than the others (29 μm). The second segment is thick and with many small papillae. The last two segments are spine-like.
- The second row (sr_2) has either four spinoscalids in the young larvae or six as in the older larvae. The four scalids (s2a–a′, s2c–c′), which are found in all larvae, are elongated (21 μm) and two- or three-segmented, whereas the two additional scalids (s2b–b′) in the older larvae are short (9 μm) and reduced.
- The third row consists of 15 elongated (21 μm) two- or three-segmented spinoscalids. In some of the two-segmented scalids (s3c–c′, s3f–f′), there are two rigid hairs between the two segments. Ventrally the second pair of spinoscalids (s3b–b′) is not illustrated (see the round circles in Figs. 5, 8A). The dorsal three-segmented spinoscalids (s3g–g′) have a pronounced knee between the second and third segments and end in a curved tip similar to

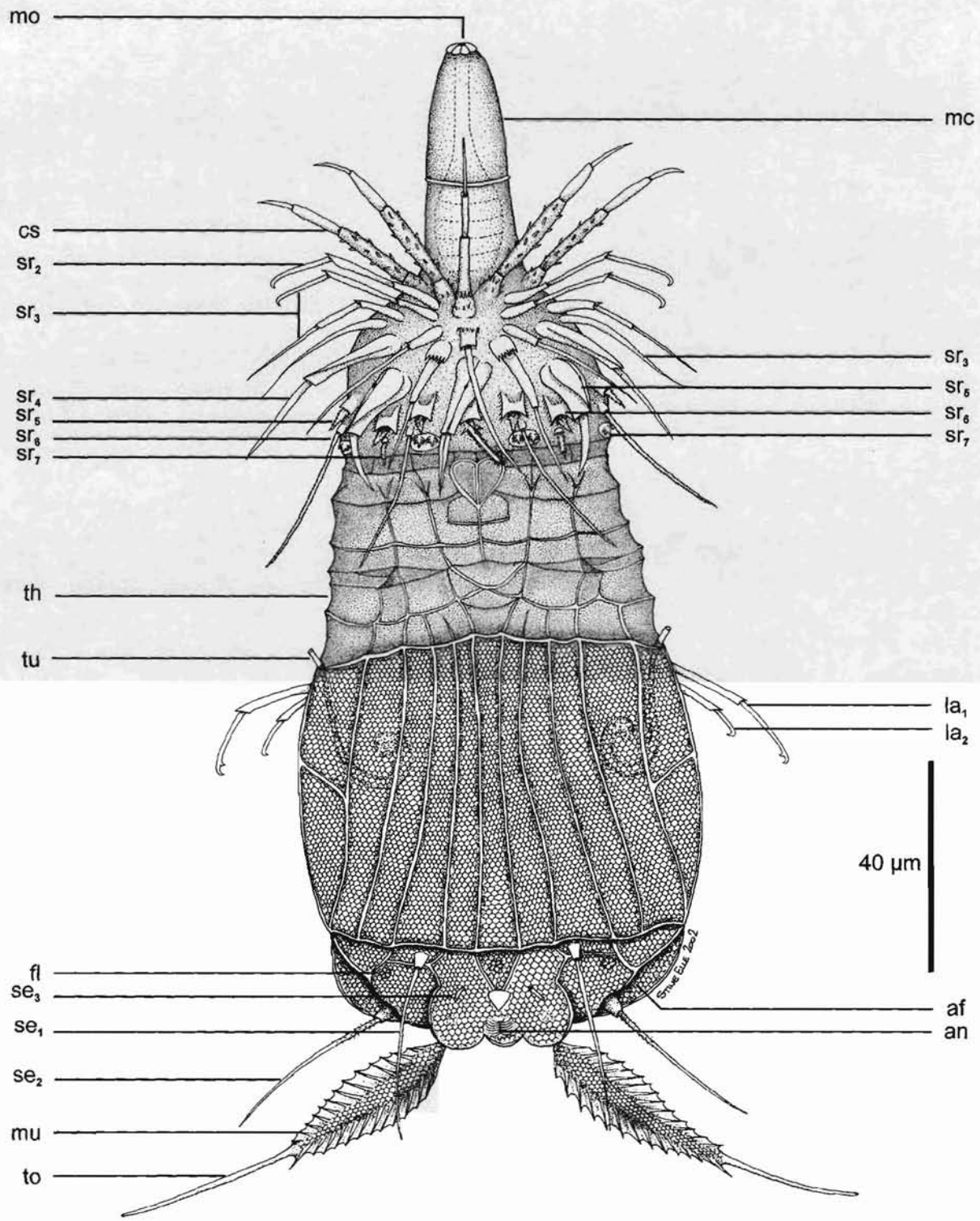

Fig. 6. Drawing of *Nanaloricus gwenae* sp. nov. paratypic Higgins larva (ZMUC LOR 460), dorsal view.

