

# Morphology of *Xenodasys* (Gastrotricha): the first species from the Mediterranean Sea and the establishment of *Chordodasiopsis* gen. nov. and Xenodasyidae fam. nov.

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During a survey of the Italian marine meiofauna, several specimens of the rare gastrotrich genus *Xenodasys* were found in a submarine cave along the Ionian coast of Apulia. The finding represents the first record of the genus for the Mediterranean Sea and reinforces the consideration of marine caves as habitats of high naturalistic value. The specimens, analysed using different microscopy techniques, showed a new species, named *Xenodasys eknomios*. Scanning electron microscopy, unveiling the astonishing morphology of this unusual gastrotrich, indicates that, due to technical artefacts, light microscopy may generate unreal features, which in the past may have led to the misinterpretation of the anatomical traits of these creatures. Transmission electron microscopy indicated that the ‘Seitenfüsschen’, are genuine elements of the adhesive apparatus, in contrast with previous investigation, which attributed an exclusive sensorial function to these organs. Confocal laser scanning microscopy, combined with actin-binding fluorochromes, revealed muscular elements in a region where originally the muscular chordoid organ was reported for gastrotrich species belonging to the genus *Chordodasys*. A taxonomic revision of the species currently allocated to the genus *Xenodasys* led to the establishment of *Chordodasiopsis* gen. nov. to integrate the former *Xenodasys* (= *Chordodasys*) *antennatus* and to the drafting of emended diagnosis of the genus *Xenodasys*. An overview of the high-rank systematization of these genera is also provided, with the establishment of Xenodasyidae fam. nov. to allocate both *Xenodasys* and *Chordodasiopsis*.

## INTRODUCTION

This study is part of a larger research programme aimed at shedding light on the diversity of marine meiofauna of Italy and the Mediterranean Sea. During a survey of the meiobenthos of some semi-submerged marine caves along the Ionian coast of Apulia, southern Italy (see Todaro & Shirley, 2003; Todaro et al., 2006a), in the sandy sediment of one of the caves, we found about 50 gastrotrich specimens belonging to the rare genus *Xenodasys* Swedmark, 1967, that was previously unreported from the Mediterranean Sea.

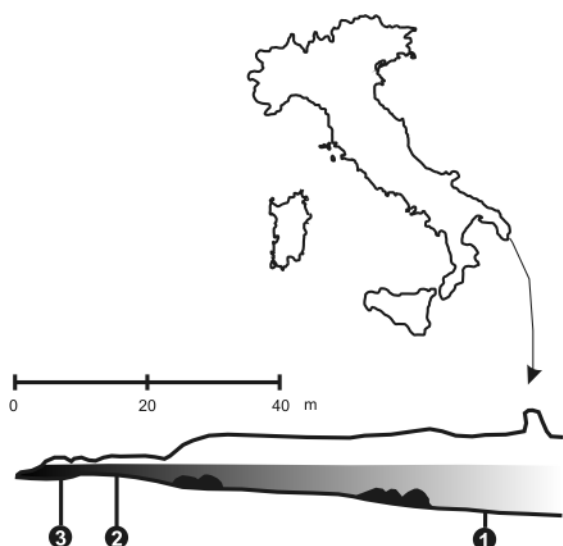
The genus includes the type species, *X. sanctigoulveni* Swedmark, 1967 from northern Europe (Roscoff, France: Swedmark, 1967; Kisielewski, 1987; Faroe Islands: Hummon, 2001; Clausen, 2004), and two, mainly western Atlantic species (see distribution below) originally affiliated with the genus *Chordodasys* Schoepfer-Sterrer, 1969 namely: *X. riedli* (Schoepfer-Sterrer, 1969) and *X. antennatus* (Rieger, Ruppert, Rieger & Schoepfer-Sterrer, 1974). However, the affiliation to *Xenodasys* of at least one of the last two species (see Hummon, 1982; Kisielewski, 1987) casts some doubts.

The finding of numerous specimens belonging to this unusual gastrotrich genus prompted us to prepare them for different microscopy analyses, especially scanning electron microscopy (SEM), aiming to clarify the nature

and organization of the complex morphology of this ‘aberrant type’ of gastrotrich (Rieger et al., 1974). As an SEM survey has never been carried out on these animals, it is hoped that electron microscopy, along with differential interference contrast (DIC) and confocal investigation, can bring us to propose a more natural grouping of the taxa that were originally spread into the two genera *Xenodasys* and *Chordodasys* and therefore present more appropriate diagnoses for both taxa.

## MATERIALS AND METHODS

Gastrotrichs were found in sandy sediment collected on the 21 June 2001 in an 80-m long cave near Santa Maria di Leuca, Lecce, Italy. Quantitative samples in four replicates were collected by SCUBA diving, coring the sediment with a hand-held 2.37 cm i.d. piston corer, at three sites located at 20, 60 and 75 m from the entrance of the caves, at 10.5, 6.0 and 5.0 m water depths, respectively; the last two sites were completely light-free (Figure 1). At each site an additional 500-ml sediment sample was collected for qualitative analysis. Qualitative samples were taken to the laboratory and analysed within ten days of collection, whereas quantitative samples were fixed on site with a 10% buffered formalin solution and stored for later checking.



**Figure 1.** Location of 'Grotta della Principessa' and sampling sites within the submarine cave. Specimens of the new species were only found in the innermost collecting site (3).

Living gastrotrichs were extracted using the narcotization–decantation technique with a 7%  $MgCl_2$  solution. Six relaxed adult specimens were observed with a Leitz 20 Dialux equipped with DIC (Nomarski) optics and photographed with a Nikon Coolpix 995 digital camera while still alive; 15 additional adult specimens were fixed overnight in a 1.0 M phosphate buffered (pH 7.3) solution of paraformaldehyde, glutaraldehyde and picric acid (see Balsamo et al., 2002), and prepared for EM analysis. For SEM, the worms were rinsed in 0.1 M phosphate-buffered saline (PBS), dehydrated through a graded ethanol series, critical point-dried using  $CO_2$ , mounted on aluminium stubs, sputter coated with gold-palladium and observed with a Philips XL 30 scanning electron microscope. For transmission electron microscopy (TEM) study, after washing in 0.1 M PBS, the gastrotrichs were postfixed in 2% osmium tetroxide solution in the same buffer, then rinsed in PBS again, dehydrated in a graded acetone series, stained en bloc in uranyl acetate in 70% acetone, and embedded in Araldite. Ultra-thin sections were cut with a LKB Ultratome 2088V, contrasted with lead citrate solution, and observed under a Philips 300 and a Zeiss 902 transmission electron microscope.

A single *Xenodasys*, collected in the cave on 9 June 2005, was surveyed by confocal microscopy; to this end the specimen was incubated for 1.5 h in 4% paraformaldehyde solution (0.1 M PBS, pH 7.4), subsequently washed with PBS and permeabilized for 1 h in a 0.2% Triton X-100 solution, 0.25% bovine serum albumin and 0.05%  $NaN_3$  in PBS and incubated in TRITC-phalloidin (Sigma) for 1 h. The gastrotrich was then rinsed in PBS and embedded in Citifluor (Plano, Wetzlar) on microscopic slides and observed using a Leica DM IRE 2 confocal laser scanning microscope; a series of 'optical sections' were projected in one maximum projection image.

