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Abstract: Except for the noticeable investigations carried out in 1928 by Karl Zelinka in the Gulf of Naples and Gulf of Trieste, research on the Italian kinorhynch fauna has been rather erratic in space and time. According to the current checklist of the Italian marine biota, 48 species of Kinorhyncha were reported up to 2008 along the Italian coastlines. However, 31 of them are considered nomina dubia and hence of doubtful utility. Here we point out those taxa and provide new information based on recent publications and on novel investigations carried out in selected areas of the Adriatic Sea (3 localities), Ligurian Sea (4), Tyrrhenian Sea (8), and Ionian Sea (1). New data derives from qualitative as well as from quantitative samples. The analysis of the new samples yielded 6 families, 9 genera, and 29 species, of which only 14 were previously recorded from peninsular waters. In summary, we recorded one new genus and two new species for Italy, together with 13 additional species that appear new to science. Particularly interesting is the finding of two new species belonging to rare genus Condyloderes, as it represents the first records of this taxon in the Mediterranean Sea. The most speciose genus is Echinoderes, followed by Pycnophyes with 10 and 8 species, respectively. The former genus includes the taxon showing the highest abundance, E. capitatus, with recorded densities up to 184 ind./10 cm2, while the latter includes the most common species P. communis, found in 7 out of the 16 newinvestigated localities. New faunistic information prompted the revision of the checklist, which in the new version includes 36 species in 9 genera and 6 families. Old and new data were utilized for a preliminary discussion on the geographic distribution of the recorded fauna, from which it appeared that five species only can be considered ubiquitous in the four Italian sea basins, whereas the others taxa appear to be restricted to one or two seas. However, many sectors of the Italian coastline remain unexplored. Besides those areas (e.g., mid Tyrrhenian and Ionian coasts), future research should be focused on peculiar habitats, such as submarine caves, lagoons, and coarse biogenic sediments, as many species and species records come from these neglected biotopes, often representing biodiversity hotspots.

# Kinorhyncha from Italy, a revision of the current checklist and an account of the recent investigations

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#### **Abstract**

Except for the noticeable investigations carried out in 1928 by Karl Zelinka in the Gulf of Naples and Gulf of Trieste, research on the Italian kinorhynch fauna has been rather erratic in space and time. According to the current checklist of the Italian marine biota, 48 species of Kinorhyncha were reported up to 2008 along the Italian coastlines. However, 31 of them are considered nomina dubia and hence of doubtful utility. Here we point out those taxa and provide new information based on recent publications and on novel investigations carried out in selected areas of the Adriatic Sea (3 localities), Ligurian Sea (4), Tyrrhenian Sea (8), and Ionian Sea (1). New data derives from qualitative as well as from quantitative samples. The analysis of the new samples yielded 6 families, 9 genera, and 29 species, of which only 14 were previously recorded from peninsular waters. In summary, we recorded one new genus and two new species for Italy, together with 13 additional species that appear new to science. Particularly interesting is the finding of two new species belonging to rare genus *Condyloderes*, as it represents the first records of this taxon in the Mediterranean Sea. The most speciose genus is *Echinoderes*, followed by *Pycnophyes* with 10 and 8 species, respectively. The former genus includes the taxon showing the highest abundance, E. capitatus, with recorded densities up to 184 ind./10 cm<sup>2</sup>, while the latter includes the most common species P. communis, found in 7 out of the 16 new investigated localities. New faunistic information prompted the revision of the checklist, which in the new version includes 36 species in 9 genera and 6 families. Old and new data were utilized for a preliminary discussion on the geographic

distribution of the recorded fauna, from which it appeared that five species only can be considered ubiquitous in the four Italian sea basins, whereas the others taxa appear to be restricted to one or two seas. However, many sectors of the Italian coastline remain unexplored. Besides those areas (e.g., mid Tyrrhenian and Ionian coasts), future research should be focused on peculiar habitats, such as submarine caves, lagoons, and coarse biogenic sediments, as many species and species records come from these neglected biotopes, often representing biodiversity hotspots.

#### 1. Introduction

The number of new kinorhynch taxa is growing at a fast pace. Especially during the last two decades the effort by groups of international researchers, backed by new microscopical techniques, has led to the discovery and description of several new species and particular interesting new higher taxa. One hundred and seven species, almost half of those currently known, have been described in the timespan 1995-2015. New records come from different areas of the World, and often relevant discoveries are from previously neglected geographic regions (e.g., Dal Zotto et al., 2013; Sánchez et al., 2014; Sørensen and Thormar, 2010; Sørensen et al., 2000).

A modern approach to the taxonomy of the kinorhynchs is changing also the long-standing thoughts about the in-group relationships of the traditional higher taxa. This is best testified by the recent inclusive revision of the systematics of the Kinorhyncha based on a phylogenetic approach (Sørensen et al., 2015). As for September 2015, the phylum comprises more than 220 species and 23 genera, whose geographic distribution appears to be largely patchy. This fact likely reflects more the limited number of investigated areas and the relatively low number of taxonomists with expertise on Kinorhyncha rather a real biogeographical phenomenon. The most species rich genera (Echinoderes, Pycnophyes, and Kinorhynchus) exhibit a cosmopolitan distribution (sensu Sterrer, 1973), while the genera characterized by a low number of species have been reported solely from one or two ecoregions (see Neuhaus, 2013). Nevertheless, new studies point out that taxa apparently endemic to a given region often reveal a wider distribution. Paradigmatic in this regard is the recent discovery of Sphenoderes in Korean waters (Sørensen et al., 2010a) after its original report from India (Higgins, 1969). A further clue of the cosmopolitan nature of most genera derives from the genus Meristoderes recently described from the Mediterranean sea and the Salomon islands (Herranz et al., 2012) and subsequently found also in Florida (Herranz and Pardos, 2013) and Korea (Sørensen et al., 2013). Similar considerations may apply to Antygomonas, Campyloderes, Condyloderes, Zelinkaderes, and Dracoderes (see Neuhaus, 2013; Neuhaus and Sørensen, 2013, and references therein). Furthermore, a survey of poorly studied habitats in relatively well studied

areas may reveal the copious presence of taxa reported as rare in the original type localities. For instance, the genus *Antygomonas* originally described based on a single male specimen found in medium sand in the upper Adriatic Sea (Nebelsick, 1990), has been recently reported to be abundant in coralline sand in the Ionian sea (Sørensen et al., 2009) and in coarse sand in US waters (Herranz et al., 2014; Sørensen, 2007).

Based on the number of species, the Italian kinorhynch fauna appears as one of the best known in the World. However, investigations have been rather erratic in space and time. The 2008 checklist of the Italian marine species includes 48 species, in six genera and five families representative of both the traditional (see below) orders Cycloragida and Homaloragida (Sandulli and de Zio Grimaldi, 2008). As requested by Italian marine checklist project (see Relini and La Posta, 2005), the distribution of the species according to 9 marine biogeographic zones (BZ) (see Fig. 1) was provided. In the case of the Kinorhyncha, species presence and distribution records come almost entirely from the seminal works carried out in the Gulf of Naples (BZ 3) and Gulf of Trieste (BZ 9) by Zelinka (1928), comprehensive of few previous records, e.g., *Echinoderes dujardinii* found in the Gulf of Salerno (Metschnikoff, 1865), at Ischia Island (Panceri, 1878), and near Brindisi (Schepotieff, 1907), the latter one falling within the BZ 7.