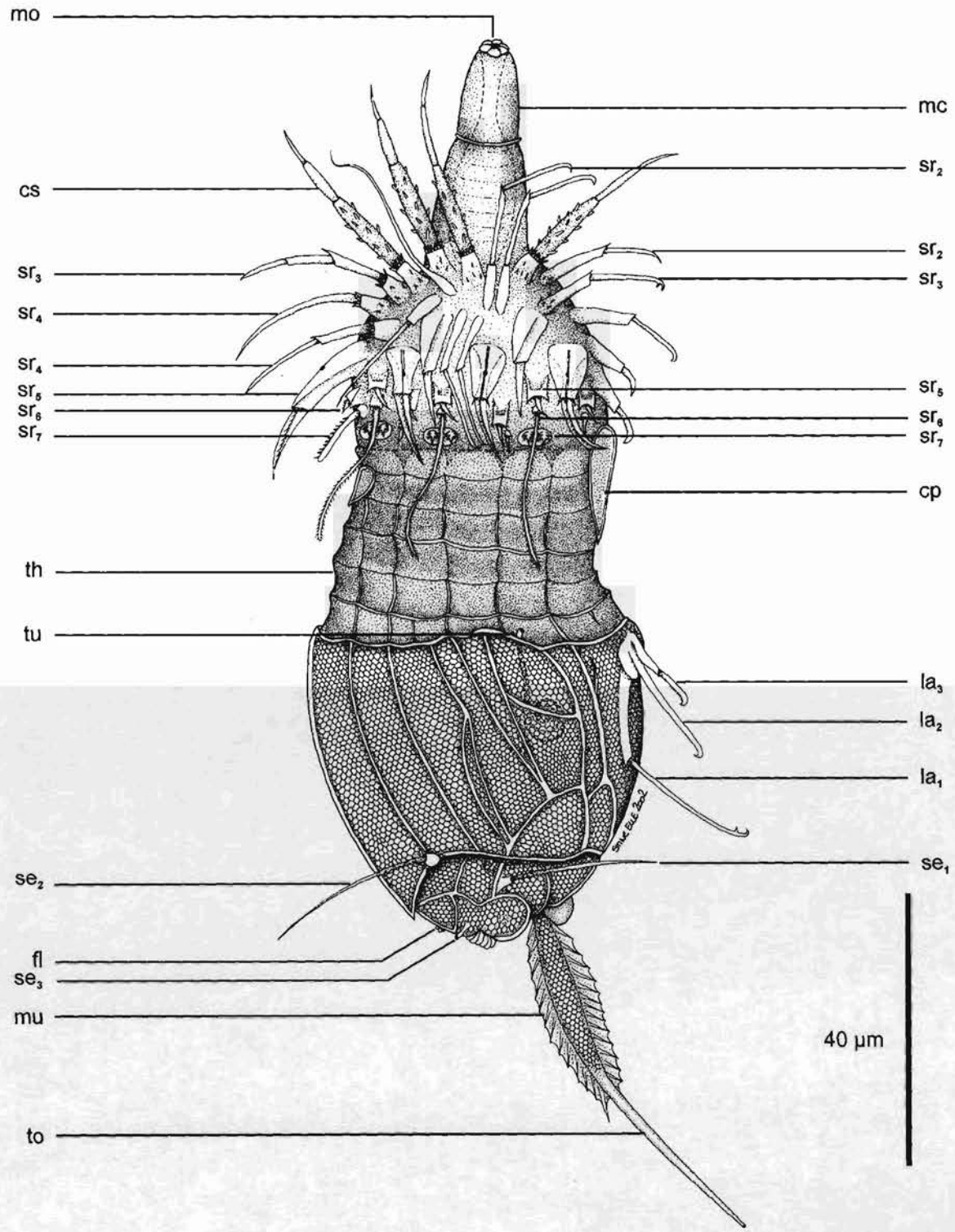

Fig. 7. Drawing of *Nanaloricus gwenae* sp. nov. paratypic Higgins larva (ZMUC LOR 459), lateral view.