Measurements were made with an ocular micrometer or derived from micrographs; in the description of the new species the locations of some morphological characteristics along the body are given in percentage units (U) of the total body length measured from anterior to posterior;

the description is based mostly on SEM prepared specimens.

Fauna from quantitative samples were extracted by the centrifugation–decantation technique using Ludox-A30 colloidal silica,  $d=1.210$ . The supernatant was filtered through a 63- $\mu m$  mesh size sieve and the retained fauna was sorted by major taxa and counted under a Wild M8 stereomicroscope.

The granulometric analysis of the substrata was determined according to Giere et al. (1988), and the parameters (mean grain size, sorting coefficient, kurtosis and skewness) were calculated by a computerized program (Todaro, 1992) based on the equation of Seward-Thompson & Hails (1973). The organic content of the sediment was determined by percentage 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 48 h.

## RESULTS

Gastrotrichs were found in all the investigated sites of the cave: however, since specimens belonging to the genus *Xenodasys* were only found at Site 3 (about 70 m from the cave entrance at 4.0 m water depth), hereafter data will be presented only for this station. A more comprehensive account regarding the Gastrotricha, the whole meio-benthos and the physical–chemical characteristics of the cave will be published in a forthcoming paper. At Site 3, the sediment was composed of moderately sorted medium–coarse sand (mean grain size, 1.001; sorting coefficient, 0.60; kurtosis 2.41; skewness, 0.196) containing 0.8% dry weight of organic matter. The water temperature was 21°C and salinity equal to 38 psu. The gastrotrich population, comprising representatives of 14 species (*Dendrodasys gracilis*, *Xenodasys* sp., *Macrodasys caudatus*, *Thaumastoderma mediterraneum*, *Diplodasys ankei*, *Diplodasys minor*, *Chaetonotus apolemmus*, *C. atrox*, *Chaetonotus* sp., *Halichaetonotus spinosus*, *Musellifer delamarei*, *Draculiciteria tessellata*, *Heteroxenotrichula pygmaea* and *Xenotrichula* sp.) reached a total density of  $7.6 \pm 1.7$  ind/10 cm<sup>2</sup>, (3.2% of the total meiobenthic population), whereas the density of *Xenodasys* specimens reached  $1.3 \pm 1.6$  ind/10 cm<sup>2</sup>.

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

Order MACRODASYIDA Remane, 1925

[Rao & Clausen, 1970]

Family XENODASYIDAE fam. nov.

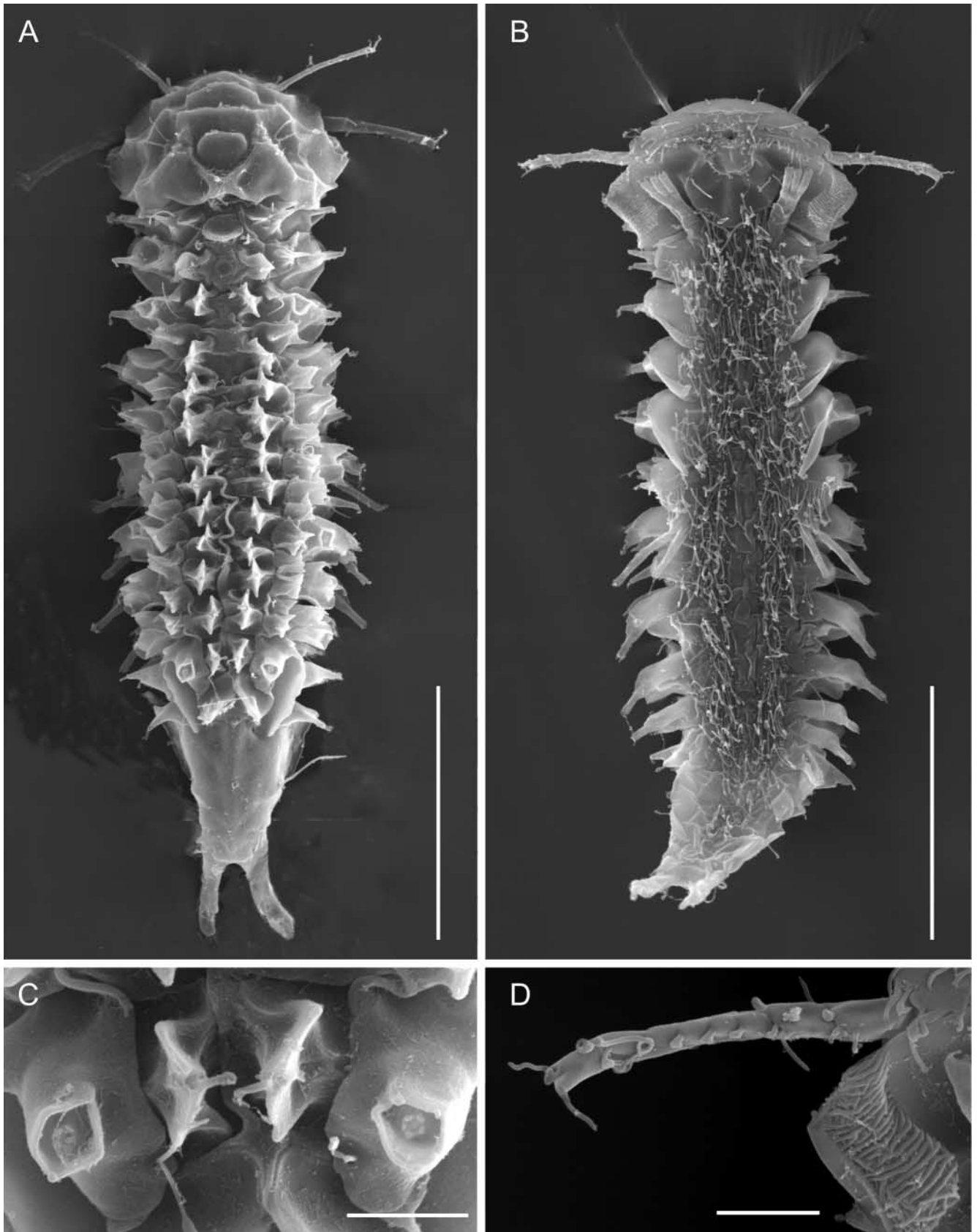
Genus *Xenodasys* Swedmark, 1967

*Xenodasys eknomios* sp. nov.