While the effort of compiling such a checklist is acknowledged, it should be stressed out that several of the reported species are considered *nomina dubia* (i.e., a doubtful application). Consequently the checklist itself is misleading or of a little help in frameworks that see these kind of lists as a useful tool in, e.g., the process of species identification or metadata analyses focusing on global patterns. Incorrect checklists are especially misleading when these kind of analyses are carried out by personnel not specifically trained in the taxonomy of the involved groups. The aim of the present work is to revise the 2008 checklist, providing: i) indication of the problematic taxa, ii) update it with additional records from articles published meanwhile and iii) expand the taxonomic spectrum by adding of new taxa and/or enlarge the geographic range of previously reported species with unpublished data from our own research carried out mostly in previously unexplored areas. As for the latter, a full set of information circumstantiating the finds is provided. For the purposes of this work, among the new information is also to include yet unpublished recordings of the Italian species from which genetic sequences were used for the phylogenetic study of Dal Zotto et al. (2013, Table 1).

#### 2. Material and methods

Identification of the problematic taxa in the available checklist by Sandulli and de Zio Grimaldi (2008) has been performed by comparing the reported taxa with information present in comprehensive papers such as e.g., Adrianov and Malakhov (1999), Higgins (1983), Neuhaus (2013), and authoritative electronic repositories such as the World Register of Marine Species (<a href="http://www.marinespecies.org/aphia.php?p=taxdetails&id=101060">http://www.marinespecies.org/aphia.php?p=taxdetails&id=101060</a>, accessed on 25 July 2015). The high ranking systematization, phylum to families, used in the emended checklist follows that of Sørensen et al. (2015).

Published information on kinorhynch species found along the Italian coasts after the publication of the 2008 checklist derives from: Dal Zotto (2015), Dal Zotto and Todaro (2009), Herranz et al. (2012), Sánchez (2015), and Sørensen et al. (2009). While browsing the 'grey' literature on Italian marine fauna, we came across the neglected paper by Mari and Morselli (1987), who reported two species of Kinorhyncha from the Laguna Veneta, so we decided to include these records in the new version of the checklist.

New, unpublished information were obtained by us from qualitative as well as quantitative samples. Qualitative samples from eleven localities (loc. 1, 2, and 4: BZ 1; loc. 5, 6, 7, 8, 9, and 10 – BZ 2; loc. 13 – BZ 6; loc. 16 – BZ 9; Figure 1) were collected manually by skin or SCUBA diving using 500 ml plastic jars (see Todaro et al., 2015). Quantitative samples from five different areas (loc. 3 – BZ 1; loc. 11 and 12 – BZ 3; loc. 14, 15 – BZ 9; Figure 1) were collected as three-four replicate cores (3.4 cm in diameter x 5 cm in height) obtained directly by SCUBA diving or through subsampling the material collected by a Van Veen grab. A list with the names of the sampled localities, together with information on depth, sediment type (according to Giere et al., 1988) and sampling date is provided in Table 1.

Regarding the qualitative samples, specimens were extracted using the narcotization-decantation technique with 7% MgCl<sub>2</sub> solution (Pfannkuche and Thiel, 1988). Kinorhynchs were removed with a micropipette, mounted on slides and observed *in vivo* with Nomarski optics using a Nikon, Eclipse 90i microscope. During observation specimens were photographed using a DS-5M digital camera and measured using the ACT-2U v.4 Nikon software. After observation, most of the surveyed specimens were removed from the slide and stored in formalin for future check. Some specimens were transferred to vials containing 95% ethanol and kept at -20°C for forthcoming molecular analyses.

Quantitative samples were treated as follows. The fauna was narcotized using a 7% magnesium chloride solution, fixed on site with a 10% buffered formalin solution pre-stained with rose Bengal and stored for later checking (Todaro et al., 2006). In the laboratory, each sample was filtered using two sieves, 1 and 0.045 mm, respectively, laid one upon the other and fauna extracted

trice from the finer fraction using the silica gel gradient centrifugation technique (LUDOX AM, d = 1.210; Pfannkuche and Thiel 1988; Todaro et al., 2001).

Kinorhynchs were isolated and transferred to HS slides and identified like the specimens from qualitative samples. Additional animals from both qualitative and quantitative samples were prepared for SEM. Specimens for SEM were formalin-fixed, dehydrated through a graded ethanol series, critical point dried using carbon dioxide with a CPD 010-Balzer, mounted on aluminum stubs, sputter-coated with Au/Pd (Sputter Coater E24, Balzer) and observed with a Philips XL 40 scanning electron microscope. Measurements were obtained from both living and fixed animals using ACT-2U software.

#### 3. Results and discussion

#### 3.1. Revision of the current checklist

The available checklist of the Italian kinorhynchs includes 48 species, , allocated to six genera, and five families (Sandulli and de Zio Grimaldi, 2008). Most of the species reported in the list were described by Karl Zelinka based on fauna collected in the gulfs of Naples and Trieste (Zelinka, 1928). However, subsequent authors (notably, Adrianov and Malakhov, 1999 and Higgins, 1983), have pointed out that many species described by Zelinka (1928) should be considered as nomina dubia because their description was based on juvenile specimens. It should be stressed that the description of a new species of kinorhynchs is usually based on adult specimens and that the morphology of the juveniles stages of most species is unknown or undescribed. In this context, it is worth mentioning that kinorhynch juveniles exhibit morphological traits that may differ from those characterizing the adult stages (male or female; see e.g., Neuhaus, 1995; Sørensen et al., 2010b), and that juveniles of different species may appear morphologically very similar. Consequently, it is virtually impossible to say if the juveniles described by Zelinka (1928) belong to good species or juveniles of already known species (very likely). Hence, the indication of nomina dubia for those species appears appropriate. The doubtful application of these names is probably the reason why none of the species erected by Zelinka (1928) based on juvenile stages has been considered in recent taxonomic works.

With regard to the species listed in the current checklist, 29 taxa are considered *nomina dubia* by Higgins (1983) and/or, Adrianov and Malakhov (1999) and also by the Neuhaus (2013); these are: *Echinoderes erinaceus* (Zelinka, 1928) (original name: *Habroderes erinaceus*); *Echinoderes erucus* Panceri, 1878 (reported as *Echinoderes erucus* Zelinka, 1928; original name: *E.* 