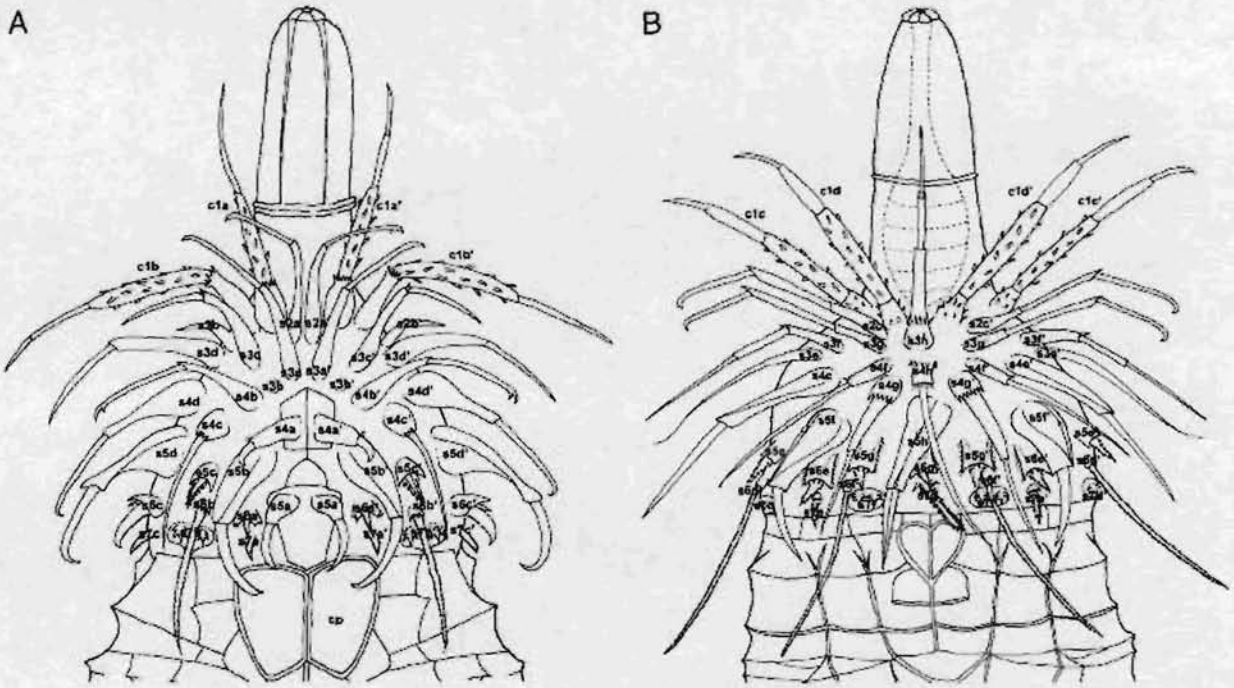

Fig. 8. Schematic drawing of the introvert of the Higgins larva of *Nanaloricus gwenae* sp. nov. **A.** Ventral. **B.** Dorsal.

s2c–c′. The lateral pair (s3e–e′) is whip shaped without any segmentation. The middorsal scalid (s3h) is three-segmented with several spines near the base.