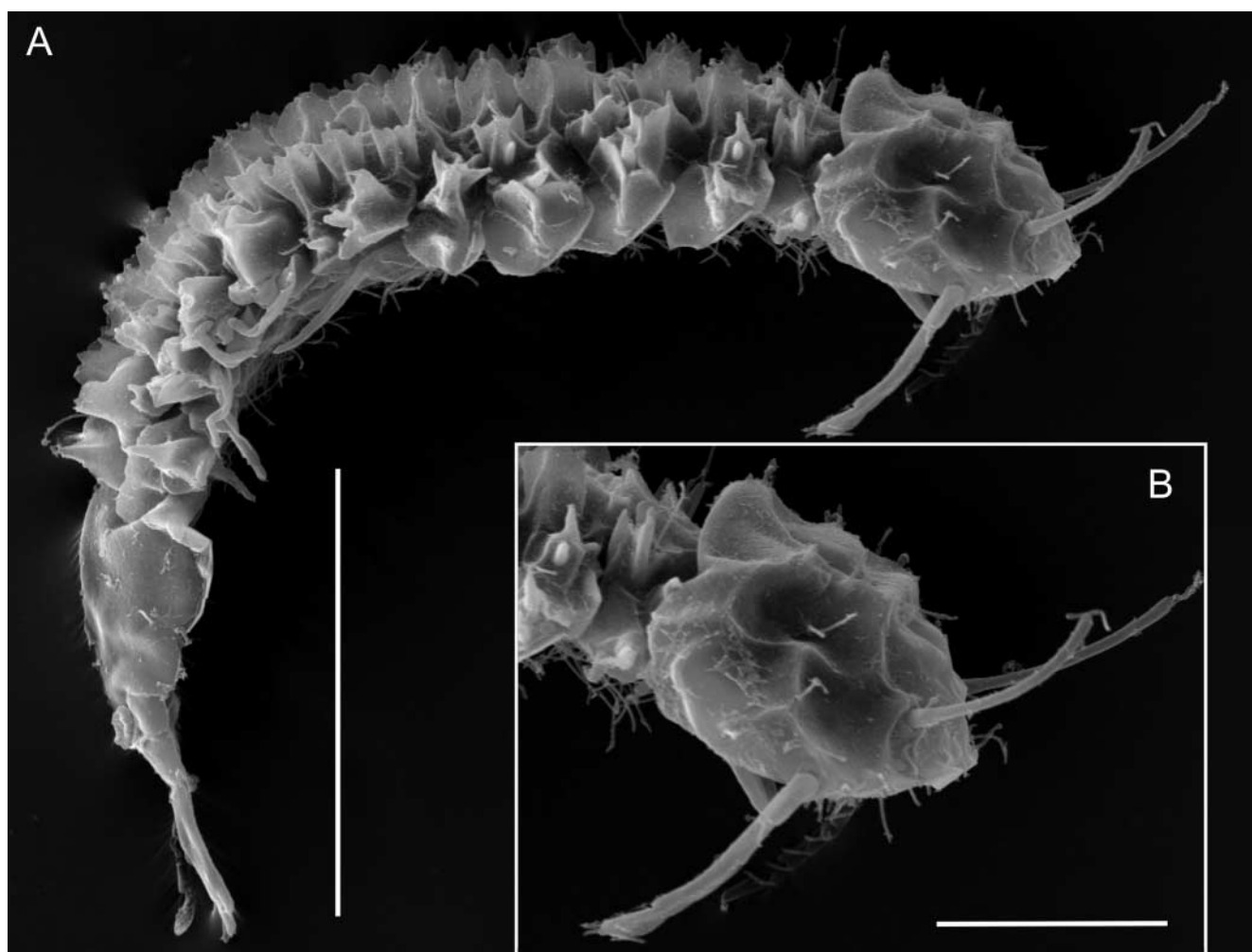
(Figures 2–5)

### Diagnosis

A small *Xenodasys* with body up to 260  $\mu m$  in length, with well-defined head, corrugated trunk and branched posterior end, indenting to U89; head roughly hexagonal in shape up to 40  $\mu m$  long and 62  $\mu m$  wide, with two pairs of dorsal and lateral tentacles; lateral tentacles showing external articulations; head ventrally bearing numerous



**Figure 2.** *Xenodasys eknomios* sp. nov., habitus, scanning electron microscopy micrographs. (A) Dorsal view; (B) ventral view; (C) close-up of the dorsal posterior trunk, showing the calyx-like formation bearing inside a possible sensorial organ; and (D) close-up of the segmented cephalic tentacle and detail of the head ventrolateral plate, covered by transverse cuticular ridges. Scale bars: A, B, 50  $\mu$ m; C, D, 5  $\mu$ m.



**Figure 3.** *Xenodasys eknomios* sp. nov., habitus, scanning electron microscopy micrographs. (A) Lateral view; and (B) close-up of the head. Scale bars: A, 50  $\mu\text{m}$ ; B, 20  $\mu\text{m}$ .

transverse ridges on sides. Trunk showing 11–12 conspicuous prominences (indentations) along the lateral margins but turning smooth caudally; prominences ending with conical structures or cylindrical adhesive tubes. Dorsal armature made up of distinct three-dimensional protrusions with strong axial thorns rising from a quadrangular base, directly from the body. Adhesive apparatus made up, on each side, of three anterior tubes, born from an extensible fleshy base, seven lateral tubes, two of which, at mid body, borne from a common base ('Seitenfüßchen'); three posterior tubes at the distal end of each caudal branch. Mouth, subterminal, 2–4  $\mu\text{m}$  in diameter, surrounded by cuticular teeth. Pharynx up to 62  $\mu\text{m}$  in length, slightly broader and ciliated at its posterior end; pharyngeal pores at the base, pharyngo-intestinal junction at U24.6; intestine straight, roughly the same width along its length; anus ventral at U76. Simultaneous hermaphrodite, with paired testes and ovaries lateral to the intestine. Testes begin wider at U40 and narrow posteriorly, male pores on the ventral side at U59; spermatozoa filiform, with coiled head and flagellate tail. Ovaries posterior to the testes, with oocytes maturing anteriorly, starting at U60; frontal and posterior organs unknown. Chordoid organ present.

