

This article was downloaded by:

On: 15 January 2010

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Italian Journal of Zoology

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t741771159>

### **A new meiobenthic priapulid (Priapulida, Tubiluchidae) from a Mediterranean submarine cave**

M. Antonio Todaro <sup>a</sup>; Thomas C. Shirley <sup>b</sup>

<sup>a</sup> Dipartimento di Biologia Animale, Università di Modena e Reggio Emilia, Modena, Italy <sup>b</sup> Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Juneau, AK, USA

**To cite this Article** Todaro, M. Antonio and Shirley, Thomas C.(2003) 'A new meiobenthic priapulid (Priapulida, Tubiluchidae) from a Mediterranean submarine cave', *Italian Journal of Zoology*, 70: 1, 79 — 87

**To link to this Article:** DOI: 10.1080/11250000309356499

**URL:** <http://dx.doi.org/10.1080/11250000309356499>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.



# A new meiobenthic priapulid (Priapulida, Tubiluchidae) from a Mediterranean submarine cave

M. ANTONIO TODARO

Dipartimento di Biologia Animale, Università di Modena e Reggio Emilia,  
Via Campi 231/d, I-41100 Modena (Italy)  
E-mail: todaro.antonio@unimo.it

THOMAS C. SHIRLEY

Juneau Center, School of Fisheries and Ocean Sciences,  
University of Alaska Fairbanks,  
Juneau, AK 99801 (USA)

## ABSTRACT

A new priapulid species belonging to genus *Tubiluchus* is described on the basis of specimens collected in November, 2000 and June, 2001 in a submarine cave along the Ionian coast of Apulia, Southern Italy. The new species closely resembles *T. corallicola* that inhabits the tropical-subtropical areas of the Western Atlantic Ocean but can be distinguished by the smaller size of the adults, and the smaller size and the shape of the pharyngeal teeth of the larval stages. A careful microscopical survey of the sensorial elements led to the discovery of a central bristle in the flosculi and a small hole at the tip of the associated setae, allowing speculation about a tactile sensorial function for the former and a chemoreceptive function for the latter. While *T. troglodytes* n. sp. represents the first fully described species of this genus from the Mediterranean Sea, the population densities recorded, particularly those found in June, 2001 ( $83.8 \pm 40.1$  SD ind./10 cm<sup>2</sup>) rank among the highest value reported for priapulids from any location in the world. On both sampling dates the sex ratio (female/total adults) in the *T. troglodytes* n. sp. population averaged about 0.6. Presence of larval stages of different ages during both sampling periods could imply that *Tubiluchus* spp reproduce the year around.

**KEY WORDS:** Priapulid - Mediterranean meiofauna - Submarine caves - Benthos - Taxonomy.

## ACKNOWLEDGEMENTS

Several friends and colleagues helped in ways large and small: we are grateful to G. Belmonte (University of Lecce) for providing us with invaluable information on Grotta del Cielo and priceless logistic help during sampling. D. Mosci (University of Urbino) assisted by SCUBA divers of the 'Gruppo Speleologico Neretino' (Lecce) collected the samples; F. Leasi (University of Modena e Reggio Emilia) helped to carry out the sorting; Mikhail Blikshteyn (University of Alaska Fairbanks) translated the Russian articles; M. Ferraguti (University of Milan) provided some of the literature; P. Tongiorgi read an early draft of the manuscript. We thank two anonymous reviewers for their insightful comments. The research was supported by a grant to MAT (MIUR, Scientific Research Fund 2000-2001). The study benefited also from a grant to F. Boero COPI (Cofin Project 1999/2000 "Aspetti descrittivi e metodologici della biodiversità animale in Italia").

## INTRODUCTION

Several adult of dwarf priapulids along with larval stages were found while the senior Author was engaged in a survey concerning the meiobenthos of a submarine caves along the Ionian coast of Apulia, Italy. The presence of the tail in the adults allowed identification of specimens to the genus *Tubiluchus* (see Todaro *et al.*, 2001, *Abstract* in Atti 63° Congr. U.Z.I.: 49). Subsequent to the first description of a tail-bearing priapulid, i.e., *T. corallicola* Land 1968, five additional species have been described from a variety of regions and habitats, including the tropical-subtropical Atlantic Ocean, the Red Sea, the Philippines area and the Arctic, testifying to the cosmopolitan nature of the genus (Land, 1982, 1985; Adrianov *et al.*, 1989; Adrianov & Malahov, 1991). Although representatives of the genus *Tubiluchus* previously have been reported to occur in the Mediterranean (Land, 1985; G. Gunnard, pers. commun.), the specimens from either the Levantine or the Western Basins have not been identified, leaving open the question of how many species exist in the region. The finding of numerous specimens including males, females and larval stages, should allow a species level identification of the Apulian worms, and therefore begin to illuminate the Mediterranean *Tubiluchus* fauna. In a larger framework, additional information on this "poorly-known" taxon should contribute to future debates on priapulid biology and their global biogeography.

## MATERIALS AND METHODS

Females, males and larval stages were found in sediment collected in November, 2000 and June, 2001 in the 120-m long cave "Grotta Piccola del Cielo" near Santa Maria di Leuca, Lecce (Italy). Quantitative samples in four replicates were collected by SCUBA diving, coring the sediment with a hand-held piston corer 2.37 cm

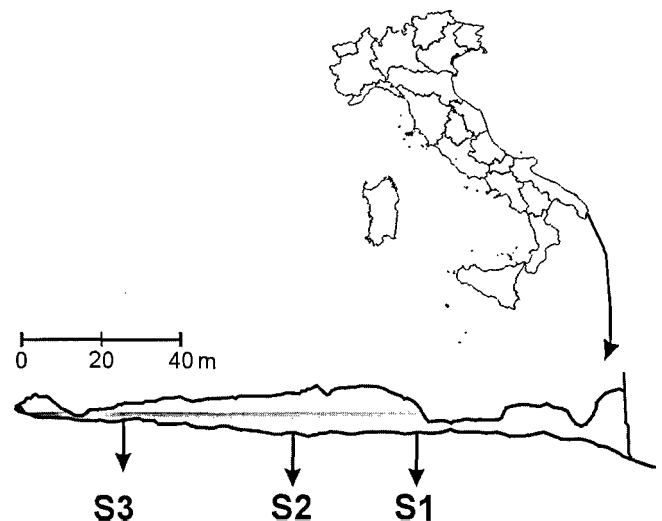


Fig. 1 - Localization of grotta del Cielo and sampling sites within the submarine cave.

i.d. in three light-free sites located at 55, 75 and 90 m from the entrance of the caves, at 4.2, 3.6 and 3.2 m water depth respectively (Fig. 1). In each site an additional 500-ml sediment sample was collected for qualitative analysis. Quantitative samples were fixed on site with a 10% buffered formalin solution and stored for later checking; qualitative samples were taken to the lab and analysed within 10 days from collection.

