



A new species and first report of the genus *Nanaloricus* (Loricifera, Nanaloricida, Nanaloricidae) from the Mediterranean Sea

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ABSTRACT

Several loriciferans representing different developmental stages were recovered during a meiobenthic survey of the coarse, organogenic, sand of the Meloria shoals, Livorno, Italy. Loriciferans were found in low numbers and only in few of the monthly samples collected over a 15-month period. Adults and postlarvae were always found in the upper 15 cm of the sediment, while the presence of larvae was noted down to 30-35 cm. A taxonomic analysis revealed that all specimens belong to an undescribed species of the genus *Nanaloricus*. Diagnosis and description of *Nanaloricus kbaitatus* n. sp. is given based on the adult, postlarval, and larval stages. This is the first report ever of loriciferans from Italian waters, and the first record of the genus *Nanaloricus* in the Mediterranean Sea. The presence of these rare animals is indicative of the high naturalistic value of this coastal area of Tuscany.

KEY WORDS: Loricifera - *Nanaloricus* - Benthos - Mediterranean Sea - Meiofauna - Biodiversity.

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INTRODUCTION

Loriciferans are multicellular organisms that by virtue of their small size and their way of life are considered, by benthic researchers, as part of the permanent marine meiofauna. In loriciferans, sexes are separated with male and female displaying similar size but some degree of dimorphism (cf. Kristensen, 1991a). Although most species are gonochoristic (dioecious) with assumed internal fertilization, parthenogenesis also occurs in some instances (Kristensen, 1991b). Offsprings hatch from eggs laid singly; development is indirect and the adult status is reached by molting through several juvenile stages, including the 4-5 instars of the peculiar Higgins larva (Kristensen, 1991a).

The phylum Loricifera was instituted in the first half of the 1980's to fit *Nanaloricus mysticus*, a petite animal with morphological characteristics recalling the kinorhynchs or the larval stage of the priapulids, yet distinct from these (Kristensen, 1983). The 'unique' organisms were extracted from shelly sand collected at 25 m water depth in the bay of Roscoff, France. The first documented finding of these Metazoa dates back to 1974 when a single animal of this type was recovered off the coast of North Carolina, U.S.A., from medium siliceous sand collected at about 400 m water depth. The specimen was first recognized as being a male loriciferan in 1983 at the time of the description of Loricifera. Later its affiliation was related to the newly described taxon *Pliciloricus enigmaticus* (see Higgins & Kristensen, 1986). Simultaneously with *P. enigmaticus*, the authors described seven additional mesobenthic species of Loricifera, all recovered from medium-coarse sand collected along the Atlantic coast of the U.S.A.; to note the profound morphological differences existing between the latter species and *Nanaloricus mysticus*, the American taxa were allocated in a newly established family, the Pliciloricidae (Higgins & Kristensen, 1986).

Overall, only 10 loriciferan species have been described to date, yet at least 70 other species have been reported from different oceans of the world, testifying to the cosmopolitan nature of the phylum (Kristensen & Meier, 1986; Kristensen, unpublished data). Surprisingly, although nine out of the ten described species inhabit coarse bottoms, most of the records pertain to finer sediments including the muddy ones typical of the hadal depths (Hubbard *et al.*, 1988; Kristensen & Shirayama, 1988). Indeed, it is in silt-clay bottoms that the only four species of loriciferans known from the Mediterranean were found (Soetaert *et al.*, 1984; Balduzzi *et al.*, 1995).

Until now, loriciferans were unknown for the Italian waters although these animals have been actively searched for during many meiofaunal surveys along the peninsular coast (S. Grimaldi-De Zio, pers. com.). Here we report on a new species of this phylum based on observation of several specimens, in different life stages, found while the senior author was engaged in a survey concerning the meiobenthos of the Meloria

shoals, near Livorno, Italy (Grimaldi-De Zio *et al.*, 1997; Huys & Todaro, 1997; Todaro, 1997a).