eruca); Echinoderes ferox (Zelinka, 1928) (original name: Habroderella ferox); Echinoderes gracilis Zelinka, 1928 (original name: Hapaloderes gracilis); Echinoderes hyalinus (Zelinka, 1928) (original name: Habroderella hyalina); Echinoderes meridionalis Panceri, 1878; Echinoderes minax (Zelinka, 1928) (original name: Habroderes minax); Echinoderes minimus (Zelinka, 1928) (original name: Hapaloderes minimus); Echinoderes minutus Panceri, 1878; Echinoderes monocercus Claparède, 1863; Echinoderes pallidus (Zelinka, 1928) (original name: Centropsis pallida); Echinoderes parallelus (Zelinka, 1928) (original name: Centropsis parallela); Echinoderes pulchellus (Zelinka, 1928) (original name: Centropsis pulchella); Echinoderes pusillus (Zelinka, 1928) (original name: Centropsis pusilla); Echinoderes rosaceus (Zelinka, 1928) (reported as E. rosaceus Remane, 1936; original name: Centropsis rosacea); Echinoderes spinosus Panceri, 1878; Echinoderes splendidus (Zelinka, 1928) (original name: Habroderes splendidus); Echinoderes trispinosus (Zelinka, 1928) (original name: Habroderella trispinosa); Pycnophyes biserratus (Zelinka, 1928) (original name: Centrophyes biserratus); Pycnophyes conspicuus (Zelinka, 1928) (original name: *Hyalophyes conspicuus*); *Pycnophyes curvatus* (Zelinka, 1928) (original name: Centroderes curvatus); Pycnophyes denticulatus (Zelinka, 1928) (original name: Centrophyes denticulatus); Pycnophyes diffusus (Zelinka, 1928) (reported as P. diffusus; original name: Centrophyes diffusus); Pycnophyes longihastatus (Zelinka, 1928) (original name: Centrophyes longihastatus); Pycnophyes moderatus (Zelinka, 1928) (original name: Centrophyes moderatus); Pycnophyes rectilineatus (Zelinka, 1928) (original name: Centrophyes rectilineatus); Pycnophyes solidus (Zelinka, 1928) (original name: Hyalophyes solidus); Pycnophyes tenuis (Zelinka, 1928) (original name: Centrophyes tenuis); Pycnophyes validus (Zelinka, 1928) (original name: Centrophyes validus).

How to deal with the species considered *nomina dubia* in the framework of the present work? According to the International Code of Zoological Nomenclature (ICZN, 1999) the scientific names remain valid. However, in the Fauna Europaea project (de Jong et al., 2014), species considered *nomina dubia* have been ignored (i.e., omitted from the database), even though some authors do not agree with this general rule (see van Helsdingen, 2003). In our opinion the assembly, and the final goal of a checklist, approaches that of the fauna Europaea project; consequently, we decide not to include taxa considered *nomina dubia* in this updated version of the checklist (see below). Regardless, we hope that future investigation in the gulf of Naples and Trieste may help to better clarify the status of the juvenile specimens described by Zelinka (1928).

In addition to the *nomina dubia* listed above, two other species listed in the 2008 checklist appear problematic, namely *Centroderes eisigii* Zelinka, 1928 and *Pycnophyes flagellatus* Zelinka,

1928 as they have been considered as junior synonyms of *Centroderes spinosus* (Reinhard, 1881) and *Paracentrophyes quadridentatus* Zelinka, 1928 respectively (see Higgins, 1983; Neuhaus et al., 2013). We agree with the view of the latter authors and, consequently, do not include these species in the emended version of the checklist (see below). As far as the distribution of species is concerned, we could not find the original reference accounting for the presence of *Centroderes spinosus* in the Italian marine biogeographic zone n. 5 (southeast coast of Sicily), consequently we could not confirm the datum reported by Sandulli and de Zio Grimaldi (2008) regarding the presence of this taxon in that marine biogeographic zone. By contrast, two records not included in the 2008 checklist are acknowledged: *Echinoderes dujardinii* (Fam. Echinoderidae) and *Semnoderes armiger* (Fam. Semnoderidae), reported by Mari and Morselli (1987) to be associated with the sponge *Hymeniacidon sanguinea* (Grant, 1826) collected in the southern side of the Laguna Veneta, near Chioggia (BZ 9).

As for the high ranking systematization, Sørensen et al. (2015), based on a phylogenetic approach, proposed the phylum Kinorhyncha to be subdivided in two classes, Allomalorhagida and Cyclorhagida, the latter divided in three orders (Kentrorhagata, Echinorhagata, and Xenosomata). Allomalorhagida currently do not include any taxon of order rank, due the low statistical support at the nodes of interest shown on the phylogenetic trees derived from the analyses (see Sørensen et al. 2015). We accept this solution and consequently in the preparation of the new checklist we transfer: i) the families Antygomonidae, Centroderidae and Semnoderidae and related taxa, traditionally affiliated with the order Cyclorhagida, to the new established order Kentrorhagata (class Cyclorhagida), ii) the family Echinoderidae and related taxa, previously affiliated with the order Cyclorhagida, to order Echinorhagata (class Cyclorhagida), and iii) the families Neocentrophyidae and Pycnophyidae and related taxa, traditionally affiliated with the order Homalorhagida, to the class Allomalorhagida.

# 3.2 Kinorhynch taxa reported from Italy after 2008

Based on the examined literature the following taxa should be added to the list.

Class ALLOMALORHAGIDA Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Family Pycnophyidae Zelinka, 1896

Genus Pycnophyes Zelinka, 1907

Pycnophyes zelinkaei Southern, 1914

This species was recently found by Sánchez (2015) in muddy sand at 22-53 m depth from the Gulf of Naples. The species was originally described from Irish coastal waters (Southern, 1914), subsequently reported from other Atlantic localities (Bamber, 1989; McIntyre, 1962), and more recently from Gibraltar (Sánchez et al, 2012).

The presence of this species along the Tyrrhenian coast of Italy points out its broad distribution within the Mediterranean Sea and along the European coasts.

Class CYCLORHAGIDA Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Order Echinorhagata Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Family Echinoderidae Butschli, 1876

Genus Meristoderes Herranz, Thormar, Benito, Sánchez and Pardos, 2012

Meristoderes macracanthus Herranz, Thormar, Benito, Sánchez and Pardos, 2012

A written report of this species from Italian waters (Sardinia) can be found in Herranz et al. (2012); direct contact with M. Herranz and M. V. Sørensen allowed us to confirm that samples were collected in September 2010 from La Maddalena Archipelago during a workshop on meiofauna (see Curini-Galletti et al., 2012). By crossing works by Neuhaus (2013) and Curini-Galletti et al. (2012), the exact location appears to be Cala Ferrigno, where coarse sandy sediment was collected at 4 m depth. The species was originally described from the vicinity of Blanes (Gerona, NE Spain), hence it appears so far to be endemic to the Mediterranean basin.

Order Kentrorhagata Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Family Antygomonidae Adrianov and Malakhov, 1994

Genus Antygomonas Nebelsick, 1990

Antygomonas caeciliae Dal Zotto, 2015

A relatively high number of specimens (> 20) of this species was found in October 2008 in the medium-coarse, biogenic sand collected at about 1.5-3.5 m water depth in the Meloria shoals, off of Leghorn, Tuscany (Dal Zotto, 2015). Prior to its formal description, a short account on the specimens from the Meloria shoals was provided by Dal Zotto and Todaro (2009). Recently specimens apparently belonging to this species have been found by us near the type locality, in the island of Pianosa (BZ 2) in similar sediment and water depth (see below).

Antygomonas incomitata Nebelsick, 1990

Several individuals, juveniles and adults of both sex and of this species were found in coarse coralline debris mixed with mud, collected at 14 m water depth near Porto Cesareo, Taranto, South Italy (Sørensen et al., 2009, and personal communication). Strictly speaking, this is the first finding of the species in Italian waters (BZ 6, see below); however the species was originally described from the Bay of Vestar (Croatia), which falls within the boundaries of the BZ 9 of the biogeographic zones of the Italian seas. Consequently, we will include also the Croatian record in the updated version of the checklist (see below).

#### 3.3. New data

A total of 29 species in nine genera and six families of Kinorhyncha were recorded from 16 Italian localities mostly surveyed over the years 2004-2015 during research on meiofaunal organisms (see e.g., Dal Zotto et al., 2007, 2008; Guidi et al., 2011; Leasi and Todaro, 2010; Marotta et al., 2005; Todaro et al., 2004, 2006; see also Dal Zotto et al., 2016). Information about localities, environmental parameters and kinorhynch fauna are summarized in Tables 1-4. Information on each recorded species follows below.