Living priapulids were extracted by a narcotization-decantation techniques using a 7% MgCl<sub>2</sub> solution. Five males and five females were observed with a Leitz 20 Dialux DIC optic microscope while still alive, and about 25 adults and 5 larvae were fixed overnight in a 1.0 M phosphate buffered (pH 7.3) solution of paraformaldehyde, glutaraldehyde and picric acid, following Ermak & Eakin (1976) and prepared for SEM analysis. To this end, worms were rinsed in 0.2 M cacodylate buffer, dehydrated through a graded ethanol series, critical point-dried using CO<sub>2</sub>, mounted on aluminium stubs, sputter coated with gold-palladium and observed with a Philips XL 30 scanning electron microscope. Fauna from quantitative samples was extracted by the centrifugation-decantation technique (Pfannkuche & Thiel, 1988) using Ludox-A30 colloidal silica, density = 1.210. Supernatant was filtered through a 63 µm mesh size sieve and the retained fauna was sorted by major taxa and counted with a Wild M8 stereomicroscope. Priapulids were identified by life stage and sex. Measurements were made with an ocular micrometer or derived from SEM micrographs.

Granulometric analysis of the substrata was determined according to Giere *et al.* (1988). Mean grain size, sorting coefficient, kurtosis and skewness were calculated by a computerized program (Todaro, 1992) based on the equation of Seward-Thompson & Hail (1973). The organic content of the sediment was determined by percent weight loss after combustion of 100 g of sediment at 480 °C for 4 h after the sediment had been dried in an oven at 60 °C for 24 h.

## TAXONOMIC ACCOUNTS

On both sampling occasions priapulids were found only at site 1 (about 55 m from the cave entrance at 4.2 m water depth); hereafter data will be presented only for this station. A more comprehensive account regard-

ing the meiobenthos and the physical-chemical characteristics of the cave will be published in a forthcoming paper (see also Todaro *et al.*, 2001, *Abstract* in Atti 63° Congr. U.Z.I.: 49). At site 1, on both sampling dates, sediment was composed of moderately sorted fine sand, containing about 5% dry weight of organic matter (Table I). The priapulid population comprising both adults and larvae reached a higher density in June, 2001 (total density  $83.8 \pm 40.1$  SD ind./10 cm<sup>2</sup>), compared to November, 2001 (total density  $37.0 \pm 15.0$  ind./10 cm<sup>2</sup>); on both occasions the priapulid sex ratio (female/total adults) averaged about 0.6 (Table II).

Preliminary morphological analysis set apart the present specimens from any other previously described species, leading to establishment of the following new taxon.

### Order PRIAPULOMORPHA

#### Family TUBILUCHIDAE

#### Genus *Tubiluchus*

[Type species *Tubiluchus corallicola* Land, 1968]

*Tubiluchus troglodytes* n. sp.

(Figs 2-8)

### Diagnosis

Adults: male and female of similar size; body excluding the tail  $1618.8 \pm 173$  µm ( $n = 10$ ) long, with the introvert fully extended; tail up to three times the body length; buccal tubes about 89 µm in length and 86 µm in width, bearing weak longitudinal ridges and 3-4 circlets of papillae. Anterior pharynx up to 132 µm in length, armed with comb-like teeth, 4-6 µm long and 7-9 µm wide; introvert  $215 \pm 89$  µm long and  $282 \pm 17$  µm wide ( $n = 10$ ), bearing conical to triangular, furry

TABLE I - Characteristics of the microhabitat in site 1 of the Grotta Piccola del Ciolo.

Sample	Temperature (°C)	Salinity (‰)	Grain size (phi)	Sorting	Kurtosis	Skewness	Organic matter (% d.w.)
07-Nov-2000	19	38	2.89	0.86	3.13	-0.34	4.8
05-Jun-2001	21	38	2.37	0.85	2.97	-0.33	5.2

TABLE II - Priapulid density, mean  $\pm$  SD (ind./10 cm<sup>2</sup>), in site 1 of the Grotta Piccola del Ciolo.

Date	Male	Female	Total adults	Sex ratio	Larve	Total
07-Nov-2000	$3.9 \pm 3.5$	$5.6 \pm 1.9$	$9.5 \pm 2.6$	$0.63 \pm 0.34$	$27.5 \pm 16.2$	$37.0 \pm 15.0$
05-Jun-2001	$9.7 \pm 6.0$	$13.9 \pm 6.8$	$23.6 \pm 12.7$	$0.60 \pm 0.05$	$60.2 \pm 28.7$	$83.8 \pm 40.1$