MATERIALS AND METHODS

Sediment samples were collected monthly, March 1996 to May 1997, at a 7-m-deep site located 300 m NW of the lighthouse of the Meloria shoals, Livorno, Italy. Samples in four replicates were obtained by SCUBA divers hand coring 15 cm into the sediment with Plexiglas corers, 3.55 cm internal diameter (10 cm² area). On 12 December 1996, an additional 35-cm-long corer (4.2 cm i.d.) was used to check for fauna deeper in the sediment. Meiofauna was fixed *in situ* with 5% formalin after being narcotized using an isotonic MgCl₂ solution. At the time of the sampling, the temperature at the sediment-water interface and the salinity of the interstitial water were measured. In the laboratory, the sediment was analysed for granulometry and total organic content according to Todaro (1992, 1997a). Meiofaunal taxa were extracted from sand by the multiple decantations technique (Pfannkuche & Thiel, 1988) and collected on a 63-µm mesh sieve. Loriciferans were sorted out using a Zeiss STEMI DV4 stereomicroscope at 40x magnification, and subsequently prepared for taxonomic analysis. Most of the specimens were observed with a Phillips XL 40 S.E.M. after being dehydrated through an ethanol series, critical point dried, mounted on aluminum stubs, and sputter-coated with gold-palladium. One Higgins larva and a postlarva were mounted in glycerin and observed using a Zeiss interference contrast microscope. Measurements were derived from photos or taken directly at the microscope, using an ocular micrometer. Since the only two adult specimens found were prepared for S.E.M. observation, we were forced to design one of them as the holotype.

RESULTS

The specimens found are reported in Table I along with their developmental stage, the sampling date, and the characteristics of the microhabitat at the time of collection, while the measurements of some of the main morphological traits are reported in Table II. In all, ten

specimens of Loricifera we found: two fully developed females (Fig. 1A, B), four postlarvae (Fig. 1C, D), and four Higgins larvae (Fig. 5A). Unfortunately, during the preparation for SEM observation two Higgins larvae and one postlarva were lost; therefore this, study considers only the remaining seven specimens.

Phylum LORICIFERA

Order **Nanaloricida**

Family NANALORICIDAE

Genus *Nanaloricus*

[Type species *Nanaloricus mysticus* Kristensen, 1983]

Nanaloricus khaitatus nov. sp.

Figs 1-5; Tables I, II

Diagnosis

Adults: 210-220 µm long with the mouth cone and tube fully extended. Loric length 101-130 µm. Mouth cone with tube 50-57 µm long. The sixth loric plates without longitudinal ridges. 4+4+2 loric flosculi. Very fine honey-comb sculpture of the loric cuticle. 15 loric hollow spikes, 11 of which bearing 3 transverse arched bridges. Middorsal primary trichoscalid 17 µm in length; secondary trichoscalid 35 µm in length.

Postlarvae: Loric length excluding spikes 102-132 µm (all postlarvae are retracted). One of the rows of the spinoscalids, which is present in the adults, is lacking. Each of the six loric plates has 2-4 longitudinal ridges as in the Higgins-larva. All 15 hollow spikes of loric smooth. 4+4+1 loric flosculi.

Higgins-larva: 112-125 µm in length (mouth cone and introvert retracted). The two caudal toes (72-92.5 µm long) have only mucro proximal (25-30 µm), each with a very long hollow spine, which have a gland opening at the distal end. 1+1+1 loric flosculi.

Type material

The holotype is an adult female. It was collected by M. Antonio Todaro on 10 April 1997 from a bottom pit

TABLE I - *Nanaloricus khaitatus* nov. sp.. Type and number of the specimens, sediment depth at which they were found, and microhabitat characteristics at the time of sampling.

| Parameter | 1996 | | December | January | 1997 | |
|---------------------------|-----------------------|------------------|-------------------|----------------------|----------------------|--|
| | April | October | | | February | April |
| specimen | postlarva* (n = 1) | larva (n = 1) | larva* (n = 2) | postlarva (n = 1) | postlarva (n = 1) | adult postlarva larva (n = 2, 1, 1) |
| Sediment depth (cm) | 0-15 | 2.4-4.8 | 30-35 | 0-15 | 0-15 | 1-15 |
| Mean grain size (phi) | 0.48 | 0.21 | 0.05 | 0.39 | -0.02 | 0.32 |
| Sorting | 0.54 | 0.51 | 0.48 | 0.42 | 0.53 | 0.47 |
| Skewness | 9.8 | -0.15 | -0.01 | -0.78 | 0.08 | -0.65 |
| Kurtosis | 3.67 | 2.60 | 2.84 | 4.11 | 2.19 | 3.32 |
| Total organic content (%) | 2.16 | 1.57 | 1.01 | 1.44 | 1.64 | 1.2 |
| Water temperature (°C) | 13.00 | 19.00 | 15.00 | 13.00 | 13.00 | 15.00 |
| Salinity (‰) | 38.00 | 38.00 | 38.00 | 38.00 | 38.00 | 38.00 |

*, specimen lost.