- The fourth row (sr_4) consists of 15 spinoscalids of various shapes. Midventrally there is a pair of hook-shaped scalids (s4a–a′) with three stiff hairs between the segments and positioned on two rectangular plates. This pair, together with two other pairs in the next row, comprises a complex of scalids surrounding the thoracic closing plates (cp). The next pair (s4b–b′) is spinose with a pronounced knee between the two segments and terminates as a double claw. Pair (s4c–c′) is two-segmented with a large bulbous base and three spines. In this row, there are two large claw-shaped pairs (~21 μm); the lateral pair (s4d–d′) has a very large base but without a diagonal ridge, and the more dorsal pair (s4e–e′) is smaller but with diagonal ridges. The next pair (s4f–f′) is simple, two-segmented and with a small spine. The last pair (s4g–g′) resembles the s4c pair on the ventral side, except that it is three-segmented and has several spines on the bulbous first segment. Middorsally there is a single scalid (s4h), which has a rectangular base with five spines near the base and several fine hairs between the two segments.
- The fifth row (sr_5) consists of 15 spinoscalids. Midventrally there is a pair of short (9 μm) and simple hook-shaped spinoscalids (s5a–a′) on a large triangular plate. Additionally, in this row, there are seven large claw-shaped scalids (19–21 μm) alternating with six short serrated scalids (6 μm) positioned on basal plates. The seven large claw-shaped scalids (s5b–b′, s5d–d′, s5f–f′, s5h) have a very large first segment with two very pronounced diagonal ridges and one central ridge. The ventral pair (s5c–c′) of the serrated scalids has a triangular basal plate, whereas the two dorsal pairs (s5e–e′, s5g–g′) have rectangular basal plates with several fine hairs along the edges on s5g–g′ and smooth on s5e–e′.
- The sixth row (sr_6) consists of 13 spinoscalids. Six long-serrated scalids (s6b–b′, s6d–d′, s6f–f′) alternate with seven short-serrated scalids (3 μm) (s6a–a′, s6c–c′, s6e–e′, s6g). The ventral pair of the long-serrated scalids (s6b–b′) is shorter (18 μm) than the dorsal ones (23 μm). The pair of short-serrated scalids (s6a–a′) positioned ventrally is situated on basal plates that have been split into two and are also

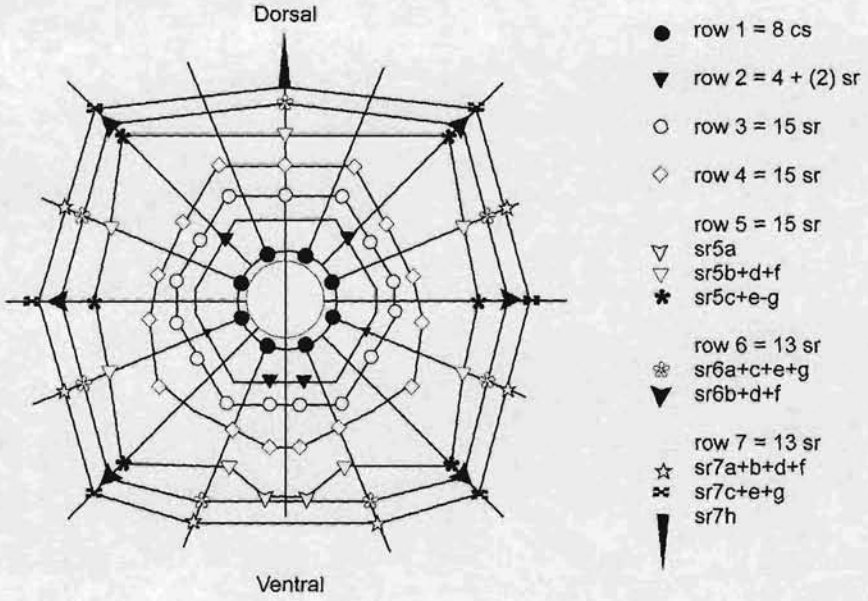

Fig. 9. Scalid diagram of the introvert of the Higgins larva of *Nanaloricus gwenae* sp. nov.

serrated. In the lateral pair (s6c–c′), the basal plates are not serrated but have two large spines. The short-serrated scalids on the dorsal side (s6e–e′, s6g) resemble those of the previous row.

- The seventh row (sr_7) consists of 13 scalids where six tooth-like scalids alternate with six simple serrated scalids and one leaf-like spine. The three pairs of tooth-like scalids (s7b–b′, s7d–d′, s7f–f′) have a large oval basal plate with two tooth-like scalids on each plate. The leaf-like scalid (s7g) is a clavoid sensory organ with a large flattened spine and serrated margins.

The thorax (th) is flexible and with five to seven transversal folds. Ventrally there are two large thoracic closing plates (cp) resembling a butterfly and dorsally there are two small heart-shaped plates. The large ventral closing plates (cp) are used as a closing apparatus when the introvert is withdrawn into the body.

Between the thorax and the abdomen, there are three pairs of ventral locomotory setae. The setae form a common base and are basally fused together. The most ventrolateral setae (la_1) are very long (50 μm) with a prominent knee and terminating in a double claw. The middle pair of setae (la_2) resembles the ventrolateral pair, but the former are only half the length (26 μm) of the latter. A variation in the distal part of the middle pair (la_2) is seen, because later-stage Higgins larvae have a double claw (Fig. 5) and earlier stages have a single claw (Figs. 6, 7). The most ventral pair of setae (la_3) is slightly shorter (20 μm) than the middle pair. Additionally, between the thorax and the abdomen there is a pair of lateral tubes (tu) opening up into two large spiraled multicellular glands.