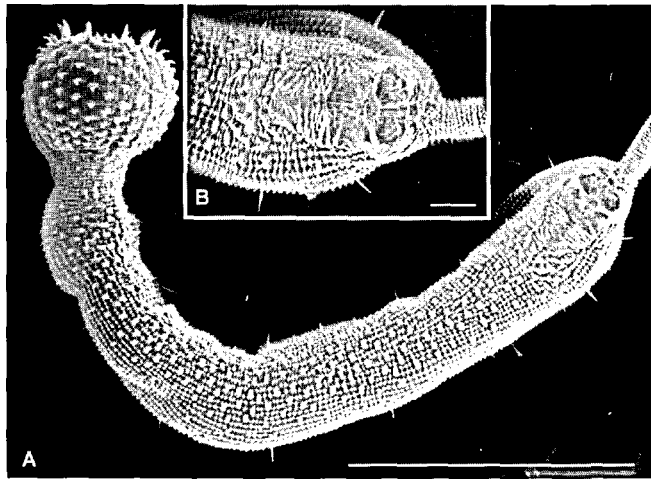


Fig. 2 - *Tubiluchus troglodytes* n. sp., male, SEM micrographs. **A**, habitus, ventral view. **B**, close up of the genital region. Scale bar: A = 500  $\mu$ m; B = 50  $\mu$ m.

scalids, 14-18  $\mu$ m long and 11-16  $\mu$ m wide at base; scalids of the first circles carry two terminal cones up to 5  $\mu$ m long; scalids of the 2<sup>nd</sup> to 16<sup>th</sup> circlets carry one terminal cone each, up to 6  $\mu$ m long; scalids of circlets 17 to 23 deprived of terminal cone; neck region 136  $\pm$  23  $\mu$ m long, narrowing to 131  $\pm$  7.9  $\mu$ m ( $n$  = 8) at mid length and carrying cupped, flower-like flosculi having 7-11 petals; abdomen roughly rounded, 1266  $\pm$  121  $\mu$ m long and 165  $\pm$  23  $\mu$ m wide ( $n$  = 10), covered with tumuli up to 10  $\times$  9  $\mu$ m in size reinforced by 7-11 cuticular ridges; tubuli generally scattered sparsely all the entire abdomen, but more densely packed on the male ventral surface together with numerous setae; genital area almost devoid of setae/and or tubuli in females, ornamented by 60-80 setae and tubuli in the male.

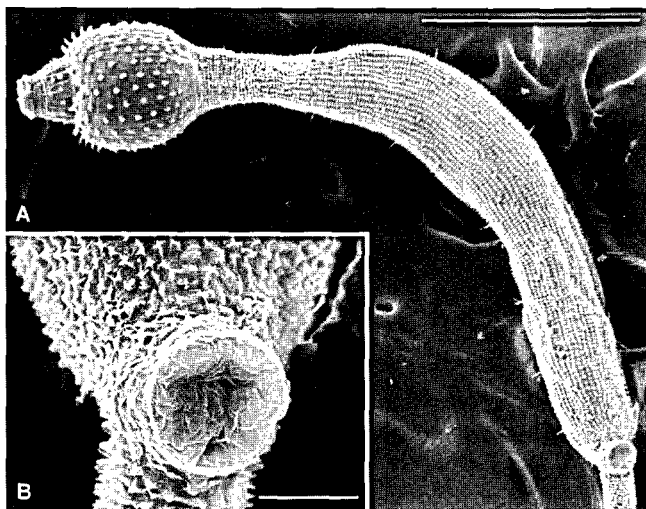


Fig. 3 - *Tubiluchus troglodytes* n. sp., female, SEM micrographs. **A**, habitus, ventral view. **B**, close up of the genital region. Scale bar: A = 500  $\mu$ m; B = 50  $\mu$ m.

### Type material

Holotype: an adult male specimen with the introvert fully extended, formalin-glycerin wholemount, deposited at The Natural History Museum, London, U.K. (NHM 2002.1). Additional material: one paratypic female specimen with the introvert fully extended, formalin-glycerin wholemount (NHM 2002.2), and two paratypic larval stages formalin-glycerin wholemount (NHM 2002.3). Other specimens on SEM stubs are kept in the meiofauna collection of the senior Author (ref. n. It-2001: 6-10).

### Type locality

Grotta del Ciolo, Lecce, Italy (Lat. 39°38' N; Long. 74°11' W). At about 55 m from the entrance and at 4 m water depth, in fine carbonatic sand rich in detritus.

### Etymology

The name alludes to the habitat (*trogle*, Gk cave, hole and *dytes*, Gk diver, enterer) of the new species.

### Description

#### Adults

Mature animals are 1381-1740  $\mu$ m in length, measured from the tip of the fully everted introvert to the base of the tail (Figs 2, 3). Total body length results from the extension of the of the following anatomical regions: introvert 204-290  $\mu$ m, neck 102-160  $\mu$ m, and abdomen 1227-1489  $\mu$ m. The widths of introvert, neck and abdomen are 263-310  $\mu$ m, 126-147  $\mu$ m, and 206-290  $\mu$ m, respectively. The tail is up to three times the length of the body and may reach 50-55  $\mu$ m in width. In living specimens during movement the body proportions change and the neck especially is capable of extending and narrowing significantly. The colour of the animals is yellowish white with the darker intestine and the ripe gonads, particularly the ovaries, visible through the body wall.

The buccal tube, the region connecting the introvert with the anterior pharynx, is cylindrically shaped, up to 89  $\mu$ m long and about 86  $\mu$ m wide; its surface bears weak longitudinal ridges and papillae arranged in several circlets (Fig. 4A). Elements of the first two or three circlets resemble the anterior scalids of the introvert (see below), but those in the circlets closer to the anterior pharynx are broad and triangular shaped. The anterior pharynx region is 132  $\mu$ m long, resembling a truncated cone 118  $\mu$ m wide at the base and 86  $\mu$ m wide at the distal end (Fig. 4A). The proximal part of the anterior pharynx carries 8-9 rows of pectinate teeth: comb-like cuticular elements 4-6  $\mu$ m long and 7-9  $\mu$ m wide consisting of two curved hooks laterally with up to 16 delicate spine in-between. Each tooth is connected by means of a very short stalk, the manubrium, to a basal plate; a series of very fine spinelets arranged in a sec-