#### *Type material*

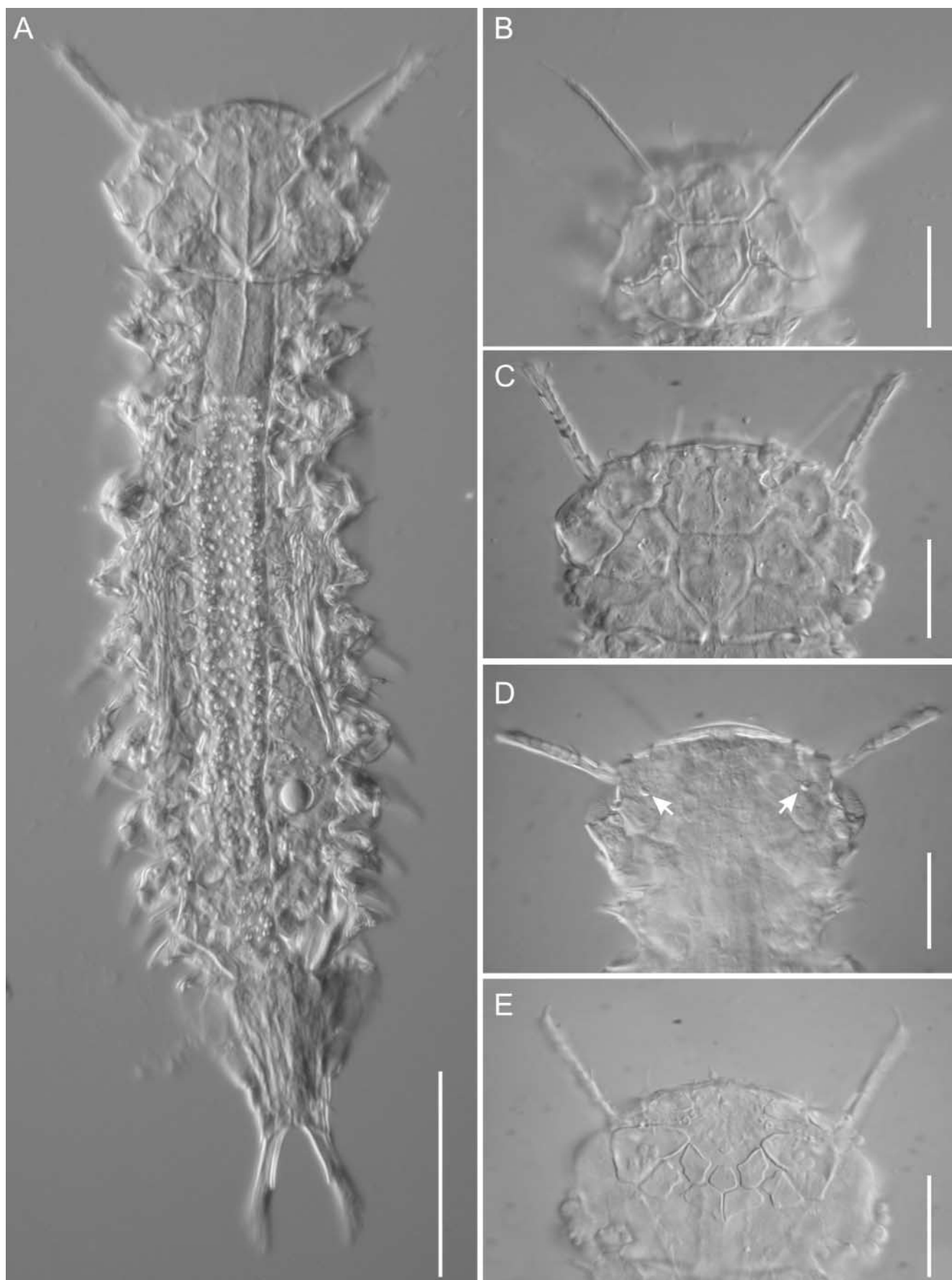
**Holotype:** an adult specimen on an SEM stub showing the ventral side (Figure 2B), deposited at the Natural History Museum, London, UK (NHM ref. no. 2006.1122). **Additional material:** two paratypic adult specimens on an SEM stub showing the dorsal and the lateral side respectively (Figures 2A & 3; NHM ref. no. 2006.1123–1124). Other specimens on SEM stubs are kept in the meiofauna collection of the senior author (ref. no. It-2001–12, 13). Five other specimens prepared on slides for DIC observation, but not extant. About 15 specimens used for DNA extraction and amplification of partial 18S rDNA gene (European Molecular Biology Laboratory accession number AY228133 *Xenodasys* sp.; Todaro et al., 2003a).

#### *Type locality*

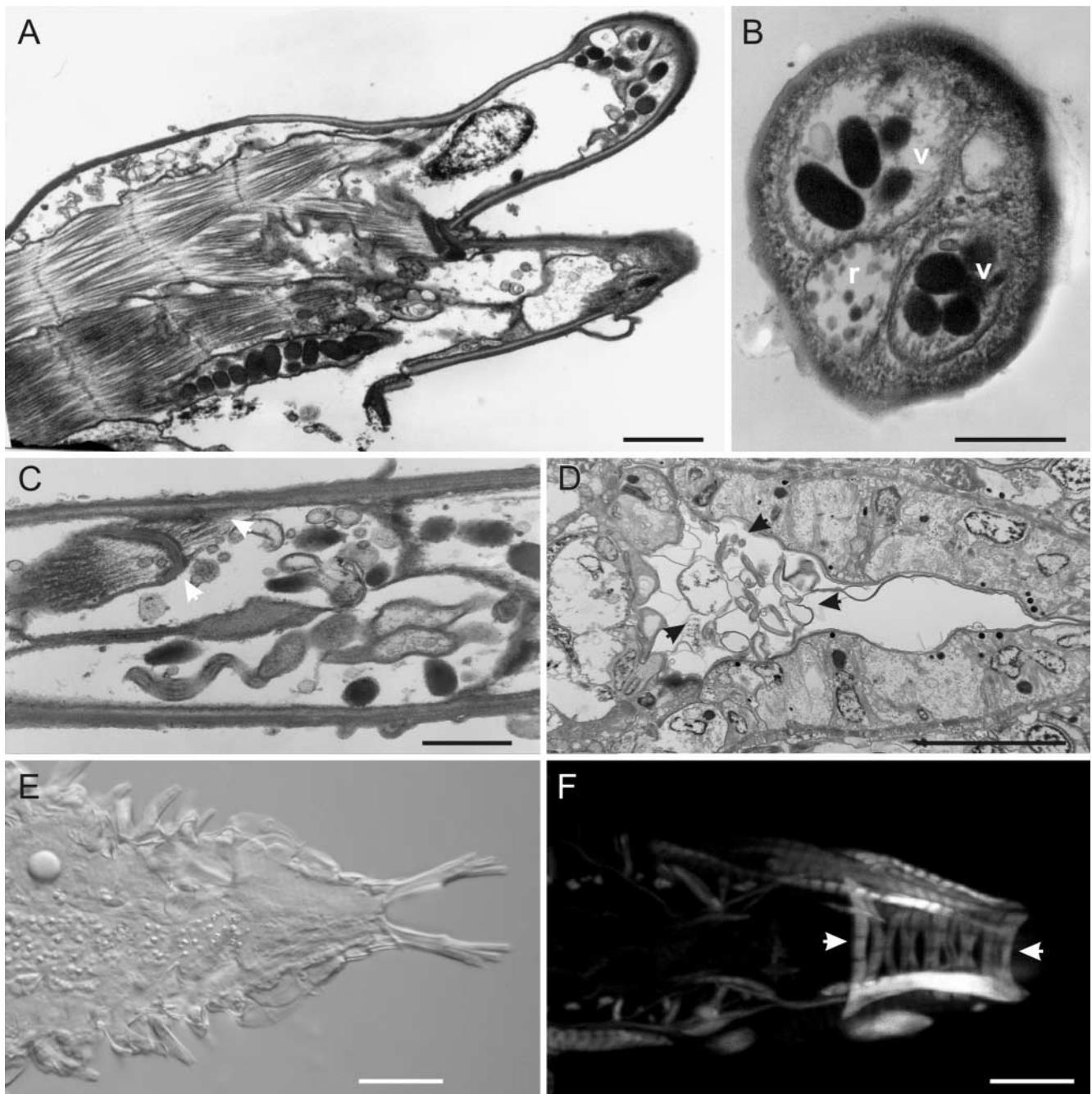
'Grotta della Principessa', Lecce, Italy (Lat. 39°48'30" N, Long. 18°22'71"E). At about 75 m from the entrance and at approximately 5 m water depth, in clean, medium, siliceous sand.