Class ALLOMALORHAGIDA Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Family Neocentrophyidae Higgins, 1983

Genus Paracentrophyes Higgins, 1983

Paracentrophyes quadridentatus (Zelinka, 1928) (Figure 2)

We found specimens of this taxon in silty samples collected at 30-50 m depth from the Gulf of Castellammare (Sicily, loc.11 and 12.). The densities ranged from 1 to 4 ind./10 cm<sup>2</sup>. Previously, in Italy, the species was known for the Gulf of Naples (Zelinka, 1928). Outside Italy the species appears to be widespread along the Atlantic and North Sea coasts of Europe (Bamber 1989; McIntyre, 1962; Nyholm 1947; Sánchez et al., 2012). SEM survey of some of the Sicilian specimens revealed interesting details (e.g., number and position of sensory spots) useful for intraspecific comparisons and/or comparisons with the only two other species allocated in this genus.

During a recent survey, at about 100 m depth, in front of Leghorn (Ligurian Sea, loc. 3), we found a single female adult specimen of a *Paracentrophyes* whose morphological characters differ from the original description of *P. quadridentatus* (Zelinka, 1928) in showing, e.g., greater TL (500 vs 370  $\mu$ m), longer middorsal spine of segment 11 (75 vs 65  $\mu$ m), and longer lateral terminal spines (126 vs 96  $\mu$ m). These morphometric features appear similar to those of *P.* cf. *quadridentatus* reported from Spain and from the Faroe islands (see Sørensen et al., 2010c).

Family Pycnophyidae Zelinka, 1896

Genus Kinorhynchus Sheremetevskij, 1974

Kinorhynchus giganteus Zelinka, 1908

This species has been found in the Northern Adriatic (Chioggia, loc. 14) and Tyrrhenian Sea (Gulf of Castellammare, loc. 11 and 12) and also in the Ligurian Sea (Leghorn, loc. 3). *K. giganteus* population densities were of up to 2 ind./10 cm<sup>2</sup> at Chioggia and Leghorn and up to 7 ind./10 cm<sup>2</sup> in the Gulf of Castellammare.

## Kinorhynchus sp. 1

Three specimens of this taxon were found in the Upper Adriatic Sea, at Chioggia (loc. 14), in sediment made of coarse silt collected at 20-29 m water depth were salinity is about 30-32 psu. The presence of pointed middorsal processes, together with much shorter trunk length, clearly differentiate this species from *K. giganteus* and from *Kinorhynchus* sp. 2 (see below), the only two other species belonging to this genus known so far the Mediterranean Sea.

# Kinorhynchus sp. 2

Two specimens of this taxon were found in silty sediment of the Northern Adriatic region, however, they were collected from the Vallona Lagoon (loc. 15), at 1-3 m depth, where salinity ranges from 18 to 31 psu (ARPAV, 2011-2015). A smaller size, blunt middorsal processes, and presence of posterolateral indented margins of segment 10 are the main traits that distinguish this species from the other two congeners known from the Mediterranean Sea.

Genus Pycnophyes Zelinka, 1907

Pycnophyes carinatus Zelinka, 1928

This species has been found by us in the Gulf of Castellammare (Sicily, loc. 11 and 12), and previously in the Gulf of Naples by Zelinka (1928). Population densities were of up to 17 ind./10 cm<sup>2</sup>. Recently the species has been reported from the Mediterranean coast of Spain (Sánchez *et al.*, 2012). Some morphological details of our specimens, like the shape of tergal margin and lateral projections of the segment 1, appear somewhat different from those reported in the original description by Zelinka (1928) but similar to those reported in the Spanish specimens.

# Pycnophyes communis Zelinka, 1908

We found this species in the Ligurian (loc. 3), Tyrrhenian (loc. 7, 11 and 12), and Northern Adriatic seas (loc. 14, 15 and 16). Previously it was known from the Gulf of Naples and Trieste (Zelinka, 1928). Recently, this species has been found also in muddy sediment collected at 16 m depth near Ancona (Adriatic Sea, Southern boundary of the BZ 9; F. Semprucci and M.V. Sørensen, pers. comm.). *P. communis* appears as one of the most common Italian kinorhynch species, it inhabits subtidal muddy sediments down to 100 m. In contrast with its ample distribution, populations density of this taxon appears, at the investigated locations, relatively low, ranging from 1 to 2 ind./10 cm<sup>2</sup>. A bit surprising was the discovery of this species form the Vallona Lagoon where salinity may be relatively low; however, the reported presence in the Baltic Sea (see Neuhaus 2013) should testify that *P. communis* is an eurhyaline species. Future study based on molecular markers could clear out doubts about the present of cryptic species in the currently recognized taxon, as some details of the specimens from the Vallona Lagoon seem to suggest.

#### Pycnophyes flaveolatus Zelinka, 1928

Four specimens resembling *P. flaveolatus* closely were found in the Vallona Lagoon (Northern Adriatic Sea, loc. 15). Differences with the specimens reported from the Gulfs of Trieste and Naples by Zelinka (1928) regard, for instance, the lateral terminal spines, which in our specimens are longer (130 µm vs 80-120 µm). Outside Italy, the species has been found in Germany (Kiel) and Sweden (west and East coast).

#### Pycnophyes robustus Zelinka, 1928 (Figure 3)

The species has been found in the Gulf of Castellammare (Sicily, loc. 11 and 12), showing population densities ranging from 1 to 3 ind./10 cm<sup>2</sup>. Previously it was known from the Gulfs of Naples and Trieste (Zelinka, 1928). Our SEM observations show for the first time the presence of spermatophore on a male specimen (Fig. 3C). Previously, spermatophores in Pycnophyidae were recorded only on female specimens (see Brown, 1983; Neuhaus and Higgins, 2002; Neuhaus, 2013).

Pycnophyes rugosus Zelinka, 1928

Individuals ascribed to *P. rugosus* were found by us in the Ligurian Sea (Leghorn, loc. 3), even though some morphological details differ from the original description. For example, the total body length in our specimens appears longer (680 µm vs 620 µm) compared to the specimens described from the Gulf of Naples by Zelinka (1928). *P. rugosus* has so far been collected in Italian waters only.

# Pycnophyes sp. 1

This species was found at Chioggia (Upper Adriatic sea, loc. 14); the general appearance approaches that of *P. communis*, but in contrast with the latter species our specimens show e.g., longer lateral terminal spines (ca. 130  $\mu$ m vs 70-100  $\mu$ m).

# Pycnophyes sp. 2

Two specimens were collected from the Vallona Lagoon (Upper Adriatic Sea, loc. 15); the general appearance is clearly different from that of *Pycnophyes* sp. 1 and approach more *P. robustus*; the new species appears to be of a smaller size (trunk length ca. 530 μm vs 570-640 μm) and to possess shorter and thinner lateral terminal spines (180 μm vs 210 μm).

# Pycnophyes sp. 3

Specimens were found in silty sediment collected at about 100 m depth off Leghorn (Ligurian Sea, loc. 3). The general appearance approaches that of *P. flaveolatus* but in contrast with the latter species our specimens show e.g., much longer and stouter lateral terminal spines (ca. 170 μm vs 80-120 μm).