The abdomen or lorica (lo) has a very distinct honeycomb sculpture. There are 25 longitudinal folds, although some of them do not extend throughout the lorica. There are also several transversal folds. On the dorsal side there is a large anal field (af) consisting of several anal plates (Fig. 6). Positioned on the anal field is a pair of toes, three pairs of setae, three flosculi, and an anus (an). The toes (to) are relatively longer (64–65 μm) in the older larvae (Figs. 5, 6) than in the younger larvae (56 μm) (Fig. 7). The toes connect to the anal field by ball and socket joints. Basally, the toes are enlarged with flipper-like structures called mucrones (mu). The mucrones are leaf shaped and flattened with 14–15 transverse ridges. A pore is located terminally on the toes and the pore opens up into the toe glands. The first pair of sensory setae (se_1) is positioned on the dorsal side between the lorica folds and the anal field (Fig. 6). The setae are 31 μm long, thin, and have a round base. The second pair of posterior setae (se_2) is positioned more laterally on the anal field. The setae are of the same length (31 μm) as the first pair, but they are thicker, have a larger rounded base, and have several hairs on the spine. The third pair (se_3) is located on the large dorsal anal plate. The setae are short (4 μm), without any base, and are probably glandular due to the terminal pores. Additionally, three flower-shaped flosculi (fl) and the anus (an) are located on the anal field.

Discussion

Diagnostic features

Family Nanaloricidae. Since the description of *Nanaloricus mysticus* (Kristensen 1983), several new morphological characters have been observed and described, and these are now included in the emended definition of Nanaloricidae. Additionally, over the years, some specific characters have been reevaluated and discussed by several authors (e.g., Heiner 2004; Kristensen & Gad 2004). These characters are discussed again to justify their inclusion or exclusion in the emended definition of Nanaloricidae.

Concerning the introvert, there have been several discussions about the number of scalid rows (Heiner 2004). Originally, in the *N. mysticus* description (Kristensen 1983), the introvert was described as having nine rows of scalids. However, the ninth row described by Kristensen (1983) was not a spinoscalid row, but the first trichoscalid basal plate row. The trichoscalid basal plate row was thought to be a spinoscalid row, because it is situated more anteriorly than the other two basal plate rows and just beneath the last spinoscalid row. Kristensen & Gad (2004) therefore concluded that all species of Nanaloricidae have eight spinoscalid rows compared with nine in Pliciloricidae. Heiner (2004) concluded that there are a total of nine rows of scalids on the introvert in Nanaloricidae. A reevaluation of the different scalids showed that the second row described in *N. mysticus* (Kristensen 1983), *Armorloricus elegans,* and *A. davidi* (see Kristensen & Gad 2004) should be divided into two rows. Originally, the second row (sr_2) consisted of 16 scalids of two different types (Kristensen 1983; Kristensen & Gad 2004). Now, with the division of the second row (sr_2) into two separate rows (sr_2, sr_3), these two scalid types (nine leg-shaped scalids, seven feather-shaped scalids) are divided between the second and third rows, respectively, and the corresponding rows (sr_{4-8}) attain a higher number (sr_{5-9}) in the new interpretation. Hence, there are now nine rows of scalids in the species of both Nanaloricidae and Pliciloricidae. For more detailed discussion concerning the number of rows, see Heiner (2004).

Another discussion is on the divisions of body regions, especially the region between the introvert and the abdomen. In Kristensen & Gad (2004), the neck and thorax regions were merged together into a thoracic region and subsequently divided into three subregions: antero-, inter-, and posterothorax. However, the old divisions of the body regions (neck and thorax) are withheld here, so that the two subregions, antero- and interthorax, are referred to as the neck and the posterothorax as the thorax. A more thorough investigation is needed to say anything decisive about the number of regions in the thoracic area.

The word "mucrones" is used here instead of the word "mucros" used in Kristensen (1983), Todaro & Kristensen (1998), and Heiner (2004). The word "mucrones," and not "mucros," is the right plural Latin form of "mucro," and therefore the word should be changed.