#### *Etymology*

The specific name alludes to the astonishing appearance of the new species (Gr. *Eknomos*, *Eknomios*: wonderful, marvellous or terrible).



**Figure 4.** *Xenodasys eknomios* sp. nov., differential interference contrast micrographs. (A) Habitus; (B) head, dorsal view showing false cuticular plates; (C) as above but with different focal plane (see text for details); (D) head, focal plane below the cuticle, showing two eye spots (arrows); and (E) head, ventral view showing false cuticular plates (see text for details). Scale bars: A, 50  $\mu$ m; B–E, 20  $\mu$ m.



**Figure 5.** *Xenodasys eknomios* sp. nov.—(A–C), transmission electron microscopy (TEM) micrographs of the ‘Seitenfüsschen’. (A) Longitudinal section; (B) transverse section (v, viscid gland; r, releasing gland); (C) longitudinal section (partial) showing junctions between two longitudinal myocytes and between myocyte and cuticle (arrows); (D) TEM longitudinal section through the pharynx, showing the lumen covered by cilia (arrows); (E) posterior end of the body seen under differential interference contrast optics, showing the peduncular region and the posterior adhesive tubes; and (F) peduncular region seen under confocal laser scanning microscope showing muscular fibres of the chordoid organ (arrows). Scale bars: A, 1  $\mu\text{m}$ ; B, C, 0.5  $\mu\text{m}$ ; D, 5  $\mu\text{m}$ ; E, F, 20  $\mu\text{m}$ .

#### Description

##### General appearance

The body is tenpin-shaped, up to 260  $\mu\text{m}$  in total length ( $254 \pm 6.1 \mu\text{m}$ ), with well-defined head, corrugated trunk and branched posterior end; head roughly hexagonal in shape,  $37.7 \pm 3.3 \mu\text{m}$  long and  $58.8 \pm 5.8 \mu\text{m}$  wide, with anterior margin rounded and two pairs of cephalic tentacles. One pair, anterodorsal in position, originating at U2.3, made up of thin,  $25.1 \pm 2.9 \mu\text{m}$  long rods devoid of articulations and lines of suture, showing short bristles only at the distal end. One pair, lateral in position, origi-

nating at U2.5, made up of thick  $26.5 \pm 1.9 \mu\text{m}$  long rods, with 6–7 external articulations and irregularly rugged terminal end, showing few, short, bristles along the shaft and tips. Trunk shows 11–12 conspicuous prominences (indentations) along the lateral margins from U14.5 to U75.5 but turning smooth further back, developing into a region often called the caudal peduncle. The first seven prominences, three of which are particularly wide, each end with one (2 to 5) or two (1, 6) conical structures (possible adhesive tubes), whereas the following four each end with a cylindrical adhesive tube. The last prominence

lacks any terminal structure. The posterior extremity of the body is furcated; indenting to U89 and each furcal branch carries three terminal adhesive tubes.

#### *Cuticular armature*

Head: the SEM analysis indicates the head as a sort of cephalic capsule made up of cuticular thickenings and crests, variously shaped and completely fused to each other. Dorsally, at the top of the capsule is a heart-shaped thickening, surrounded by strong ridges emerging from a deep, pentagonal hollow. Anterior to this plate is a rectangular one, which in turn is preceded anteriorly by two trapezoidal plates with convex anterior margins. Posterior to the central plate there are two areas, roughly triangular in shape, delimited by prominent margins. In lateral view, the cephalic capsule appears to be run across by a series of crests delimiting shallow hollows and small plates. There are five major hollows on each side, one is posterior, located next to the triangular areas, one is dorsolateral, two are lateral and one runs from the apex to the anterior margin of the head. It is from the lateral portion of this last area that, on each side, the 'smooth', anterodorsal tentacle emerges. The thicker lateral tentacle is implanted next to the anterior corner of the triangular plate.

The ventral side of the cephalic capsule is made up of a wide, trapezoidal plate, having convex anterior margins and a concave posterior margin. The surface of this plate, far from being flat, bears several ornamentations of different shape and size. Posterior to the mouth, which opens medially on the anterior margin of the plate, there is a semi-rounded cuticular thickening, which arises from another plate of larger size and of roughly similar form but with a posterior margin shaped like a curly brace bracket. The tipped margin of this plate is directed backward, beyond the head, and overlaps the anterior margin of the trunk. The entire surfaces of the lateral sides of the ventral plate show densely packed transverse ridges (corresponding with densely scratched ventrolateral plates of *X. sanctigoulveni* described by Kisielewski, 1987).

Trunk: the armature on the dorsal and lateral trunk region is made up of very distinct three-dimensional protrusions with strong axial thorns rising from a quadrangular base, directly from the body. From the top of most (all?) of the protrusions a tiny, conical structure emerges. Locally, protrusions can depart from this general shape showing three or only two axial thorns instead of the usual four. On each side, these cuticular elements are arranged in three columns, dorsal, dorsolateral and lateral, respectively.

The dorsal column includes 13 protrusions; the first one originating at U30, more posteriorly compared to the corresponding elements of the dorsolateral and lateral columns. That is, because at U18, between the first pair of protrusions lies a large oval-shaped plate. The posterior-most cuticular element (at U70) of the dorsal column appears as a strong keel, oriented from the median line diagonally to the rear.

The dorsolateral column is made up of 14 elements; the first one originates at U16.5 and is of the most common shape, whereas the posterior-most protrusion (at U73) is larger and peculiarly shaped, resembling a sort of three-

dimensional comma. It includes an anterior and more medial portion fashioned into a columnar calyx-like formation, with a round-to polygonal opening, connected to a lateral fin-like lamina that extends posteriorly. It is worth noting that inside the calyx-like formation there is a structure that resembles the flosculi, the sensorial organs described for loriciferans and priapulids (e.g. Todaro & Shirley, 2003).

The lateral column is made up of 12 elements, single or paired; the paired elements (i.e. 3, 7, 8, and 9) consist of a ventral four-thorn protrusion and a dorsal columnar calyx-like formation. The second to fifth lateral protrusions originate from large, three-side free, basal plates, which slightly overlap each other.

#### *Adhesive apparatus*

Anterior series, three stout tubes per side ( $5.4 \pm 1.4 \mu\text{m}$  long), originating in a hand-like fashion from a cylindrical, fleshy base,  $13.5 \pm 1.0 \mu\text{m}$  long. The base originates from the anterior trunk region at U15, and is directed anteriorly and obliquely toward the sides. Lateral series, seven tubes per side including one tube,  $5 \mu\text{m}$  long, in the anterior region at U14.5, originating ventrolaterally from the furrow separating the head from the trunk. Two evident ventrolateral tubes are located in the mid trunk region at U50, roughly at the level of the sixth lateral prominence; these tubes, of roughly the same length,  $14.2 \pm 0.7 \mu\text{m}$ , share a common base and are generally referred to as 'Seitenfüßchen' [=lateral pedicle] (see Remane, 1927; Reiger et al., 1974). Four additional tubes,  $5\text{--}8 \mu\text{m}$  in length, originating from the tips of elongate lateral prominences at U57.5, U66, U70.5 and U75 respectively. Posterior series, three stout tubes per side  $5\text{--}8 \mu\text{m}$  long, at the distal end of each caudal branch; longer tube toward the body-midline, shorter toward the sides.