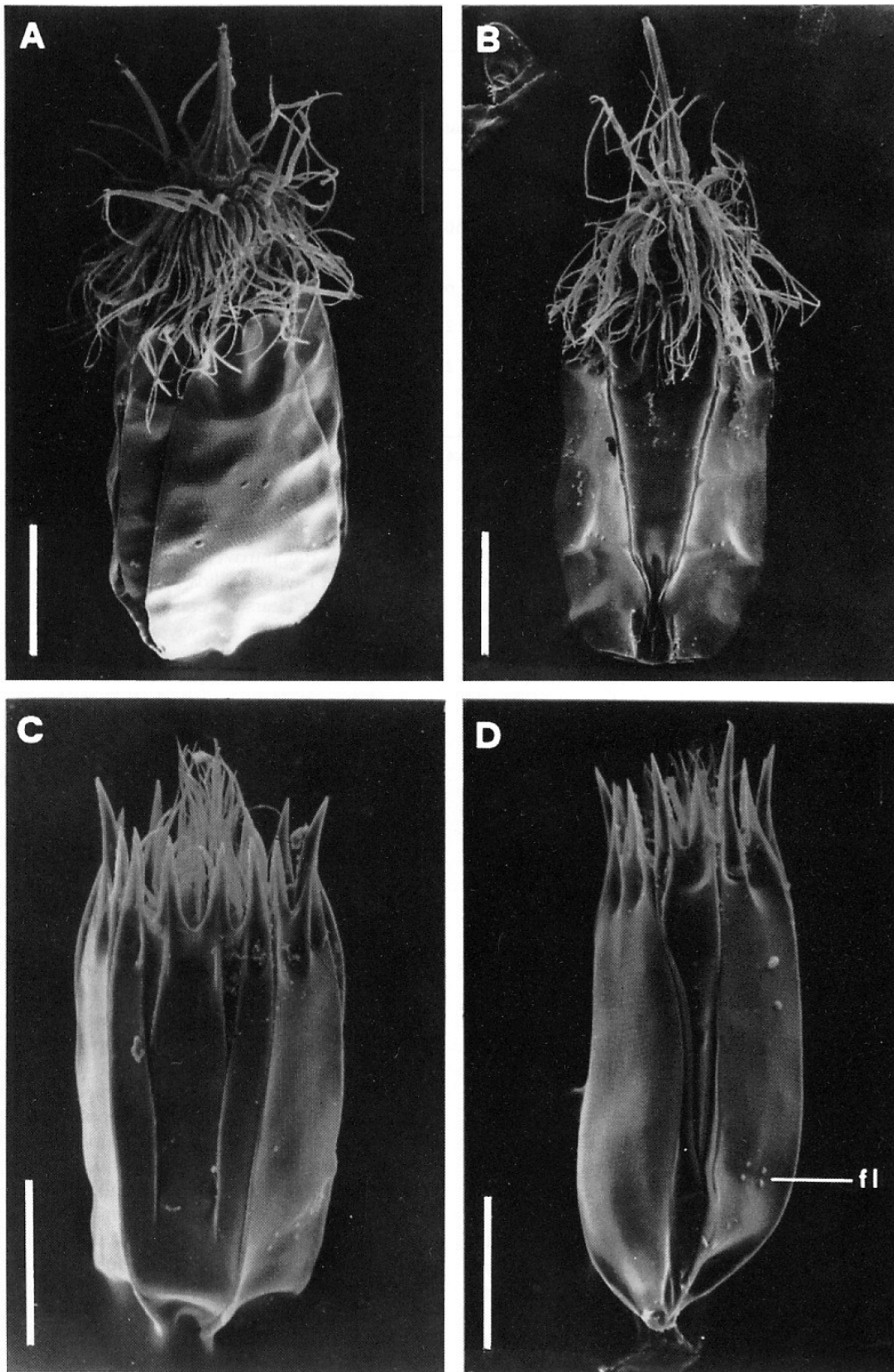


Fig. 1 - *Nanaloricus kbaitatus* nov. sp. - **A**, paratype, adult female (specimen 1), ventral; **B**, holotype, adult female (specimen 2), dorsal; **C**, paratype, postlarva (specimen 3) with the introvert retracted inside the lorica, dorsal; **D**, paratype, postlarva (specimen 4) with introvert retracted inside the lorica, showing the loriciflosculi (fl), dorsal. Scale bars, 40 μ m.