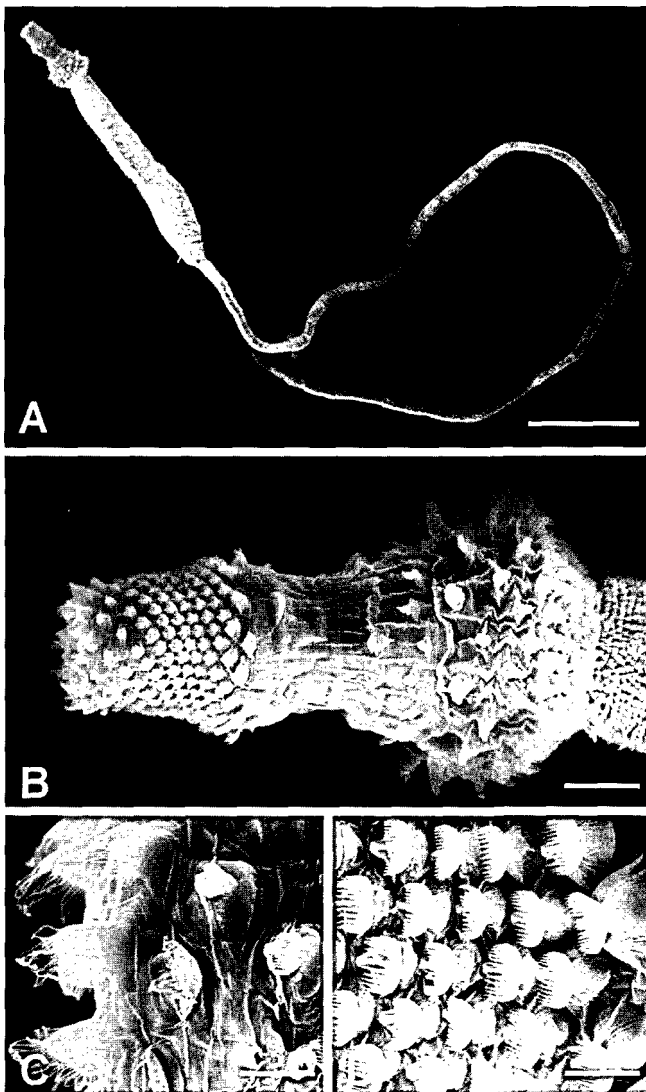


Fig. 4 - *Tubiluchus troglodytes* n. sp., female, SEM micrographs. A, habitus, showing most of the tail. B, introvert, showing the everted buccal cone and the anterior portion of the pharynx. C, close-up of the fimbriae. D, close-up of the pharyngeal pectinate teeth. Scale bar: A = 500 µm; B = 50 µm; C, D = 10 µm.

ondary comb arising from the basal plate ornate the emergence of each manubrium from the anterior side (Fig 4C). The narrower distal portion of the pharynx bears fimbriae, crescent shaped cuticular elements whose free margin are ornated with a fringe of delicate, long hairs (Fig. 4B).

The introvert is provided with 23-25 longitudinal row of leaf-like scalids (cf. Adrianov & Malakhov, 2001a). Eight scalids, equally spaced, are placed around the oral opening comprising the anterior, the first circlet of scalids; eight scalids are present also in following circlets with ordinal number  $3 + 3x$  and  $4 + 4x$  ( $x = 0, 1, 2, 3, \dots$ ), while nine scalids are present in the second and in circlets with ordinal number  $2 + 3x$  (Figs. 2A, 3A, 4A). Scalids of the first circlet are the largest, up to 38 µm long, almost conical in shape, with a rounded distal end

provided with two short, 5 µm, apical glandular cones (Fig. 5A). Scalids of the second circlet are morphologically similar to those of the first, but shorter (20-24 µm) and carry only a single terminal cone (Fig. 5B). Scalids of the subsequent circlets are hairy (i.e., external side covered with microsetae), almost triangular in shape, 14-18 µm long and 11-16 µm wide (Fig. 5C-I); they gradually decrease in size from the front to the back and for the most part carry a terminal single glandular cone up to 6 µm long (Fig. 5C-F). In scalids of the 4-6 posteriormost circlets the cone is lacking altogether (Fig. 5G-I).

The neck region (cervix) bears numerous cuticular paillae collected in 12-16 rings or circlets; rings are separated from each other by folding lines (Figs 2A, 3A). Interspersed amid paillae of each ring are sensory organs, the flosculi. Their morphology resembles a cup-like flower ( $3.6 \times 4.5$  µm) having 7-11 petals (Fig. 6); the petals are actually prominent bulges, which run vertically on the outside of the calyx and continue as short projections above its rims. In all cases one bristle (ca 5 µm long) arises from the central portion of the cup (Fig. 6H). In most SEM prepared specimens the bristle is missing and is visible only at its insertion point in the form of a small rounded hole. An accessory seta, slender conical to club-shaped, is inserted at the base of most (but not all) flosculi. The length of the seta (up to 10 µm) generally exceeds that of the companion flosculus, and its tip bears a small opening. A dual sensory function for the flosculus-setae system can reasonably be envisioned, perhaps a tactile function for the bristle and a chemoreceptive function for the setae. In both sexes flosculi similar in shape and size to those visible in the neck region may be found along the trunk, especially on the anterior ventrolateral sides; yet, in female specimens they are particularly abundant on the proximal 1/3 portion of the tail. While flosculi of the tail, similar to those of the neck, originate directly from the animal body, in several instances the flosculi of the trunk arise from the basal portion of large setae.

The abdomen and the tail are covered by longitudinal rows of tumuli, small papillae up to  $10 \times 9$  µm in size, peripherally supported by 7-11 cuticular ridges (Fig. 7A). The adhesive organs ('tubuli') are up to 27.5 µm in length; they resemble small tube-shaped organs, having a proximal, bulbous base joined to a stiff, elongate, distally-tapering tube marked by constrictions near its base and distal end (Fig. 7C). One or two very tiny distally adjoined tread-like elements, the 'tentacles', emerge from the concavity at the end of the tubulus; their function is still obscure. The accessory bristle reported for tubuli of other congeneric species (i.e., *T. corallicola* and *T. remanei*) occasionally may not be present.