#### *Ventral ciliation*

Non-locomotor cilia appear to be found on the ventral trapezoidal plate of the cephalic capsule. Locomotor ciliation is confined to the trunk. It appears as a single field from U14 to U30, which then splits into two bands throughout most of the intestinal region, joining again (U76.5) just past the anus, and continues as a single field under the anterior portion of the caudal peduncle. Ciliation extends over the ventral sides of the first five to six lateral protrusions.

#### *Digestive tract*

Small, subterminal mouth,  $2.4 \pm 1.4 \mu\text{m}$  in diameter, surrounded by 14–15 triangular teeth. Laterally to the mouth are two furrows partially occupied by two small and elongate cuticular structures. The pharynx is  $60 \pm 1.4 \mu\text{m}$  in length, slightly broader at its posterior end, and extends to U24.6, approximately at the level of the third lateral protrusion; pharyngeal pores, which are difficult to see, open near the base at U24. Transmission electron microscopy analysis shows that the terminal portion of the pharynx lumen is provided with sparse ciliation of relatively short cilia. The intestine is straight, of roughly the same width along its length; the anus opens ventrally at the level of the last lateral protrusion, at U76.

*Reproductive tract*

Simultaneous hermaphrodite, with paired testes and ovaries lateral to the intestine. Testes begin wider at the level of the fourth lateral protrusions (U40) and get narrower posteriorly. Each vas deferens ends at the level of the eighth lateral protrusion (U59) with a pore located on the ventral side; spermatozoa filiform, with coiled head and flagellate tail. Ovaries posterior to the testes, with several oocytes maturing anteriorly, starting at U60. In a single specimen a very large egg was observed, occupying the posterior 2/3 dorso-lateral side of the trunk. Accessory sexual structures i.e. frontal and caudal organs were not seen.

*Remarks*

It should be emphasized that the external morphological traits of these specimens appear different when animals are observed using SEM or DIC optics. For instance, the strongly cuticularized ridges of the cephalic capsule are resolved by DIC optics (light microscopy in general) as the outlines of polygonal scales and plates, and as such have been described and figured previously in other species of this genus (i.e. Swedmark, 1967; Kisielewski, 1987). The shape of these unreal features, as well as their peculiar arrangement on the dorsal and ventral sides of the head, in *X. eknomios* sp. nov. is shown in Figure 4B, C and E, respectively. However, while light microscopy might generate false images, causing misleading interpretation of the morphology of this species, it has some advantages over SEM as it allows the observation of internal organs (e.g. reproductive apparatus) and structures positioned below the cuticle, which cannot be documented using the SEM technique. In this regard, light microscopy showed the presence, on the anterolateral sides of the head of the new species, of a pair of rounded, brilliant elements (Figure 4D) not seen during SEM observation. Based on their position and by inference from other gastrotrich species, these elements could be sensorial organs involved in the recognition of light (i.e. ocelli). So it appears that the use of different microscopy techniques is advisable when dealing with specimens characterized by particularly complex morphology, such as the one belonging to the genus *Xenodasys*.

Transmission electron microscopy investigation has also proven to be extremely useful in the case of *X. eknomios* sp. nov., for example the TEM survey of its 'Seitenfüsschen', indicates these structures as genuine elements of the adhesive apparatus (see Figure 5A–C), in contrast with Tyler & Rieger (1980) who, investigating *X. antennatus*, indicated an exclusive sensorial function for these organs. Further, our TEM analysis resolved muscular fibres, myo-cuticular junctions and junction between myocytes within the tubes of the lateral pedicles 'Seitenfüsschen' (Figure 5C); similar elements were also recorded along the basal shaft of the anterior adhesive tubes, leading us to hypothesize the active movement of these structures. It should be emphasized that this is the first record ever of muscular elements within the adhesive tubes of any gastrotrich species (cf. Tyler & Rieger, 1980). Transmission electron microscopy survey showed also few scattered cilia in posterior luminal region of the pharynx (Figure 5D), not seen under Nomarski optics.

## DISCUSSION

The genus *Xenodasys* currently includes three species: *X. sanctigoulveni* Swedmark 1967, *X. riedli* (Schöpfer-Sterrer, 1969) and *X. antennatus* (Rieger, Ruppert, Rieger & Schoepfer-Sterrer, 1974). The type species was described from sandy material collected in the Roscoff area, France (Swedmark, 1967), a location where it was also found later by Kisielewski (1987); more recently a single specimen of this species has been reported from coarse shell gravel collected at 90 m water depth on the Faroe Bank, Denmark (Clausen, 2004). According to W.D. Hummon (Ohio University), another specimen has been recovered and photographed by D. Murison from material collected from the Faroe–Iceland sill (cf. Clausen, 2004; see also Hummon, 2001).

The second species was found at first in clean coarse sand with shell fragments, off Beaufort, NC, USA at 40–41 m water depth; subsequent records come from other North Carolina locations, Florida, Bermuda, the Virgin Islands, the Scottish Shetlands and possibly South Africa (Hummon, 2001 and personal communication).

In virtue of an axial notochord-like rod, the 'chordoid organ', located in the posterior region of the trunk, the presence of anterior and ventrolateral adhesive tubes, the existence of a single pair of cephalic tentacles and the presence of cilia in the pharynx and gut lumen, the first 14 specimens analysed were originally allocated to a new genus named *Chordodasys* (see Schoepfer-Sterrer, 1969). For the same reasons, also the two specimens of the third species, found respectively at 90 m water depth in sandy shell sediment off Beaufort, NC, USA and at 43 m water depth in heterogeneous shelf sediment off the southern coast of Georgia, USA (Rieger et al., 1974), were originally affiliated to *Chordodasys*.

The re-evaluations of the morphological traits of the French specimens led the name *Chordodasys* to be considered as a junior synonym of *Xenodasys*, consequently moving both the American species to the latter taxon (cf. Hummon, 1982; Kisielewski, 1987). We agree only partially with this view. In particular, we consider it appropriate to move only the first discovered American species (i.e. *C. riedli*) to *Xenodasys*. In this regard, it is worth mentioning that our survey under confocal microscopy, carried out on a single specimen of *X. eknomios* sp. nov. stained with actin-binding fluorochromes, revealed muscular elements in the peduncular region (Figure 5E,F) where the muscular chordoid organ was reported for *Chordodasys* species, thence eliminating the last crucial taxonomic difference between forms of the two genera. On the other hand, since the second American discovered species (i.e. *C. antennatus*) shows several taxonomically relevant differences from *X. sanctigoulveni*, *X. riedli* and *X. eknomios* sp. nov., its accommodation in the genus *Xenodasys* does not appear circumstantiated, and may be a source of taxonomic confusion as it grossly extends the generic boundary of the latter taxon.