Class CYCLORHAGIDA Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Order Echinorhagata Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Family Echinoderidae Bütschli, 1876

Genus Echinoderes Clarapéde, 1863

#### Echinoderes agigens Bâcescu, 1968

We found several adults of *E. agigens* in silty sediment from the Northern Adriatic (Chioggia and Cesenatico, loc. 14 and 16). The taxonomic status of this taxon is somewhat controversial. Huys and

Coomans (1989) consider it as *species inquirenda* but Neuhaus (2013) listed it among the currently recognized species, and as an accepted species it is reported in the WoRMS database <a href="http://www.marinespecies.org/aphia.php?p=taxdetails&id=101103">http://www.marinespecies.org/aphia.php?p=taxdetails&id=101103</a> accessed on 24 July 2015). We follow the latter author. Hopefully, our specimens will allow a more precise description this taxon including a the report of a certain morphological variability shown by Adriatic animals (e.g., a few recorded specimens lacked some of the lateral spines reported in the original description). This is the first record for the species in the Mediterranean Sea and outside its type locality, the Black Sea (Bâcescu, 1968). The finding in the Northern Adriatic Sea can be related to the habitat characteristics, such as the relatively low salinity and other environmental features (sediment, etc.), resulting similar to those reported in the original description. A faunistic similarity between the Northern Adriatic and the Black Seas was observed also for other meiofaunal taxa, such as gastrotrichs (Todaro et al., 2003).

# Echinoderes capitatus (Zelinka, 1928)

We found the species in the Northern Adriatic Sea (14. Chioggia), the Tyrrhenian Sea (Sicily: 11. Castellammare and 12. Trappeto), and the Ligurian Sea (3. Leghorn). The species was previously reported from the Gulfs of Naples and Trieste (Zelinka, 1928), hence our findings widen its geographic range and make the species one of the most common cyclorhagids along the Italian coastlines. In our samples population densities reached up to 184 ind./10 cm<sup>2</sup> at Castellammare (see Dal Zotto et al., 2016), 153 ind./10 cm<sup>2</sup> at Trappeto, and up to 5 ind./10 cm<sup>2</sup> at Chioggia and Leghorn. We noticed that some specimens from Sicily showed a number of characters in agreement with the original description by Zelinka (1928), and not with the details provided more recently by Nebelsick (1992). For instance, we observed 4 spines on segment 2, lateroventral spines on segments 5 and 8, paradorsal spines on segment 8, two small paradorsal spines on segment 10. In general the number of spines, especially on segment 8, appears to be lower than the one reported by Nebelsick (1992). The discrepancies between the original description by Zelinka (1928) and the redescription by Nebelsick (1992), together with the contrasting information reported by Adrianov and Malakhov (1999), who, for instance, refer to a dorsal spine on segment 4 only in an identification key, but not in the species description, stimulate some hypotheses. Probably the absence of dorsal spines derives from the accidental damaging of specimens (see Pardos et al., 1998). Alternatively, this taxon could either show a very high intraspecific variability, or represent a cluster of cryptic species. Further analyses are needed to resolve the doubts related to the morphology of this species. Molecular data from specimens of Adriatic and Tyrrhenian populations have been collected (see Dal Zotto, 2015)

and along with other kinorhynchs that are being gathered, they will be analyzed to evaluate intraand interpopulation divergences.

# Echinoderes ferrugineus Zelinka, 1928

This species, formerly known for the Adriatic Sea (Slovenia) and the Tyrrhenian Sea (Gulf of Naples, Zelinka, 1928), was found by us in Northern Adriatic Sea (Chioggia, loc. 14) and in Southern Tyrrhenian Sea (Sicily: Castellammare, loc. 11, and Trappeto, loc. 12). The densities of *E. ferrugineus* were of 1-3 ind./10 cm² at Chioggia, and of up to 81 ind./10 cm² in the Sicilian locations. Some of our specimens differ from the original description by Zelinka (1928), as the lateral spines on segment 9 are lacking, and the lateral terminal spines appear shorter. It should be stressed that the original (old) descriptions of some echinoderids can be rather approximate and confusing, as reported also by Higgins (1982), and this appears to be also the case for *E. ferrugineus* also.

# Echinoderes gerardi Higgins, 1978

Our findings of *E. gerardi* in the southern Tyrrhenian Sea (Sicily: Castellammare, loc. 11, and Trappeto, loc. 12) represent the first records of the species in Italian waters (see also Dal Zotto et al., 2016). and the third ever for the taxon as specimens resembling *E. gerardi* have been recently reported from the Turkish coast (Sönmez et al., 2016). *E. gerardi* reached a maximum population density of 22 ind./10 cm<sup>2</sup> at Trappeto, and of 4 ind./10 cm<sup>2</sup> at Castellammare. Originally *E. gerardi* was discovered in the Gulf of Tunis (Tunisia) inside a poriferan (Higgins, 1978), while in our case it was found in silty sediment. Higgins (1985) suggested that most of the reports of *E. dujardinii* in the Mediterranean could actually represent misidentifications of *E. gerardi*; if so, our data indicate that misidentification may plague not only specimens associated with sponges (e.g., Mari and Morselli, 1987) but also sediment dwellers.

# Echinoderes sp. 1

This species has been found at Chioggia (loc. 14) in coarse silt at depths of 20-29 m, together with *E. agigens*, *E. capitatus*, and other species reported above. Size and general morphology resemble that of *E. setiger*, but the pattern of the lateroventral spines is quite different (e.g., spines on segments 8 and 9 are lacking), and the length of lateral terminal accessory spines is much shorter.

# Echinoderes sp. 2

This echinoderid has been found twice in the Ligurian Sea (Leghorn, loc. 2 and 3). Specimens are characterized by a combination of dorsal and lateroventral spines that resemble that of *E. dujardinii*,

but in contrast to the latter species, they exhibit the lateral accessory spines on segment 7 (not 8), and have much longer middorsal and lateral terminal spines.

# Echinoderes sp. 3

Specimens of this taxon have been found in the Ligurian Sea (Leghorn, loc. 3) in sympatry with the congeneric *E. capitatus* and *Echinoderes* sp. 2, and with several other species (see Table reported above). Among other morphological traits, *Echinoderes* sp. 3 bears dorsal spines on segments 5 to 8 and has rather long lateral terminal spines. The sediment in which the species has been found is made up by fine sand-coarse silt and was collected at 100 m water depth.

# Echinoderes sp. 4 (Figure 4)

Twenty-eight specimens of this echinoderid have been found off Grosseto (Tyrrhenian Sea, geographic area n. 2). This species, corresponding to *Echinoderes* sp. 5 in Dal Zotto et al. (2013) and Dal Zotto (2015), shows many characters in common with *E. peterseni*, reported from Disko Island, Greenland (Higgins and Kristensen, 1988). The Italian specimens differ from the polar species as they show a small middorsal spine and latero-subdorsal tubes on segment 2, and lack the ventral spines on the same segment. Furthermore, the lateral terminal spines and the middorsal spines appear longer in this taxon. Differences between the two species exist also concerning the microhabitats that hosts them. For instance, the Italian species inhabits coarser and deeper environment than the one characterizing the type locality of *E. peterseni* (medium sand at 37 m depth vs mud with rocks and pebbles covered with encrusting algae *Lithothamnium corallina* at 9 m depth). COI data from this taxon have been gathered (see Dal Zotto 2015) for possible future comparisons at molecular level. Published phylogenies based on molecular markers, confirm the distinctness of this taxon and of the two that follow below (Dal Zotto et al. 2013).