In female specimens tubuli are scattered over the entire abdomen, while in males they are distributed sparsely over the dorsal and dorsolateral sides but distributed, together with numerous setae, elements similar to the tubuli but tapering to a point (Fig. 7A, B), in a band-like pattern on the ventral surface, anterior to the perigenital

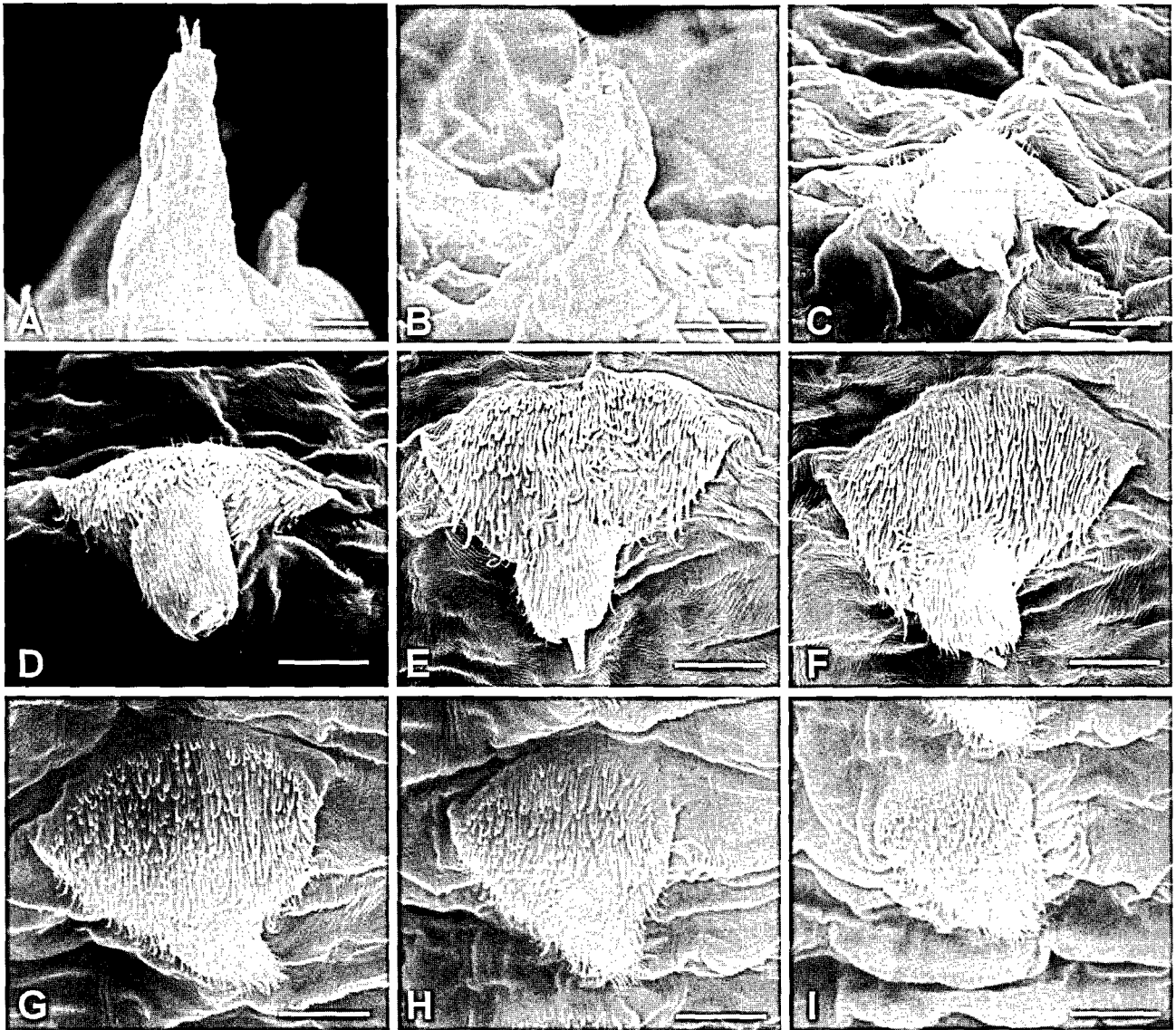


Fig. 5 - *Tubiluchus troglodytes* n. sp., male, SEM micrographs: close-up of the scalids. **A**, scalid of the 1<sup>st</sup> circlet. **B**, scalid of the 2<sup>nd</sup> circlet. **C**, scalid of circlet 3. **D**, scalid of circlet 4. **E**, scalid of circlet 7. **F**, scalid of circlet 10. **G**, scalids of circlet 17. **H**, scalids of circlet 20. **I**, scalid of circlet 23. Scale bar = 5  $\mu$ m.

area (Figs 2, 3). As in other species of the genus *Tubiluchus*, the perigenital area of the new species exhibits a marked sexual dimorphism (Figs 2B, 3B). In female specimens the area does not have evident differentiation compared to other regions of the trunk (i.e., bears only few setae and tubuli), whereas in the males this area is peculiarly differentiated: the cuticle of its posterior-most portion (genital bulge) appears elevated to make an 8-like design whose ridges are ornate with 8-10 setae of different lengths (up to 23  $\mu$ m). A clavula with stalk, 6  $\mu$ m in length, and head, 4.5  $\mu$ m in diameter, is inserted on each side between two 23  $\mu$ m-long setae inserted laterally on the anterior edge of the bulge. Up to 70 additional setae (4-25  $\mu$ m in length) are implanted more anteriorly to the 8-like feature giving a very furry appearance to the entire region (Fig. 2B).

#### Larvae

The description is based on the five SEM prepared specimens all of which have a fully extended introvert, including two specimens that have also everted the mouth cone; main metric and meristics traits are reported in Table III. Larva total body length (introvert + neck + lorica) ranges from 448  $\mu$ m to 635  $\mu$ m; a tail is missing.

The fully everted mouth cone is 120  $\mu$ m in length and 86  $\mu$ m in width; it bears pharyngeal teeth arranged in 8-9 circlets (Fig. 7A). Only teeth of the first five or perhaps six circlets are armed with well differentiated spines or cuticular processes, while other are in the form of strong cuticularized, smooth plates (Fig 7D-G).