***Nanaloricus gwenae* sp. nov.** *Nanaloricus gwenae* sp. nov. can clearly be assigned to the genus *Nanaloricus* due to the following characteristics in adults: a mouth cone with a short mouth tube, no oral stylets present, teeth-like scalids positioned on basal plates in the ninth row, and a lorica with six lorica plates and honeycomb sculpture. The genus-specific characters for the Higgins larvae are a mouth cone without internal or external armature, four to seven spinoscalids in the second row (sr_2), teeth-like scalids positioned on basal plates in the seventh row (sr_7), ventral locomotory setae curved, fused basally and la_3 long, and toes with leaf-shaped mucrones.

This new species, *N. gwenae* sp. nov., from Fort Pierce, USA, resembles the type species of Loricifera, *N. mysticus,* so much that, not surprisingly, the two species were thought to be conspecific in 1983 (Kristensen 1983). However, there are several very distinct differences between the two adult species, especially concerning the mouth cone, the thickness and shape of the spinoscalids, the honeycomb sculpture of the lorica, and the different shape of the body and lorica. The characters that distinguish the adult of *N. gwenae* sp. nov. from *N. mysticus* include the following: (1) a long and flexible mouth cone compared with short and non-flexible; (2) a larger difference between the primary and secondary oral ridges; (3) clavoscalids (cs) with several small papillae on the first segment; (4) generally thinner spinoscalids, less robust leg-shaped scalids (sr_2) with a smaller first segment, and smaller teeth-like scalids (sr_9); (5) a longer lorica which is more oval (compared with more rectangular in *N. mysticus*); and (6) a less distinct honeycomb sculpture.

There are also several differences between the two species concerning the Higgins larvae. The characters that distinguish the Higgins larva of *N. gwenae* sp. nov. from *N. mysticus* are as follows: (1) the mouth cone is more cone shaped than rectangular; (2) clavoscalids have papillae on the second segment compared with smooth and spinose; (3) only four or six spinoscalids are there in the second row (sr_2) (the middorsal has been reduced), compared with seven in *N. mysticus;* (4) the ventrolateral setae (la_1) are one-third longer and more curved; (5) anteriorly, the

lorica is not as wide, but more oval in shape; and (6) the toes are slightly longer and the mucrones are not as wide and large as in *N. mysticus*.

A comparison between both the adults and Higgins larvae of *N. gwenae* sp. nov. and *N. khaitatus* is difficult, because no drawings and only a few SEM pictures have been published of *N. khaitatus* (see Todaro & Kristensen 1998). In adults, similarities include a long mouth cone, clavoscalids with papillae on the proximal part, and 14–15 lorica spikes anterior on the six lorica plates. Todaro & Kristensen (1998) describe the mouth cone as short with a longer mouth tube. This is not correct. The mouth cone is long and the mouth tube is short, because there are four very weakly defined oral ridges (see Todaro & Kristensen 1998: fig. 3A, for details), which were not observed during the description of *N. khaitatus*. Concerning the number of spikes in the holotype of *N. khaitatus*, the midventral spike is reduced, whereas in the others there is a small ventral spike. Differences between the adults of the two species are transverse cuticular bridges in the lorica spikes, a very distinct honeycomb, and two flosculi positioned posteriorly on the dorsal plate in *N. khaitatus*. The differences between the Higgins larvae of *N. gwenae* sp. nov. and *N. khaitatus* include the following: the Higgins larva of *N. khaitatus* has a more rectangular body shape with a more distinct honeycomb sculpture and the mucrones are larger and more triangular in shape.

Life cycle of Nanaloricidae

The life cycle of Nanaloricidae was first described by Kristensen (1991a), based on observations of the type species *N. mysticus;* later, Kristensen & Brooke (2002) added a few new observations. In 2004, two new genera of Nanaloricidae, *Armorloricus* (Heiner 2004; Kristensen & Gad 2004) and *Phoeniciloricus* (Gad 2004a), were described. These descriptions, together with additional observations, concluded that all species of Nanaloricidae have a simple life cycle with only sexual reproduction. The adults are gonochoristic (dioecious) and the males show sexual dimorphism in the clavoscalids and the primary appendages of the ventral pair of trichoscalids (Kristensen 1991a,b). Both adults have paired gonads. The female has two asymmetrically maturing ovaries, because the oocytes only mature in one ovary at a time. The eggs are released one at a time and fertilization is either external or internal.

The fertilized egg hatches to form a Higgins larva, which molts several times (Kristensen 1991a). The number of larval stages (instars) probably varies considerably between species, and no definite observations have yet been made to verify that there are two to four larval stages. The number of larval stages mentioned in Kristensen (1991a) is a result of categorizing the different larvae into length groups and not of specific observations of characteristics.