# Echinoderes sp. 5

Two specimens of this species, were found in a medium-fine sandy sediment at a 7 m depth from Budelli Island (Sardinia, loc. 8). It shows some characters resembling *Echinoderes* sp. 4, but differs in having long middorsal spines on segments 4, 6, and 8, ventral tubes on segment 2, very long lateral terminal spines (ca. 150  $\mu$ m), and different shape of tergal extensions but, apparently, lacks the lateral accessory tubes on segment 8. This species is also reported as *E.* sp. 6 in Dal Zotto et al. (2013). In the same sample was found also *Meristoderes macrachantus* (see below).

# Echinoderes sp. 6

This species has been reported from two locations, namely Lo Scollione (Capraia Island, loc. 4) Le Bombarde (Sardinia, loc 10). In both case the specimens were found in medium sand, but collected at two different depths, 30 m and 1.5-3 m respectively. In the first case were found 2 specimens, while in the second 5 specimens were recorded, showing a possible preference for sandy sediments. One of the traits that characterizes this taxon is the presence of rather long middorsal spines on segments 5, 6 and 7. This taxon corresponds to *E.* sp. 7 in Dal Zotto et al. (2013).

Genus Meristoderes Herranz, Thormar, Benito, Sánchez and Pardos, 2012

*Meristoderes macracanthus* Herranz, Thormar, Benito, Sánchez and Pardos, 2012 (Figure 5) Three specimens of *M. macracanthus* were found in a 500 ml sandy sample collected in 2009 from the sublittoral (-7 m) of the famous pink beach of Budelli Island (Sardinia, loc. 8). In the same sample there was also *Echinoderes* sp. 6. As reported above, additional specimens of this taxon have been collected in 2010 from the same island, as reported by Herranz et al. (2012).

Three more specimens of *M. macracanthus* have been found in a peculiar habitat: the Nereo Cave located at about 20 m depth, near Capo Caccia (Sardinia, loc. 9). The reports of kinorhynchs from troglobial habitats are very few (e.g., Sørensen et al., 2000). Cave sediments have been considered biodiversity hotspots for other meiofaunal taxa (e.g., priapulids: Todaro and Shirley, 2003; gastrotrichs: Todaro et al., 2006; tardigrades: Villora-Moreno, 1996).

Order Kentrorhagata Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos and Yamasaki, 2015

Family Antygomonidae Adrianov and Malakhov, 1994

Genus Antygomonas Nebelsick, 1990

Antygomonas caeciliae Dal Zotto, 2015

Beside the original record (2008, see above), 12 specimens of this species have been found again at the Meloria Shoals (loc. 1), in 2009 in medium to coarse biogenic sediment, collected at 5-7 m water depth. Furthermore, some specimens resembling *A. caeciliae* have been collected from the nearby island of Pianosa (loc. 5), in medium to fine sand at 6-7 m.

Antygomonas incomitata Nebelsick, 1990

Specimens of this species have been found by us in the Ligurian, Tyrrhenian and Ionian Sea (loc. 1, 5 and 13 respectively), in sediment types ranging from fine to coarse sand, collected at different water depths (2-7 m). *A. incomitata* has been found in sympatry with *A. caeciliae* at the Meloria

Shoals and at the near Pianosa Island. Considering the information from Nebelsick (1990) and Sørensen et al. (2009), the species appears to be widely distributed along the Italian coasts, and apparently not restricted to any specific type of sediment, as long as it is made of clean sand (i.e., without mud).

Family Centroderidae Zelinka, 1896

Genus Centroderes Zelinka, 1907

Centroderes spinosus (Reinhardt, 1881)

We found this species at Chioggia (loc. 14), in sediment made of coarse silt, collected at 20-30 meters depth, where it reached densities up to 2 ind./10 cm<sup>2</sup>. In Italy the species was previously reported for the Gulf of Trieste and, as *C. eisigii*, in the Gulf of Naples (Zelinka, 1928).

Genus Condyloderes Higgins, 1969

Condyloderes sp. 1 (Figure 6)

This is the first time that species of the genus *Condyloderes* Higgins, 1969 are reported from the Mediterranean Sea, and the third time from European waters (McIntyre, 1962; Sánchez et al., 2012). We found several specimens (densities ranging from 1 to 6 ind./10 cm²) of this taxon in Sicily (Lower Tyrrhenian sea, loc. 11 and 12) while engaged in studies monitoring the effects of some fish farms on meiofauna (see e.g., Dal Zotto et al., 2016). *Condyloderes* sp. 1 shows several morphological traits (e.g., number and position of cuspidate spines: paradorsal ones on segments 3 and 7, subdorsal on segment 7, couples of lateroventral ones on segments 2, 8, and 9, and ventral cuspidate spines on segment 5), which clearly differentiate it from the other congeneric species.

# Condyloderes sp. 2

We found a second apparently new species of *Condyloderes* at Leghorn (Ligurian Sea, loc. 3) and in sympatry with *Condyloderes* sp. 1 in Sicily (loc. 12). Specimens of this taxon resemble *C. multispinosus* (McIntyre, 1962) in the general morphology but seem to differ from it in some details (e.g., length of midterminal spine and lateral terminal spines, etc.). Additional specimens and further analyses will better clarify the taxonomic status of these kinorhynchs. In contrast with the relative high abundance showed by *Condyloderes* sp. 1 in the Gulf of Castellammare, *Condyloderes* sp. 2 was quite rare, both in this area (only two specimens found) as well as at Leghorn (four specimens).

The wider distribution range of this taxon registered during our studies indicated that distribution sometimes is decupled from abundance.

Family Semnoderidae Remane, 1929

Genus Semnoderes Zelinka, 1907

Semnoderes armiger Zelinka, 1928

This is the only species reported for the family. *S. armiger* has been found from Leghorn, Castellammare, Trappeto, and Chioggia (loc. 3, 11, 12, 14), where it has reached densities of 1-8 ind./10 cm<sup>2</sup>. Originally reported by Zelinka (1928) from the Gulf of Trieste (Upper Adriatic Sea) our findings in locations 3, 11 and 12, greatly widens the Italian distribution to include now also the Ligurian Sea and the Tyrrhenian Sea. Outside Italy the species has been reported from the Mediterranean Spain, the Black Sea, and several localities in the North-eastern Atlantic Ocean, making it one of the most widely distributed kinorhynch species in Europe (Bâcescu, 1968; Neuhaus, 2013; Sánchez et al., 2012). As far as the microhabitat concerns, *S. armiger* inhabits several kinds of sediments, from coarse sand to mud, and it has been collected at depths ranging from 15 to more than 300 m (Sánchez et al., 2012).

#### 3.4. Remarks on the new data

#### 3.4.1. Fauna

The analyses of samples from 16 new or newly investigated locations yielded 6 families, 9 genera, and 29 species, of which only 14 were previously recorded from peninsular waters (Figure 7, Table 2). Overall, we registered one new genus (*Condyloderes*) and two new species records for Italy, together with 13 additional species that appear new to science. Particularly interesting is the finding of two new species of the rare genus *Condyloderes*, as it represents the first records of this taxon in the Mediterranean Sea.