TABLE II - *Nanaloricus khaitatus nov. sp.*. Metrics of the specimens found. Measurements are in μm .

| Parameter | Specimen | | | | | | |
|--|------------|------------|----------------|----------------|----------------|------------|------------|
| | 1 adult | 2 adult | 3 postlarva | 4 postlarva | 5 postlarva | 6 larva | 7 larva |
| Total body length | 210.0 | 220.0 | - | - | - | 112.6 | - |
| Lorica length | 101.0 | 130.0 | 102.0 | 132.0 | 135.0 | - | 105.5 |
| Mouth cone length | 50.0 | 57.0 | - | - | - | - | - |
| Caudal toe length | - | - | - | - | - | 72.0 | 92.5 |
| Width at mid abdomen | 65.8 | 69.7 | 67.3 | 60.0 | 75.0 | 53.0 | - |
| Lorica's hollow spike maximum length | - | - | 25.0 | 28.0 | 30.0 | - | - |
| Lorica's hollow spike minimum length* | 12.5 | 19.5 | 21.5 | 19.6 | 20.5 | - | - |
| Sensory setae of the first set | - | - | - | - | - | 17.0 | 21.0 |
| Sensory setae of the second set | - | - | - | - | - | 32.0 | 40.0 |

*, abortive mid-ventral spike not considered; -, not applicable or not available.

at 7.0 m depth filled with coarse, organogenic sand, located 300 m NW of the lighthouse of the Meloria shoals, Livorno, Italy (43°32' N; 10°12' E). The holotype, a paratypic female, two postlarvae and one Higgins larva all on SEM-stubs are kept in the meiofauna collection of the senior author (ML 1997/ 7-38). One postlarva and one Higgins larva (both glycerol slides) have been deposited in the collection of the Zoological Museum, Copenhagen.

Etymology

The specific names alludes to the hairy appearance of the spinoscalids, Greek *khaita*, long hair.

Description

Holotype

The holotypic female (specimen 2 in Table II) is mounted ventrally on a S.E.M.-stub (Figs 1B, 2). Measured from the tip of the mouth cone to the anus, the holotype is 220 μm in length. The maximal width (of the lorica) is 70 μm . The specimen has the mouth cone with the long mouth tube and the introvert totally extended from the lorica, while the thorax is partially retracted inside the loricate armature. Due to a technical artifact, the abdomen looks a bit shrunken, so that the dorsal plates of the lorica appear more concave than is revealed by light microscopy.

The mouth cone with the fully extended mouth tube is 57 μm long and is surrounded by eight heavy ridges (also called oral stylets in Kristensen, 1983). These stylets are long (23 μm) and rigid. They cannot be retracted unlike the hexagonal mouth tube. The basal part of each stylet is furcate (external furca). The terminal mouth opening proves separated from the stylets and can be telescopically retracted inside the animal via the

mouth tube. The mouth cone narrows posteriorly and inserts onto the introvert resulting slightly separated from the first row of scalids by a naked area.

The introvert bears several hair-like appendages, the scalids, arranged in 9 rows. In our S.E.M. prepared specimen only appendages of the first row, the clavoscalids, are well discernible and easily identifiable. They appear as 8 narrow club-shaped arms encircling with their proximal portion the basal part of the mouth cone, while the distal part is oriented slightly forward. The distal end of the clavoscalid is pointed, whereas the proximal part is slightly enlarged and covered with tiny papillae giving it a velvet-like appearance. The second to sixth rows of appendages consist of spinoscalids having a bulbous base and terminal, very hairy, spine. The spinoscalids may be three-segmented as those comprised in the second and third rows or just two-segmented as those of the following four rows. The seventh row of cephalic appendages consists of serrated spinoscalids having a plate-shaped basis. The eighth and ninth rows of scalids present on the introvert are not well discernible in the holotype owing to the technique used.

The thorax is made up of two segments. The anterior portion, called the neck, bears 15 appendages, the trichoscalids, each arising from a basal plate. The posterior portion of the thorax lacks appendages, and it is totally covered by the lorica and therefore not discernible. The neck is separated anteriorly from the introvert by a constriction and a naked area without scalids. The trichoscalids emanate between the hollow spikes of the lorica (Fig. 1B). The serrated appendages consist of seven double trichoscalids alternating with eight single trichoscalids. The mid-dorsal trichoscalid is double (Fig. 2A) and consists of a short broad primary scalid (17 μm) and a long slender secondary one (35 μm). Both elements originate from two separated heart-shaped

basal plates. Seven dorsal trichoscalids of which three are double and four are single can be seen on the holotypic female.

The lorica, 130 μm long, consists of six longitudinal heavily sclerotized plates without longitudinal ridges. It is characterized externally by fine honey-comb like sculptures. Anteriorly, the lorica ends with 14 large hollow spikes (20-30 μm). A 15th spike, the mid-ventral, is abortive in this species and cannot be seen in the holotype (see below). Most hollow spikes bear three transverse cuticular bridges; in contrast, the four lateral spikes (the longest one, 30 μm) lack the bridges; instead, each of them bears a gland opening at its tip. The bridges can be seen externally by SEM-technique and the spikes consist of three compartments without gland ducts. Four flosculi are present posteriorly on each of the dorsolateral plates (Figs 1B, 2B). Two very small flosculi are present on the large triangular mid-dorsal plate at its posterior (Fig. 2C). The lorica shows several small pores ordered in a characteristic pattern on each plates. Relative to the dorsolateral plates, one pore can be seen near the flosculi and two in proximity of the anterior hollow spikes. The pores appear to be duct openings for an unicellular epidermal gland. The posterior end of the lorica is smoothly rounded and lacks an anal cone. The anal opening is covered by a small anal plate with a middorsal ridge, just behind the two middorsal flosculi (Fig. 2C)