Notable differences between *Chordodasys antennatus* and the other three species are the presence in *C. antennatus* of several long sensory structures along the body, adhesive pads in place of the caudal adhesive tubes, anterior adhesive tubes borne directly from the body and, most important, the complete absence of cuticular armature on



the dorsal side. Another difference may attain the head dorsal tentacles (=sensory processes), that appear to be composed of several articles in *Chordodasys* but unsegmented in *Xenodasys sanctigoulveni* and *X. eknomios* sp. nov. and absent in *X. riedli*. As the type species of the genus *Chordodasys* has been transferred to *Xenodasys*, in compliance with the International Code of Zoological Nomenclature, we propose the establishment of the genus *Chordodasiopsis* gen. nov. to incorporate *X. antennatus* (= *Chordodasys antennatus*), which becomes also the type species for the genus. Diagnoses of the new genus and an emended diagnosis for the genus *Xenodasys* are provided below.

The systematization above generic level of the above reported taxa has also been troubled. Swedmark (1967), provisionally classified *X. sanctigoulveni* to the order Macrodasysida in the family Dactylopodolidae, based on a supposed similarity between the adhesive apparatus of his species and that of other dactylopodolids. The same systematization was proposed for *X. riedli* (= *C. riedli*) by Schoepfer-Sterrer (1969), who highlighted two 'striking' similarities between her species and species of *Dendrodasys*, i.e. the presence of cilia in the gut lumen and deferens that end in two laterally situated male pores; the presence of cross-striated muscles was also considered as a possible homology in members of the two genera.

d'Hondt (1970) transferred *X. sanctigoulveni* to the family Neodasyidae (order Chaetonotida), due to the apparent absence of both the pharyngeal pores and anterior adhesive tubes; traits that are shared with *Neodasys*. This decision was supported by Hummon (1974); in the same paper the latter author proposed to transfer *Chordodasys riedli* from the family Dactylopodolidae to the family Turbanellidae. Rieger et al. (1974), in recognizing a close relationship between *Xenodasys sanctigoulveni* and *Chordodasys riedli* and *C. antennatus*, argued against the transfer of *Xenodasys* to the Chaetonotida, as the characteristics used for the transfer were presumptions on missing observations and because the only clear diagnostic characteristics that were useful in separating the Chaetonotida from the Macrodasysida (i.e. neither the histological structure of the pharynx nor the orientation of the pharynx lumen) were not analysed. However, Rieger et al. (1974) endorsed Hummon's second resolution regarding the transfer of *Chordodasys* (and by inference also of *Xenodasys*) to the family Turbanellidae; this was justified mainly on the basis of two supposed synapomorphies: the presence of lateral pedicles 'Seitenfüßchen' and anterior adhesive tubes arranged in a hand-like fashion.

In a later paper, Hummon (1982) considers *Chordodasys* as a junior synonym of *Xenodasys* and contra Hummon (1974) affiliated the latter genus to the family Dactylopodolidae. Kisielewski (1987) gave good arguments for Hummon's (1982) decision regarding the synonymy; however, in his opinion the affiliation of *Xenodasys* to the Turbanellidae, as suggested by Rieger et al. (1974), seemed more appropriate. Notwithstanding, Ruppert (1988) considered *Xenodasys* (= *Chordodasys*) among the Dactylopodolidae, stressing that the Dactylopodolidae are the only macrodasysidan gastrotrichs that have cross-striated muscles and cilia in the

pharyngeal and intestinal lumen. Clausen (2004) agreed, indicating further that the fact that in the Dactylopodolidae, as in *Xenodasys* (= *Chordodasys*), the vasa deferentia run straight back, each ending with a pore, favours this affiliation, in contrast with members of the Turbanellidae, where sperm ducts curve medioanteriorly, ending in a common pore (see also Balsamo et al., 2002).

Our observations on *X. eknomios* seem to confirm a relationship of *Xenodasys* (and by inference also *Chordodasys antennatus*) that is closer to the Dactylopodolidae than the Turbanellidae, as far as similarities in the vasa deferentia layout and the massive presence of cross-striated muscles are concerned. We would like to emphasize that the organization of the anterior adhesive muscles in *Xenodasys*, which are borne from a long extensible fleshy base, may be similarity with that of *Dendrodasys*, although in the latter species, the shaft ends with a single tube. On the other hand, the bilateral tufts of the anterior adhesive tubes described for *Chordodasys antennatus*, which are borne directly from the body, in many respects resemble the arrangement of the anterior tubes of *Dactylopodola*.

Notwithstanding, it cannot be overlooked that among Gastrotricha in general and Dactylopodolidae in particular only members of the genera *Xenodasys* and *Chordodasiopsis* bear the chordoid organ. The sharing of this striking, surely homologous feature, sets without doubt the two taxa on a clade distinct from all the other Gastrotricha. As a recent comprehensive cladistic analysis of Gastrotricha based on molecular traits (i.e. sequence of the 18S rRNA gene) found *Xenodasys* sp. (= *Xenodasys eknomios* sp. nov.) in a sister taxon relationship with the lepidodasyids *Dolichodasys* and *Cephalodasys* (Todaro et al., 2006b), we trust that in absence of other certain synapomorphic traits the morphological 'distance' between the *Xenodasys/Chordodasiopsis* assemblage and the remaining Gastrotricha is best recognized by formally establishing a new taxon i.e. Xenodasyidae fam. nov. to include both *Xenodasys* and *Chordodasiopsis*. A diagnosis of the new family and an emended diagnosis for the Dactylopodolidae is provided below.

As far as geographical distribution is concerned, *Xenodasys* has definitely been reported from both sides of the Atlantic Ocean. Specimens of *Xenodasys* were also reported among the fauna associated with a new marine Tardigrada from Australia (Boesgaart & Kristensen, 2001); after having seen pictures of these animals (M.A. Todaro, unpublished data), the Australian finding is confirmed here. Based on the very few records, *Xenodasys*, along with the recently described *Diuronotus* (Todaro et al., 2005), can be considered as one of the rarest gastrotrich genera. The Mediterranean finding, which is the first in one of the best studied basins in the world (cf. Hummon, 2001; Todaro et al., 2003b), supports this statement and reinforces the consideration of marine caves as habitats of high naturalistic value i.e. hot-spots for biodiversity and endemism (Todaro et al., 2006a).

#### *Taxonomic affinities*

Among the *Xenodasys* species described so far, *X. eknomios* most closely resembles *X. sanctigoulveni* in virtue of its general appearance, body size, presence of one pair of dorsal cephalic tentacles and presence of transverse ridges

on the lateral sides of the head ventrally. According to the detailed description of *X. sanctigoulveni* given by Kisielowski (1987), the similarity between the two species also regards number and arrangement of anterior and posterior adhesive tubes (three and three per side). Notwithstanding the similarities, in a personal correspondence, after having seen pictures of the Italian specimens J. Kisielowski stated: "...what is obvious and spectacular difference between your *Xenodasys* and mine it is dorsal trunk cuticular covering. In my specimen, the trunk dorsal surface was flat, without any distinct three-dimensional protrusions. I am completely sure—also on my photo IC there is no trace of any higher structure and every cuticular formations are visible on two-dimensional photograph...". Possible three-dimensional protrusions 'bosses' [=bumps] are reported in the original description of *X. sanctigoulveni* by Swedmark (1967), however, these are arranged in four columns and not in six columns as in *X. eknomios* sp. nov.