Most of the species found in this study belong to the genera *Echinoderes* and *Pycnophyes*; this datum is not surprising since these taxa are the most species-rich in the phylum. The most common species is the allomaloragidan *Pycnophyes communis* reported from seven localities out of 16: almost half of the surveyed locations. The most common cyclorhagidan was *Echinoderes capitatus* (four localities), which is also the species reaching the highest abundances (184 ind./10 cm² at Castellammare, location 11). Details on abundance of the latter species in the Sicilian locations are reported in a dedicated paper (Dal Zotto et al., this volume).

Considering the relatively low number of surveyed localities and the low species richness reported (ca. 3.6 species/site), it is noticeable that almost half of the recorded species appear to be new to science. The only previous extensive studies carried out in the Italian waters focused on the Gulfs of Trieste and Naples (Zelinka, 1928). A survey of the Gulf of Naples was recently repeated, even though only partial results have been published so far (Sánchez, 2015). Two of our sampling locations in the Gulf of Castellammare (Sicily, loc. 11 and 12) show characteristics (sediment type and water depth) similar to the Tyrrhenian sites visited by Zelinka; so it is not surprising that here we found eight species out of 11 (12 if data by Sánchez, 2015 are considered) previously reported from Naples.

By contrast, in the Upper Adriatic Sea, we found many additional species (6) not reported by Zelinka (1928). Likely this is related to the habitats we have sampled, that appear quite different from those investigated by Zelinka. In fact, our three collecting locations are situated relatively south compared to Zelinka's sites; yet, they are most likely affected by the largest Italian waterways, the Po River, and one is positioned inside a lagoon. By contrast, the locations sampled by Zelinka are restricted in the uppermost portion of the northern Adriatic Sea, in a more confined area.

Additional new species and/or species new to the Italian waters were from areas (Ligurian Sea) or habitat not investigated before e.g.: deep sediment of the Ligurian Sea, the medium—coarse sand from Sardinia and Capraia islands or the sandy sediment from the submarine Nereo Cave.

Besides the putative new taxa, it is worth noting that 14 out the 20 previously known species have been found also during our investigations, even though some of the recorded specimens show details not reported from the original material e.g., *Pycnophyes flaveolatus* and *P. rugosus* (see above). Ongoing and future taxonomic analyses including specimens from other areas and the type localities will clear out the uncertainties.

Combining our new data with the accepted species (i.e. *nomina dubia* excluded) by Zelinka (1928) the number of know species from the Italian waters amount to 37 (see Table 5), of which 22 are formally described (Figures 8,9), and 15 will be described at the end of the ongoing taxonomic survey.

#### 3.4.2. Ecological remarks

With regards to the bathymetry, most of the samples analyzed during our research were from relatively shallow coastal waters, less than 50 m deep; however the numerous samples (> 100 cores) from Leghorn (loc. 3) are much deeper (90-130 m) providing an ample bathymetric range.

Considering the scanty information about the basic ecology of the Kinorhyncha, our information

may turn out to be insightful with regard to a possible depth preference of the species found. Consequently, based on the depth at which the samples were taken, we subjectively subdivided the recorded fauna into four water depth intervals: 1-9, 10-29, 30-50, 90-130 m. The distribution of species by depth interval is shown in Table 3.

Ten species in six genera (Condyloderes, Semnoderes, Echinoderes, Paracentrophyes, Kinorhynchus, and Pycnophyes) appeared in samples from deeper waters (>90 m), though only four of them were exclusively found in this habitat, namely: Echinoderes sp. 2, Echinoderes sp. 3, Pycnophyes cf. rugosus, and Pycnophyes sp. 3. Three out of four species seem new to science; this feature stresses the potentially high kinorhynch diversity in deep waters and at the same time the scarce knowledge about this fauna in the Italian waters. Of the remaining six species found at 90-130 m depth, five (Condyloderes sp. 2, Echinoderes capitatus, Paracentrophyes quadridentatus, Kinorhynchus giganteus, and Pycnophyes communis) have a depth range that includes also shallower waters (see Neuhaus, 2013 and Table 3) while one, Semnoderes armiger, has been reported mostly from shallow waters (Sánchez et al., 2012; Sørensen et al., 2009) and rarely from water depths below 100 m (see Sánchez et al., 2012). Six species found during our surveys occurred only at 30-50 m depth, namely Condyloderes sp. 1, Echinoderes ferrugineus, E. gerardi, Echinoderes sp. 4, Pycnophyes carinatus, and P. robustus. However, previous records indicate that these taxa are not restricted to this bathymetric range. For example, E. gerardi was known for shallow waters sponges (Higgins, 1978), and was recently reported from intertidal algal washing (Sönmez et al., 2016), whilst E. ferrugineus was previously reported from 1 to 35 m (Zelinka, 1928); P. carinatus and P. robustus were found at 12-35 m and 17-35 m. respectively (Sánchez et al., 2012; Zelinka, 1928).

Other five species were found only at 10-29 m depth: *Centroderes spinosus*, *Echinoderes agigens*, *Echinoderes* sp. 1, *Kinorhynchus* sp. 1, *Pycnophyes* sp. 1, with *C. spinosus* previously found in several locations at similar depths (e.g., Gulf of Naples, 35 m; Zelinka, 1928), while *E. agigens* was known previously for depths down to 60 m, in the Black Sea type locality (Bâcescu, 1968).

Six species occurred only in our shallower samples (<10 m): *Antygomonas caeciliae*, *A. incomitata*, *Echinoderes* sp. 5, *Kinorhynchus* sp. 2, *P.* cf. *flaveolatus*, *Pycnophyes* sp. 2. For some of them, our records confirm previous findings and could indicate a potential preference for this depth. Nonetheless, *P. flaveolatus* was reported at greater depths (17-35 m; Zelinka, 1928), similarly to *A. incomitata* which has been recorded from 2-25 m deep sediments (Nebelsick, 1990; Sánchez et al., 2012; Sørensen et al., 2009).

*Meristoderes macracanthus* was reported from both shallower samples and greater depth ones (10-29 m). The latter samples were collected in a submarine cave (Nereo Cave, loc. 9), characterized by relatively coarse sediment (medium-fine sand, see below), not very different in terms of granulometry from the shallower site's one (Budelli Island, loc. 8). Previously, Herranz et al. (2012) reported this species from 11-37 m deep sandy sediments. It is likely that the sediment type influences the presence of this taxon more than depth.

To summarize, the depth range showing the highest species richness was 90-130 m (5.0 species/site), followed by the depth intervals 30-50 m (3.2 species/site), 10-29 m (3.0 species/site), and finally 1-9 m (1.3 species/site). These results underline a scarce diversity of kinorhynchs in shallow waters, and, by contrast, a high richness in deeper habitats.

The distribution of species in function of the sediment type is summarized in Table 4. Most species (23 out of 29 spp.) were found in very fine sand/coarse silt; it should be emphasized that associated to our finer sediments there was always a fraction of clay, hence confirming literature data describing the Kinorhyncha (mud dragons) as being mostly mud dwelling animals (see Neuhaus, 2013). Six species occurred only in coarser sediment (fine to coarse sand), namely: *Antygomonas caeciliae*, *A. incomitata*, *Echinoderes* sp. 4, *Echinoderes* sp. 5, *Echinoderes* sp. 6, and *Meristoderes macracanthus*. Considering the relatively high number of locations characterized by this sediment type (7 out of 16) the associated species richness is very low (ca. 0.9 species/site). By contrast, the locations showing very fine sand/coarse silt generally yielded a much higher species richness (ca. 2.6 species/site). Again, the datum confirms results from previous studies accounting for a higher species richness associated with muddy sediments compared to the clean, sandy bottoms (Neuhaus, 2013).