The introvert is rounded, almost spherical as that of the adults; it bears furry scalids, deprived of terminal



Fig. 6 - *Tubiluchus troglodytes* n. sp., SEM micrographs: different types of flosculi of the adults. Scale bar: A, F = 2  $\mu$ m; B-E, G = 2  $\mu$ m.

cone(s), arranged in several circlets (octaradial symmetry of the primary scalids and 25-symmetry of secondary scalids, cf. Adrianow & Malakhov, 2001b). Scalids are oblong-triangular in shape (max length 40  $\mu$ m).

The neck region shows 6-8 smooth plates separated by transverse ridges and bears only few flosculi (Fig. 7B).

The abdomen is enclosed in the lorical armature that spans from 268  $\mu$ m to 369  $\mu$ m in length and 128 to 167  $\mu$ m in the maximal widths. Lorica is round in cross-section and exhibits 20 longitudinal ridges of which 10 are thicker (primary ridges) and alternate with less distinct secondary ridges. On primary ridges are inserted tubuli (28-36  $\mu$ m in length) arranged in 3-5 circlets depending

on the age of the larva and flosculi, very few and only in the posterior end (Fig. 7A, B).

#### CONCLUSIVE REMARKS

To date the genus *Tubiluchus* includes six species, namely, *T. corallicola* Land, 1968, *T. remanei* Land, 1982, *T. philippinensis* Land, 1985, *T. australensis* Land 1985, *T. arcticus* Adrianov, Malakhov, Chesunov & Tsetlin, 1988 e *T. vanuatensis* Adrianov & Malakhov, 1991.

Specimens, especially females, of the six taxa show a very close morphology, particularly the females, making

TABLE III - Main metric and morphological traits of the five larvae analysed (see text for details).

Trait	Larva				
	A	B	C	D	E
Total length	635.6	597.0	555.0	615.0	448.0
Mouth cone	Na	88.6	120.0	Na	Na
Introvert	146.0	142.0	146.0	139.0	97.0
Neck	143.0	104.0	93.0	107.0	83.0
Lorica length	346.6	351.0	316.0	369.0	268.0
Lorica width	159.0	148.0	167.0	159.0	128.0
No. of circlets of tubuli	4	4	5	5	3

Na, not available.

species identification troublesome. Most used diagnostic traits used to distinguish male specimens of different species include the type, number and arrangement of the setae and tubuli in the perigenital area (Land, 1982, 1985), while female specimens have been identified to species by virtue of their body size, relative extension of the three body regions (introvert, neck and trunk) and also by the length of the tail compared to the body length (Adrianov *et al.*, 1989; Adrianov & Malkhov, 1991). Other diagnostic characters seldom used include the length of the scalids of the 1st circlet, the number of the apical glandular cones on the scalids, and the presence/absence of the manubrium in the pectinate teeth of the pharynx (Adrianov *et al.*, 1989), or the morphology of the neck region, and that of the adhesive tubuli and flosculi (Adrianov & Malkhov, 1991).

*Tubiluchus corallicola* has been the object of several morphological surveys (es. Land, 1968; Kirsteur & Land, 1970; Kirsteur & Rützel, 1973; Calloway, 1975; Kirsteur, 1976; Higgins & Storch, 1989), therefore by virtue of its much better known external anatomy this species has always been the preferential taxon against which specimens belonging to putative new species have been compared. Indeed, for their general appearance, including the number and distribution of tubuli and setae, and length of the tail, the adults specimens from the grotta del Ciolo closely resemble the adults of *T. corallicola*, from which they can, however, be distinguished mainly because of their smaller size (1.3-1.7 mm vs 1.8-2.0 mm) and perhaps the different morphology of their flosculi that bear 7-11 petals vs the only 8 petals recorded by most Authors on the flosculi of *T. corallicola* (in this regard see discussion in Kirsteur, 1976, p. 242).

In their total body length (< 2.0 mm) as well as the relative length of the body regions the Italian adult specimens approach *T. arcticus* and *T. vanuatensis*. From these two species however, they are distinguishable because of the tail length to body length ratio, 3:1

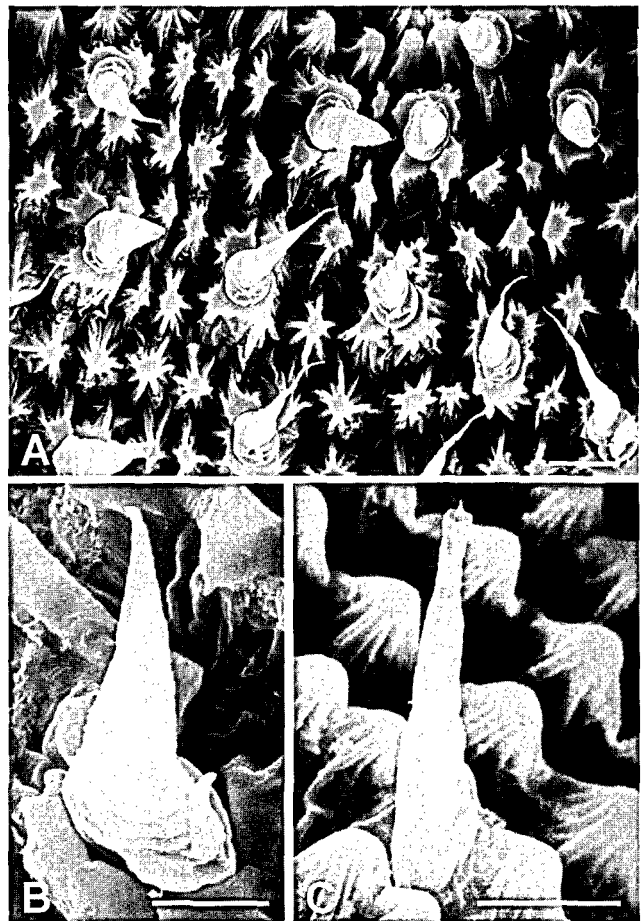


Fig. 7 - *Tubiluchus troglodytes* n. sp., adult specimen, SEM micrographs. A, trunk region, showing tumuli, tubuli and setae. B, close-up of a seta. C, close-up of a tubulus. Scale bar: A = 10 µm; B, C = 5 µm.