Paratype

An adult female, 210 μm in total body length, mounted latero-ventrally on a SEM-stub (specimen 1 in Table II; Fig. 1A). The mouth cone, not fully extended, is 50 μm long including a small everted portion of the mouth tube. The ventral morphological characters, which could not be seen in the holotypic female, include the mid-ventral plate bearing anteriorly bilateral spikes, 15 μm long, and a very short (5 μm) mid-ventral spike. All three spikes have transverse bridges as in the mid-dorsal plates (Fig. 3B). Furthermore, the mid-ventral plate bears a cuticular reinforcement in the form of a double arched elevation (like a McDonald's logo) of the lorical cuticle (Fig. 3B).

Postlarvae

The three postlarvae are all strongly retracted. Specimens 3 and 4 (see Table II) are mounted on S.E.M.-stub (Fig. 1C, D). Specimen 5 is mounted in glycerol on a microslide. All three specimens look different from the adults in several characters. It seems that only the scalids are similar except for lack of one of the spinoscalids rings (scalid ring ?). The mid-dorsal plate is nearly split in three separate plates by strong ridges, each beginning at the tip of the spike. The lorical spikes are large and without the transverse bridges (Fig. 4A). Four flosculi are present on both dorsolateral plates. A mid-dorsal flosculus was observed in speci-

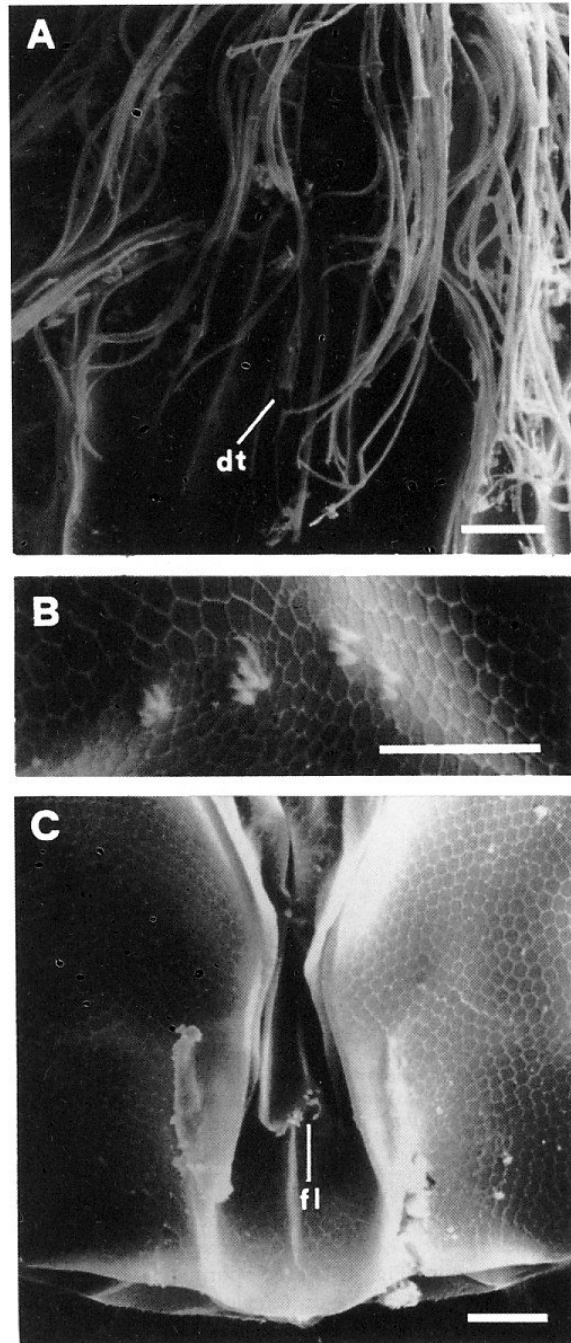


Fig. 2 - *Nanaloricus kbaitatus* nov. sp. - Holotype; **A**, close up of the mid-dorsal double trichoscalid (dt); **B**, dorsolateral lorical flosculi, right side, **C**, posterior end of the mid-dorsal lorical plate showing the flosculi (fl). Scale bars, 5 μm

men 5 (glycerol preparation) but not in specimens 3 and 4. The internal structure could also only be observed by light microscopy in specimen 5. The gonads were clearly immature and the midgut was full of many