Other differences between the two species regard the presence in *X. eknomios* of ocelli and lateral protrusions terminating with two conical structures, whereas in *X. sanctigoulveni* the ocelli are missing and all lateral protrusions end with a single tubular element. Moreover, in *X. sanctigoulveni* a pair of long adhesive tubes in place of the 'Seitenfüsschen' found in *X. eknomios* sp. nov. is reported. However, as 'Seitenfüsschen' are also reported in *X. riedli* and in the species of the related genus *Chordodasiopsis* (= *Chordodasys*), it is possible that one of the two tubes that make up these structures has been overlooked in *X. sanctigoulveni*. The other *Xenodasys* species, *X. riedli*, is distinguishable from *X. eknomios* (and also from *X. sanctigoulveni*) by a series of characteristics, among which is the larger body size (490–580 vs 250–260  $\mu\text{m}$  in total length), the absence of the dorsal cephalic tentacles and the higher number of anterior and posterior adhesive tubes on each side, respectively 6 vs 3 and 7 vs 3.

#### Diagnoses

Dactylopodolidae Strand, 1929 (emended)

Macrodasysids with well-delimited head; lateral trunk margins and cuticular covering smooth; posterior end bilobed with short and broad peduncle or bifurcated, with elongate peduncle. Adhesive apparatus consisting of two groups of anterior tubes, borne directly from the body surface or two tubes originating from long movable fleshy bases; ventrolateral tubes absent or numbering five to several pairs along the sides, posterior tubes arising from the caudal lobes margins or at apices of the bifurcated distal end of the caudal peduncle. Pharynx and intestine occasionally at least partially ciliated (*Dendrodasys*). Muscles visibly cross-striated. Reproductive apparatus hermaphroditic, composed of anterior paired elongate testes (occasionally appearing as irregular paired clusters of grape-like spermatocytes) and posterior ovaries, lateral to the intestine. Spermatozoa, filiform with a spiralized head and flagellum (*Dendrodasys*). Frontal organ and caudal organ present. The readers are warned that published information regarding the organization of the hermaphroditic reproductive apparatus of this taxon are scanty and/or in need of re-evaluation. Type genus: *Dactylopodola* Strand, 1929; other genera: *Dendrodasys* Wilke, 1954; *Dendropodola* Hummon, Todaro & Tongiorgi, 1992.

*Xenodasyidae* fam. nov.

Macrodasysids with well-delimited head, bearing one pair of well-developed, segmented tentacles laterally and, if present, one or two pairs of elongate, unsegmented tentacles/sensory processes dorsally. Lateral trunk margins smooth or bearing several prominences and indentations. Dorsal body surface with the exception of the posterior region, covered with cuticularized plates, spines and scales or with three-dimensional protrusions or smooth but provided with several pairs of segmented sensory processes, arranged in lateral and dorsolateral columns. Posterior end furcated, with elongate, bare peduncles. Adhesive apparatus consisting of two groups of anterior tubes, originating from long movable fleshy bases or borne directly from the body surface; two to seven pairs of ventrolateral tubes along the sides, at least one pair made up of two tubes borne from a common base ('Seitenfüsschen'=lateral pedicles); digitate posterior tubes or adhesive pads at the distal end of the furcated caudum. Muscles visibly cross-striated. Presence of a chordoid organ, consisting of plate-like muscle cells located posterior to the intestine. Pharynx and intestine at least partially ciliated. Reproductive apparatus hermaphroditic, composed of anterior paired elongate testes and posterior ovaries, lateral to the intestine or elongate, mixed gonad, with spermatozoa maturing anteriorly and ova posteriorly. Spermatozoa, filiform with a spiralized head and flagellum. Frontal organ present, caudal organ unknown. The readers are warned that published information regarding the organization of the hermaphroditic reproductive apparatus of this taxon are scanty and/or in need of re-evaluation. Type genus: *Xenodasys* Swedmark, 1967; other genera: *Chordodasiopsis* gen. nov.

*Xenodasys* Swedmark, 1967 (emended)

Macrodasysid with well-delimited head, bearing one pair of well-developed, segmented tentacles laterally and if present one pair of elongate, unsegmented tentacles dorsally. Lateral trunk margins bearing several prominences and indentations. Entire dorsal body surface with the exception of the posterior region, covered with cuticularized plates, spines and scales or with three-dimensional protrusions. Posterior end furcated, with elongate, bare peduncles. Adhesive apparatus consisting of digitate anterior tubes, originating from two long movable fleshy bases, implanted ventrally just posterior to the head; two to seven pairs of ventrolateral tubes along the sides, one pair made up of two tubes borne from a common base ('Seitenfüsschen'=lateral pedicles); digitate posterior tubes at the distal end of the furcated caudum. Muscles visibly cross-striated. Chordoid organ present, consisting of plate-like muscle cells located posterior to the intestine. Pharynx and intestine at least partially ciliated. Reproductive apparatus hermaphroditic, composed of paired testes and ovaries, lateral to the intestine. Testes begin wider and get narrower posteriorly; deferens end at the level of the eighth lateral protrusions with pores located on the ventral side. Spermatozoa, filiform with a spiralized head and flagellum. Ovaries posterior to the testes. Frontal organ at the left side of the gut, behind the ovary, opens via a pore on the ventrolateral surface; posterior organ unknown. The readers are warned that published information regarding the organization of the hermaphroditic

reproductive apparatus of this taxon are scanty and/or in need of re-evaluation. Type species: *Xenodasys sanctigoulveni* Swedmark, 1967 (*sensu* Kisielewski, 1984); other species *X. riedli* (Schöpfer-Sterrer, 1969); *X. eknomios* sp. nov.; *Xenodasys* sp. [Boesgaard & Kristensen, 2001].

#### *Chordodasiopsis* gen. nov.

Macrodasysid with well-delimited head, bearing one pair of well-developed, segmented tentacles laterally and two pairs of segmented sensory processes dorsally. Trunk lacking cuticular armature but provided with several pairs of segmented sensory processes, arranged in lateral and dorsolateral columns. Posterior end furcated, with elongate peduncles. Adhesive apparatus consisting of anterior tubes borne directly from the body, arranged in two groups just posterior to the head; few pairs of ventrolateral tubes along the sides, one pair made up of two tubes borne from a common base ('Seitenfüßchen'=lateral pedicles); two adhesive pads located at the distal end of the furcated caudum. Muscles visibly cross-striated. Chordoid organ present, consisting of plate-like muscle cells located posterior to the intestine. Pharynx and intestine at least partially ciliated. Reproductive apparatus hermaphroditic, composed of paired, elongate, mixed gonad, with spermatozoa maturing anteriorly and ova posteriorly; spermatozoa, filiform with a spiralized head and flagellum. Frontal organ, opens via a pore onto one side of the ventral midline; caudal organ and male pores unknown. Type species: *Chordodasiopsis antennatus* (Rieger et al., 1974).

#### Etymology

The new genus name is a combination of the now defunct genus name *Chordodasys* and the Latin suffix *opsis* (=like), alluding to the similarity between the two.

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