in *T. troglodytes* and 1:1 in the other two species; moreover the Italian specimens differ from *T. arcticus* because the scalids of the 1<sup>st</sup> circlet of its introvert bear only two terminal cones vs three cones present in the corresponding scalids of *T. arcticus*. The scalids of the ensuing 17 circlets of the new species also differ from those of the known species because they bear one terminal cone, absent in *T. arcticus*. Priapulids from Apulia differ from *T. vanuatensis*, in addition to the much greater length of the tail, also in the morphology of the neck region, which is covered with small papillae in the new species vs large, smooth plates in *T. vanuatensis*.

In addition to its smaller size, the species from the Mediterranean cave can be distinguished from *T. remanei* based on morphology of tubuli that in *T. troglodytes* are deprived of the peculiar annulations of the adhesive element of the latter species. The clavulae are also dissimilar in the two species; for instance, the head is spherical and the stalk is short in *T. troglodytes* while an oval head and a much short stalk characterize the clavulae of *T. remanei* (Land, 1982).

Male specimens from the grotta del Ciolo differ from male specimens of *T. philippinensis* in the morphology

of the genital area; e.g., they lack the peculiar perigenital setulae (cf. Land, 1985, fig. 17; Storch & Alberti, 1988).

Major differences between the new species and *T. australensis* includes the higher number of ventral setae, up to 70 in *T. troglodytes* vs "very few ventral setae" (Land, 1985), the major length of the setae (25  $\mu\text{m}$  vs up to 15  $\mu\text{m}$ ) and the absence of both the over-sized 'gigantic' flosculus and the uncinete setae reported in the posterior ventral end of the males of Australian species.

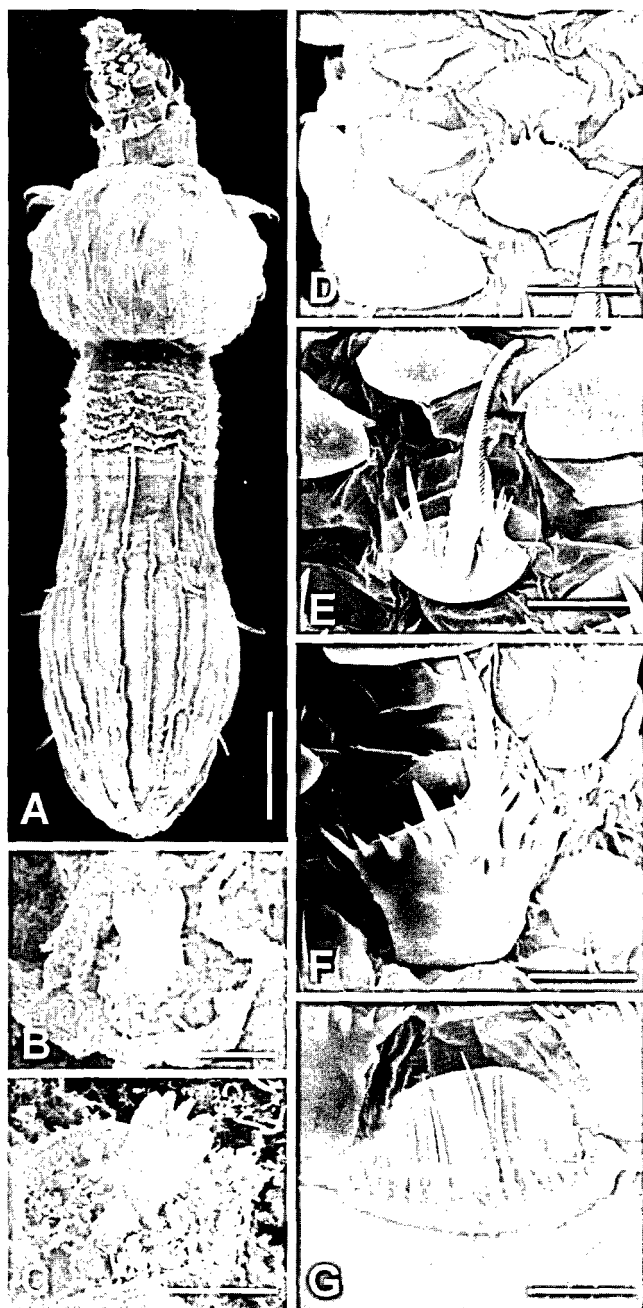


Fig. 8 - *Tubiluchus troglodytes* n. sp., larval stage, SEM micrographs. A, habitus of a larva with buccal cone and anterior pharynx region everted. B, flosculi of the neck region. C, flosculi of the lorica. D, pharyngeal teeth circlets 4-8. E, tooth of circlet 3. F, tooth of the 2<sup>nd</sup>. G, tooth of the 1<sup>st</sup> circlet. Scale bar: A = 100  $\mu\text{m}$ ; B, C = 2  $\mu\text{m}$ ; D-G = 10  $\mu\text{m}$ .

Differences in several traits allow the discrimination of the larvae of the new species from those of *T. corallicola*, *T. remanei* and *T. vanuatuensis*, the only other congeneric species for which larval stages have been described. Restricting the comparison to the shape of the pharyngeal teeth of instars of similar age, larvae of *T. troglodytes* differ from larvae of *T. corallicola* in the morphology of the teeth of the 3<sup>rd</sup> circlet in that in the new species they bear only two vs six basal spines bilateral to the strong central spine (cf. Kirsteuer, 1976, fig. 17). Larvae of the Italian species and larvae of *T. remanei*, differ because the six long spines of the teeth of the first circlet are arranged in two bilateral groups in the former, whereas they make a single group in the latter (cf. Land, 1982). Larvae of the new species differ from that of *T. vanuatuensis* because its teeth in the first circlet lack the central spine described for the latter species (cf. Adrianov & Malakhov, 1991, fig. 17).