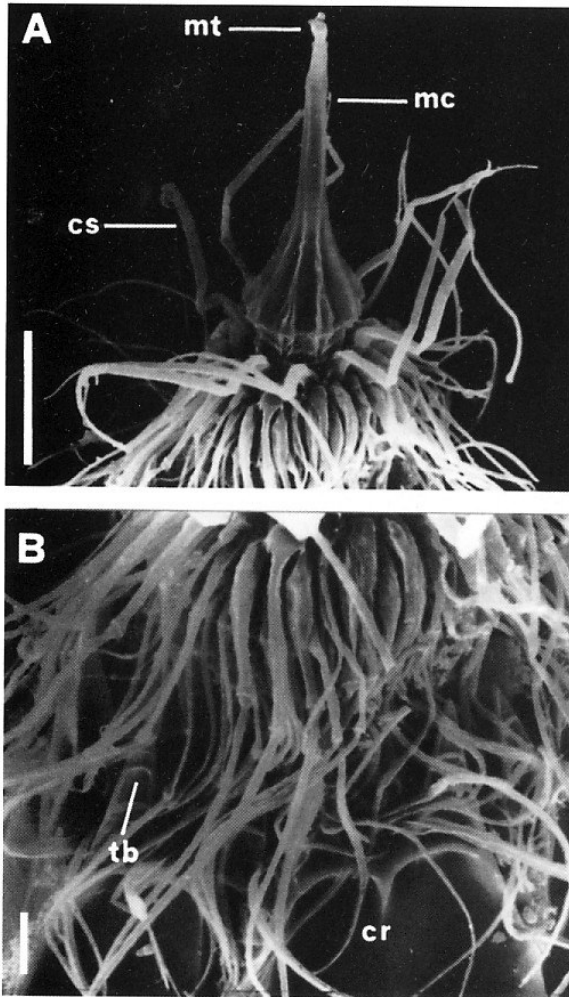


Fig. 3 - *Nanaloricus kbaitatus* nov. sp. - Paratype female, **A**, anterior end showing the mouth cone (mc), the mouth tube (mt) and the clavoscalids (cs); **B**, thorax and anterior portion of the lorica showing various types of scalids, the lorical spikes bearing transverse bridges (tb) and the two arched cuticular reinforcement (cr). Scale bars, 20 μ m

vesicles, a characteristic only found in other loriciferan postlarvae. One of the postlarvae (specimen 4) had several spermatozoa of the *Nanaloricus* type attached to the anal region (Fig. 4B). Free spermatozoa were observed at the same place on females of *N. mysticus*, but never before on postlarvae.

Higgins larvae

Specimen 6 (112 μ m in length) is mounted on the ventral site on the S.E.M. stub, so only the dorsal part could be observed (Fig. 5A). Specimen 7 is mounted in glycerol and can be observed from both the ventral and the dorsal sides. The mouth cone is retracted in both specimens and the introvert is partly inverted, too; therefore, the number and location of the scalid rings

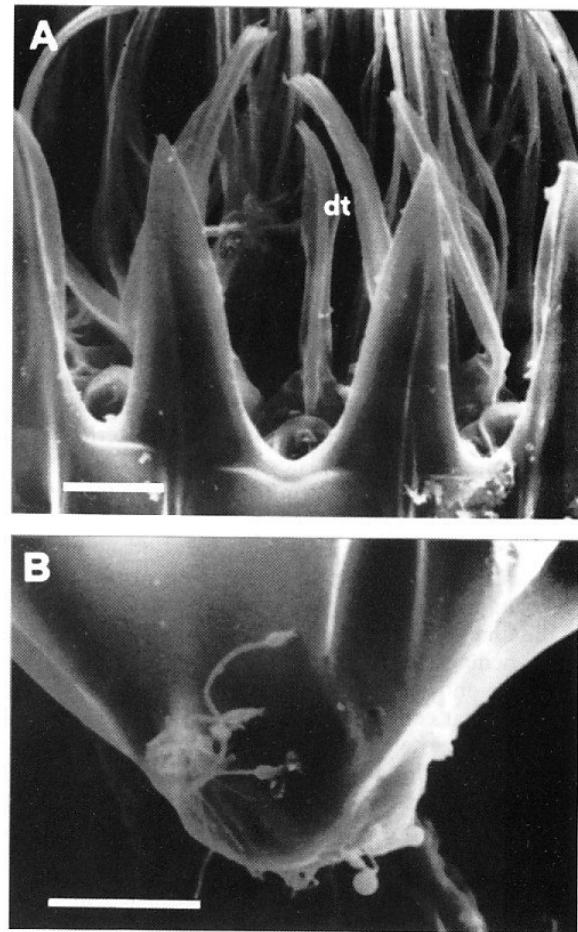


Fig. 4 - *Nanaloricus kbaitatus* nov. sp. - Postlarvae; **A**, ventral view, anterior portion of the lorica showing the spikes and a double trichoscalid (dt); **B**, close up the posterior end showing the attached spermatozoa. Scale bar, 5 μ m.

cannot be described. With regard to shape, the scalids of the present specimens resemble closely those of *Nanaloricus mysticus*. The lorica has about 20 longitudinal ridges and is characterized externally by a coarse honey-comb-like sculpture.