The last larval stage metamorphoses into a postlarva, which resembles the adult female in all Nanaloricidae species. Later, the postlarva molts into a female or male. Gad (2004a) states that postlarvae of Nanaloricidae are found rarely, because this is a short transition stage. This is not always the case. In the deep-sea material from the Galapagos Islands collected by the R/V Sonne cruise SO158, several postlarvae of a new genus with adults inside have been found. However, this, together with the postlarva of *Phoeniciloricus simplidigitatus* (see Gad 2004a), suggests that the postlarval stage is prolonged in the deep sea. Additionally, the Galapagos material reveals for the first time that adults actually come out of the postlarvae (I. Heiner, unpubl. data). This was an assumption not confirmed before.

The fertilization and embryology of Nanaloricidae is still incompletely known, because only a few species, e.g., *N. mysticus* and *A. elegans*, have been sectioned for TEM. More detailed TEM work is needed before much can be said about this subject. However, a few new observations have been made over the years. Seminal receptacles have been found in only one species, a new genus of Nanaloricidae (this is the undescribed species of *Nanaloricus* in Kristensen 1991a,b), with both seminal receptacles close to the ovaries filled with small round-headed sperms. Seminal receptacles are definitely not found in *N. mysticus* and *A. elegans*, because no sperms have been found in any of the TEM-sectioned females. The question concerning internal or external fertilization is still not resolved. However, spermatozoa have been found in the posterior end of females of *N. khaitatus* (Todaro & Kristensen 1998: see fig. 4) and *A. elegans* (Kristensen & Gad 2004: see fig. 8D), which, at least for these species, indicates internal fertilization. Additionally, the presence of claspers in the males suggests internal fertilization, because these are presumed to hold the female during copulation (Kristensen 1991b). It is difficult to say anything decisive on the kind of fertilization and life cycle of *N. gwenae* sp. nov., but all observations thus far indicate that this species may have only a sexual reproductive life cycle.

Ecological note

The sediment in which *N. gwenae* sp. nov. was found is inhabited by the lancelet *Branchiostoma*

virginiae HUBBS 1922 (see Cory & Pierce 1967); in the literature this sediment is called *Amphioxus* sand (Higgins & Thiel 1988). The associated meiofauna found in the *Amphioxus* sand of Fort Pierce is very rich, especially for tardigrades (Kristensen & Higgins 1984, 1989), gastrotrichs, polychaetes, and kinorhynchs. One of the sample sites (RMK 831004) is also the type locality for the tardigrade *Paradoxipus orzeliscoides* KRISTENSEN & HIGGINS 1989. The *Amphioxus* sand from Fort Pierce has less silt and detritus than the *Dentalium* sand from Roscoff, where *N. mysticus* was found (Kristensen 1983). However, many of the same tardigrade species are found in samples from both sites; among these, *Tholoarctus natans* KRISTENSEN & RENAUD-MORNANT 1983, described from Roscoff, is common at the Fort Pierce sites (see Kristensen & Renaud-Mornant 1983). Other tardigrade species from the "6-mile" stations at Fort Pierce are *Parastygarctus sterreri* RENAUD-MORNANT 1970, *Wingstrandarctus corallinus* KRISTENSEN 1984, *Florarctus antilliensis* VAN DER LAND 1968, *F. hulingsi* RENAUD-MORNANT 1976, *Raiarctus aureolatus* RENAUD-MORNANT 1981, *R. colurus* RENAUD-MORNANT 1981, *Tanarctus arborspinosus* LINDGREN 1971, *T. gracilis* RENAUD-MORNANT 1980, *T. heterodactylus* RENAUD-MORNANT 1980, *Batilipes dicrocercus* POLLOCK 1970, and *Orzeliscus belopus* DU BOIS RAYMOND-MARCUS 1952. Of these, *R. aureolatus, R. colurus, T. heterodactylus,* and *O. belopus* are also found on the type locality (*Dentalium* sand) of *N. mysticus* in Roscoff, France. The type locality for *A. elegans* and *A. davidi* is Trezen ar Skoden, off the coast of Roscoff (Kristensen & Gad 2004). The sediment is the so-called "*Polygordius* sand," which consists of very clean shell hash. In this sediment from 50 to 55 m depth, six species of nanaloricid loriciferans have been found during the last 30 years of research. This suggests that nanaloricids are very common in shallow water; however, many new members of this family have been found in deeper water such as seamounts (Gad 2004b) and banks (Heiner 2004).