We have no doubt that *T. troglodytes* represent a closely related but distinct species, distinguishable from other *Tubiluchus* spp also on distribution and ecological ground. *Tubiluchus arcticus* is unusual in its distribution at high latitudes, with other congeners being largely restricted to the shallow subtidal zone, predominately in coral sands within the tropics or subtropics. *Tubiluchus australensis* was found in a habitat apparently similar to that of *T. troglodytes*, in a deep cave at 6 m depth, but was from Lizard Island, in the tropical waters of the Great Barrier Reef.

In comparing *T. remanei* and *T. corallicola*, Land (1982) found it remarkable that both the adults and larvae of congeners from different oceans could be so similar morphologically. The similarity among *Tubiluchus* spp. was also noted in his descriptions of *T. australensis* and *T. philippinensis* (Land, 1985). Despite the morphological similarity of the species, Land had no difficulties in distinguishing the species, based primarily on differences in male specimens.

Similarity among priapulids is not unusual, and many extant species have changed little from their fossil relatives preserved in the Burgess shale (Conway-Morris, 1977). *Halicryptus bigginsi* is morphologically similar to its congener, *H. spinulosus*, however it has distinctive characteristics and is considerably larger (Shirley & Storch, 1999).

Shirley & Storch (1999) predicted that many additional macrobenthic and meiobenthic species of Priapulida remain to be described from a variety of arctic and tropical areas, especially in physically stressed habitats and where predation and competition might be low. The troglodytic habitat of *T. troglodytes* might be considered one with low predation. Additional specimens of *Tubiluchus* have been collected elsewhere in the Mediterranean Sea and these may represent undescribed species. An undescribed *Tubiluchus* larva was collected from the shallow subtidal of Hawaii (T. C. Shirley, unpubl. obs.). We believe that additional undescribed *Tubiluchus* species will continue to be found, even in well-sampled regions.

## REFERENCES

- Adrianov A. V., Malakhov V. V., 1991 - First finding of dwarf priapulids of the genus *Tubiluchus* (Priapulida, Tubiluchidae) in Oceania (New Hebrides Isles). *Zool. Zh.*, 70: 23-32.
- Adrianov A. V., Malakhov V. V., 2001a - Symmetry of priapulids (Priapulida). 1. Symmetry of adults. *J. Morphol.*, 247: 99-110.
- Adrianov A. V., Malakhov V. V., 2001b - Symmetry of priapulids (Priapulida). 2. Symmetry of larvae. *J. Morphol.*, 247: 111-121.
- Adrianov A. V., Malakhov V. V., Chesunov A. V., Tsetlin A. B., 1989 - *Tubiluchus arcticus* sp. n. from the White Sea (Priapulomorpha, Tubiluchidae). *Zool. Zh.*, 68: 126-131.
- Calloway C. B., 1975 - Morphology of the introvert and associated structure of the priapulid *Tubiluchus corallicola* from Bermuda. *Mar. Biol.*, 31: 161-174.
- Conways-Morris S., 1977 - Fossil priapulid worms. *Spec. Pap. Palaentol.*, 20: 1-95.
- Ermak T. H., Eakin R. M., 1976 - Fine structure of the cerebral pygidial ocelli in *Chone ecaudata* (Polychaeta: Sabellidae). *J. Ultrastruct. Res.*, 54: 243-260.
- Giere O., Eleftheriou A., Murison D. J., 1988 - Abiotic factors. *In*: R. P. Higgins & H. Thiel (eds), *Introduction to the study of meiofauna*. Smithsonian Institution Press, Washington D.C., pp. 61-78.
- Higgins R. P., Storch V., 1989 - Ultrastructural observations of the larva of *Tubiluchus corallicola* (Priapulida). *Helgol. Meeresunters.*, 43: 1-12.
- Kirsteuer E., 1976 - Notes on the adult morphology and larval development of *Tubiluchus corallicola* (Priapulida), based on in vivo and scanning electron microscopic examinations of specimens from Bermuda. *Zool. Scr.*, 5: 239-255.
- Kirsteuer E., Land J. van der, 1970 - Notes on *Tubiluchus corallicola*. *Mar. Biol.*, 7: 230-238.
- Kirsteuer E., Rützel K., 1973 - Additional notes on *Tubiluchus corallicola* (Priapulida), based on scanning electron microscope observation. *Mar. Biol.*, 20: 78-87.
- Land J. van der, 1968 - A new aschelminthe, probably related to the Priapulida. *Zool. Meded.*, 42: 237-250.
- Land J. van der, 1982 - A new species of *Tubiluchus* (Priapulida) from the Red Sea. *Neth. J. Zool.*, 32: 324-335.
- Land J. van der, 1985 - Two new species of *Tubiluchus* (Priapulida) from the Pacific Ocean. *Proc. K. Ned. Akad. Wet. Ser. C. Biol. Med. Sci.*, 88: 371-377.
- Pfannkuche O., Thiel H., 1988 - Sampling processing. *In*: R. P. Higgins & H. Thiel (eds), *Introduction to the study of meiofauna*. Smithsonian Institution Press, Washington D.C., pp. 134-145.
- Seward-Thompson B. L., Hails J. R., 1973 - An appraisal of the computation of statistical parameters in grain size analysis. *Sedimentology*, 20: 161-169.
- Shirley T. C., Storch V., 1999 - *Halicryptus bigginsi* n. sp. (Priapulida) - a giant new species from Barrow, Alaska. *Invertebr. Biol.*, 188: 404-413.
- Storch V., Alberti G., 1988 - Ultrastructural investigation of the integument of *Tubiluchus philippinensis* (Priapulida, Tubiluchidae). *Zool. Scr.*, 14: 265-272.
- Todaro M. A., 1992 - Contribution to the study of the Mediterranean meiofauna: Gastrotricha from the Island of Ponza, Italy. *Boll. Zool.*, 59: 321-333.