Dorsally, the longitudinal ridges of the lorica do not reach the caudal end. The caudal part of the larva has 7 small cuticular plates. Three of them bear a single flosculus. The middorsal plate covers the dorsal anus. The Higgins larvae carry two sets of sensory setae. One pair (17-21 μ m long) is located in the suture between the lorica and the small caudal plates. The second pair (32-40 μ m) lies more posteriorly on the dorso-lateral plates. Ventrally the four mid-ventral lorical ridges reach the caudal part of the larva. The ventro-lateral ridges form plate-like structures never observed before in any other loriciferan larva. Three pairs of locomotor spines are located between the thorax and the lorica. They have a common basis. The longest spine is about 50 μ m

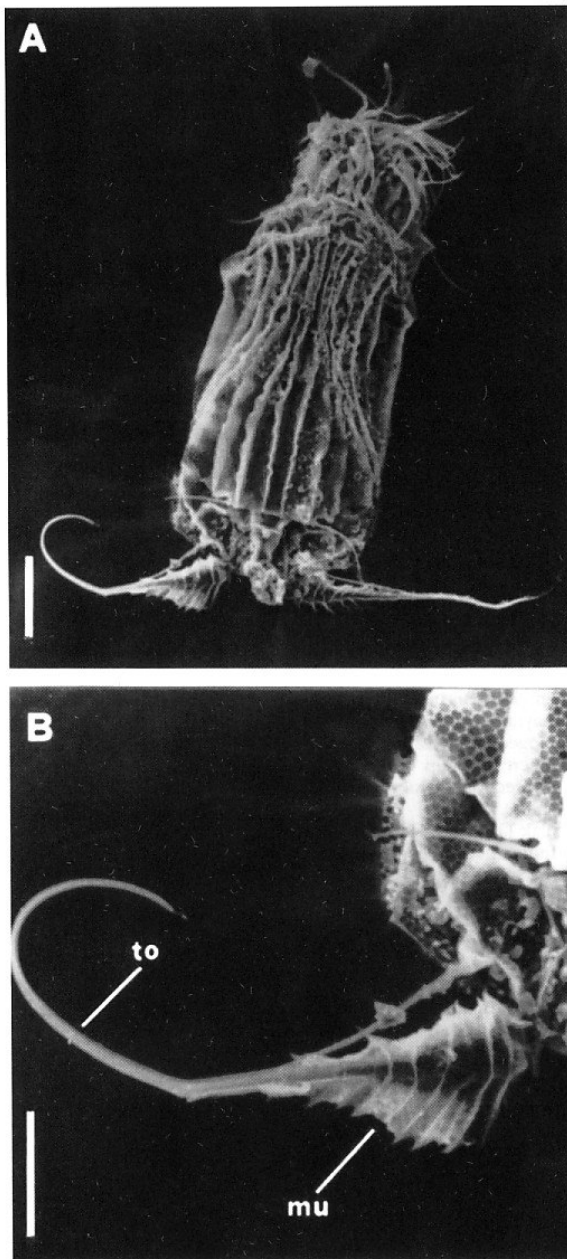


Fig. 5 - *Nanaloricus kbaitatus* nov. sp. - Higgins larva; **A**, last instar (specimen 6), dorsal; **B**, particular of the caudal toe (to) showing the mucro (mu); Scale bars, 20 μ m.

in length. The two closing plates of the thorax (for the introvert) were not observed. The most notable structures seen in the larval stages of *Nanaloricus* are two caudal toes consisting proximally of leaf-like structures, the mucro, and distally of a long cylindrical organ, open at its tip, enclosing a glandular canal. The toe of *N. kbaitatus* is very long (72-92 μ m), while the mucro-structure is only 25-30 μ m in length (Fig. 5B).