Acknowledgments. We gratefully acknowledge the staff and facilities of the Smithsonian Marine Station at Fort Pierce, Florida (contribution number 657). We also acknowledge M. Wallace, former research assistant to R.P.H., Stine B. Elle for the beautiful drawings, and our colleagues, Dr. Rick Hochberg and Dr. Fernando Pardos, who reviewed this article and made valuable suggestions. Funds for this study were provided to R.M.K. by the Danish Natural Science Research Council and Carlsberg Foundation, to both R.P.H. and R.M.K. by the Smithsonian Marine Station at Link Port, and to R.P.H. by the Sumner Gerard Foundation.

References

Cory RL & Pierce EL 1967. Distribution and ecology of lancelets (order Amphioxi) over the continental shelf of the southeastern United States. Limnol. Oceanogr. 12: 650–656.

Gad G 2004a. Discovery of Nanaloricidae (Loricifera) inhabiting sediment of volcanic origin and influenced by hydrothermal vents in the deep sea of Kilinailau Trench, Papua New Guinea (Pacific). Helgol. Mar. Res. 58: 40–53.

——— 2004b. The Loricifera fauna of the plateau of the Great Meteor Seamount. Arch. Fish. Mar. Res. 51: 9–29.

Heiner I 2004. *Armorloricus kristenseni* (Nanaloricidae, Loricifera) a new species from the Faroe Bank (north Atlantic). Helgol. Mar. Res. 58: 192–205.

Higgins RP & Kristensen RM 1986. New Loricifera from southeastern United States coastal waters. Smithson. Contrib. Zool. 438: 1–70.

Higgins RP & Thiel H 1988. Introduction to the Study of Meiofauna. Smithsonian Institution Press, Washington, DC. 487 pp.

Hubbard GF, Howard RL, & Gallaway BJ 1988. Loricifera, a recently described phylum occurring in the Northern Gulf of Mexico. Northeast Gulf Sci. 10: 49–50.

Kristensen RM 1983. Loricifera, a new phylum with Aschelminthes characters from the meiobenthos. Z. Zool. Syst. Evolut.-forsch. 21: 163–180.

——— 1991a. New higher taxa—presented by their discoverers. Loricifera—a general biological and phylogenetic overview. Verh. Dtsch. Zool. Ges. 84: 231–246.

——— 1991b. Loricifera. In: Microscopic Anatomy of Invertebrates, Vol. 4. Aschelminthes. Harrison FW & Westfall JA, eds., pp. 351–375. Wiley-Liss, New York.

Kristensen RM & Brooke S 2002. Phylum Loricifera. In: Atlas of Marine Invertebrate Larvae, Chapter 8. Young CM, Sewell MA, & Rice ME, eds., pp. 179–187. Academic Press, London.

Kristensen RM & Gad G 2004. *Armorloricus* a new genus of Loricifera (Nanaloricidae) from Trezen ar Skoden (Roscoff, France). Cah. Biol. Mar. 45: 121–156.

Kristensen RM & Higgins RP 1984. A new family of Arthrotardigrada (Tardigrada: Heterotardigrada) from the Atlantic coast of Florida, U.S.A. Trans. Am. Micros. Soc. 103: 295–311.

——— 1989. Marine Tardigrada from the southeastern United States coastal waters. I. *Paradoxipes orzeliscoides* n. gen., nov. sp. (Arthrotardigrada, Halechiniscidae). Trans. Am. Micros. Soc. 108: 262–282.

Kristensen RM & Renaud Mornant J 1983. Existence d'Arthrotardigrada semi-benthiques de genres nouveau de la sous-familie des Styraconyxinae subfam. nov. Cah. Biol. Mar. 24: 337–353.

Thomas MB, Edwards NC, & Higgins RP 1995. *Cryptohydra thieli* n. gen., nov. sp.: a meiofaunal marine hydroid (Hydroida, Athecata, Caplitata). Invert. Biol. 114: 107–118.

Todaro MA & Kristensen RM 1998. A new species and first report of the genus *Nanaloricus* (Loricifera, Nanaloricida, Nanaloricidae) from the Mediterranean Sea. Ital. J. Zool. 65: 219–226.