ECOLOGICAL REMARKS

The Meloria shoals is a shallow area (depth ranging from 2 to 25 m) of about 40 km² located some 4 km off the coast of Livorno in the lower Ligurian Sea. The core of the shoals is made up of Calcarenite, a diagenetic rock somewhat rich in calcareous material formed in the late Quaternary period (about 10⁵ b.p.). During the past glaciation events, this area emerged and the rockbed was subject to karstic phenomena. The characteristic rounded-bottom pits (called 'catini' by the local fishermen), found in good number in the area next to the Meloria lighthouse, are thought to be the result of such erosive action. Throughout time, the catini filled up with shells of small mollusks, along with the skeletal fragments of the organisms making up the coraligenous community living nearby. At 7 meters water depth, the temperature at the sediment-water interface varies according to the season from 12-13° C in winter to 25-27° C in summer; conversely, salinity remains almost constant, 37-38‰, throughout the year. These results may be due to the high energy of the local water that determine a continuous mixing of the aqueous medium; the high energy of the water column is reflected also in the coarse diameter of the sand found on the bottom of the pits. This organogenic, coarse, sand hosts a meiofauna community particularly rich (up to 4·10⁶ ind·m⁻²) and diverse (meiofaunal representative of at least 15 phyla have been found so far). Some of the multicellular meiofaunal associated taxa are: Cnidaria, *Halammobrydra* sp.; Nematoda, *Draconema* sp., *Dracognomus* sp., *Enoplolaimus* sp.; Rotifera, *Testudinella obscura*; Gastrotricha, *Diplodasys ankei*, *Platydasys ruber*, *Tetranchyroderma pachysomum*, *Chaetonotus* sp.; Kinorhyncha; Polychaeta, *Mesonerilla*; Tardigrada, *Battilipes* sp., *Chrysoarctus flabellatus*, *Parastygarctus sterreri*, *Tanarctus longisetosus*, *Actinarctus* sp.; Acarina, *Schaptognathus* sp., *Halacarus* sp.; Harpacticoida, *Amphiascus* spp., *Leptastacus* sp., *Meloriastacus ctenidis*; *Asellopsis* sp.; Amphipoda, *Idunella nana*; Ostracoda, *Pontocythere* spp., *Semicytherura* sp.

DISCUSSION AND CONCLUSIONS

On the basis of the adult abdominal region, that is covered with sclerotized lorica consisting of six plates, and of the general appearance of the larval stages, the loriciferans from Meloria fall within the family Nanaloricidae, which currently encompasses (formally) only the genus *Nanaloricus* (Higgins & Kristensen, 1986). Morphological traits of the adults' lorica and the morphology of the larval toes, which bear a series of leaf-like structures called mucros, both support the affiliation of our specimens to the genus *Nanaloricus* Kristensen, 1983. On the basis of the congruence of their morphological traits, it is reasonable to claim that all the specimens found belong to the same species taxon. Differ-

ence in the shape of the lorica, the smaller size $220 \times 70 \mu\text{m}$ vs. $235 \times 90 \mu\text{m}$, and more important the proportionally longer mouth cone ($50\text{-}57 \mu\text{m}$ vs $42\text{-}47 \mu\text{m}$), sets the Meloria specimens apart from *Nanalaricus mysticus*, to date the only species described in the genus (Kristensen, 1983). Moreover, the transverse three bridges on the lorical spikes, and the two mid-dorsal flosculi could be key-characters for the adult of *N. kbaitatus*.

At the Meloria shoals specimens were found casually during the 15-month period of research (March 1996 through May 1997), and always in low number (Table I). An attempt to increase the yield of loriferans using a different technique of meiofauna extraction (i.e., processing larger amount of sediment using freshwater osmotic shock), reported as useful in other studies (see Kristensen, 1991a) gave no better results. Therefore, our data suggest that loriferans are rare when compared to many other meiobenthic taxa and their rareness may have contributed to the late discovery of these animals in general, and in Italian waters in particular.

The finding of a representative of the genus *Nanalaricus* in the Mediterranean Sea widens the geographical range of the genus, which already comprises the North Sea (Helsingor, Denmark), the English Channel (Roscoff, France) the eastern Atlantic (Azores Islands, Portugal), and the western Atlantic (Ft. Pierce, Florida) (see Kristensen, 1983). The recovery of these rare little beasts in the Meloria shoals, pinpoints once again the prominent naturalistic value of this coastal area of Tuscany whose high meiobenthic diversity is becoming even more explicit (Todaro, 1997b, 1998; Todaro & Huys, 1997). The recovery of *Nanalaricus kbaitatus* from an easily reachable site is of additional interest since the species could be considered as a 'satellite' organism (see Schlak *et al.*, 1996). In this instance, its morphology and biology should be useful for comparisons with *N. mysticus*, the model species from which the foremost of what is now known about the phylum, and the entire data set on the family Nanalaricidae, has been drawn (cf. Kristensen, 1991a).

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