At a genus level our study confirms that *Antygomonas* tends to be restricted to clean sandy sediments, while *Echinoderes* appears to be polytopic; however the finding of three putative new species, belonging to this genus, associated with clean sand (Table 1) calls for additional surveys in localities characterized by this kind of substratum.

It must be noted for the context that the present knowledge on kinorhynch ecology doesn't allow to discriminate clearly between depth and sediment type. As these two features are generally correlated, it could be possible to observe an apparent species-depth correlation, whilst actually it would be a species-sediment type one.

#### 3.4 Geographic distribution of the Italian Kinorhyncha

Notwithstanding the addition of new 16 locations, the number of Italian localities investigated for the kinorhynch fauna remains limited. Furthermore, a large portion of the peninsular coastline appears virtually unexplored, notably: the Mid Tyrrhenian, Ionian, Mid and Southern Adriatic. Consequently, any discussion about geographic distribution if based on the 9 marine biogeographic zones recognized by the Italian checklist of marine species (see Relini and La Posta, 2005) would be particularly confounding. At this early stage, a discussion about the biogeographic distribution based on the sea basins appears more appropriate. Hence, of the 36 species know for the Italian waters some appear to be: i) restricted to a single basin: eight exclusively of the Adriatic Sea: Echinoderes agigens, E. setiger, E. subfuscus, Echinoderes sp. 1, Kinorhynchus sp. 1, Kinorhynchus sp. 2, Pycnophyes sp. 1, and Pycnophyes sp. 2. Three found only in the Ligurian Sea: Echinoderes sp. 2, Echinoderes. sp. 3, Pycnophyes sp. 3. Ten peculiar of the Tyrrhenian Sea: Condyloderes sp. 1, Echinoderes citrinus, E. ferrugineus, E. gerardi, Echinoderes sp. 4, Echinoderes sp. 5, Meristoderes macracanthus, Pycnophyes echinoderoides, P. ponticus, P. zelinkaei; ii) present in two basins (ten): Centroderes spinosus, Echinoderes dujardinii, Pycnophyes carinatus, P. flaveolatus, and P. robustus are present in the Adriatic and Tyrrhenian Seas; Antygomonas caeciliae, Condyloderes sp. 2, Echinoderes sp. 6, Paracentrophyes quadridentatus, and Pycnophyes rugosus are present in the Tyrrhenian and Ligurian Seas, and iii) virtually ubiquitous (five): Antygomonas incomitata, Semnoderes armiger, Echinoderes capitatus, Kinorhynchus giganteus, and Pycnophyes communis.

More than half of the Italian species (21 out 36) appears to be restricted to a single Italian sea basin, moreover, 11 of these taxa appear new to science. Consequently, one could think that the greatest part of the Italian kinorhynch diversity is due to species with a restricted geographic range. However, as 8 of the known species recorded for a single Italian basin have been found also outside Italy, it is likely that the range of the putative new species will be widened in the future.

One out of 36 species known for the country has been reported from all the four Italian basins: *A. incomitata*, found by us in the Ligurian, Tyrrhenian, and Ionian Sea and by Nebelsick (1990) in the Adriatic Sea. Additional four species may also be considered ubiquitous, *Semnoderes armiger*, *Echinoderes capitatus*, *Kinorhynchus giganteus*, and *Pyncophyes communis*, as the apparent absence of these species from the Ionian Sea is likely due to the fact that only a single location (two, if the study by Sørensen et al., 2009 is included) has been surveyed in this sea and moreover the investigated sediment was made up of sand, and not of the finer type inhabited by these species.

In absolute values the richest basin in terms of number of species appears to be the Tyrrhenian, followed by the Adriatic, the Ligurian, and finally by the Ionian Sea, with 25, 19, 13

and 1 species, respectively. However, if the effort of sampling (i.e., number of investigated locations by us, Sánchez, 2015, and Zelinka, 1928) is taken into account, the Ligurian basin stands out as the richest (4 locations; 3.2 species/site), followed by the Ionian Sea (1 location; 1.0 species/site), the Tyrrhenian Sea (27 locations; 0.9 species/site), and finally the Adriatic Sea (23 locations, 0.8 species/site). The richness of Ligurian Sea in terms of kinorhynch fauna is highlighted by this datum, which encourages further analyses within this basin. It must be noted for the context that 4 out of 8 locations surveyed by us in the Tyrrhenian basin yielded single species, which is likely to be related to the sediment type at these sites and also to the fact that samples often were not specifically collected for the study of the Kinorhyncha. The Ionian Sea seems to be characterized by a low kinorhynch diversity, however, it is anticipated that future investigation of deeper and finer sediments most likely will reveal a much richer fauna.

#### 4. Conclusion

Prior to this study, the number of the Italian Kinorhyncha summed to 48 (Sandulli and de Zio Grimaldi, 2008); however, a careful taxonomic revision of the taxa reported in the previous checklist revealed that 31 of them were in fact to be considered *nomina dubia* and hence of doubtful utility/application. These names were eliminated from the update checklist, and the remaining 17 taxa were accompanied with 19 further species reported in published papers but mainly found during our studies. All together the new checklist of Italian kinorhynchs includes 36 species in 9 genera and 6 families, more or less equally subdivided between the newly recognized Classes Allomalorhagida and Cyclorhagida.

Thirteen out of the 36 species appear new to science and will be described in forthcoming papers. Several interesting taxa were found in areas not previously investigated, and often characterized by peculiar habitats, such as submarine caves, lagoons, and coarse sediments. This study confirms the existence of a relatively high kinorhynch diversity along the Italian coasts, and encourage further surveys in the peninsular waters, with a particular focus on neglected habitats, often representing biodiversity hotspots. Along with qualitative sampling, quantitative surveys are particularly encouraged in order to obtain those basic ecological information. This is crucial to improve our understanding of the fascinating mud dragons – something that this study has provided only to a limited extend.

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 **Fig. 1.** Map of Italy and surrounding seas, with indication of the nine marine biogeographic zones proposed by Relini and La Posta (2005) for the checklist of the Italian marine biota. Numbers in the circles identify the 16 locations investigated during the present study. See text for further details.

**Fig. 2.** Paracentrophyes quadridentatus, adult females from the Gulf of Casellammare (Sicily). SEM photomicrographs. **A**, habitus with the introvert retracted, ventrolateral view; **B**, different specimen, close up of the introvert. oos, outer oral styles; ps, primary spinoscalids.

**Fig. 3.** *Pycnophyes robustus*, adult male from the Gulf of Castellammare (Sicily). SEM photomicrographs. **A**, habitus with the introvert retracted, ventrolateral view; **B**, close up of the anterior segments; **C**, close up of posterior region showing a spermatophore. spp, spermatophore; vt, ventral tubes.

**Fig. 4.** *Echinoderes* sp. 5, adult female from Le Formiche di Grosseto (Tuscany, Tyrrhenian Sea). DIC photomicrographs. **A**, dorsal view; **B**, close up of segments 2-5 in ventral view; **C**, close up of segments 1-2 in dorsal view. mds, middorsal spine.

**Fig. 5.** *Meristoderes macracanthus*, adult female from the submarine Nereo Cave (Sardinia). SEM photomicrographs. **A**, habitus, ventro-lateral view; **B**, close up of the introvert. ios, inner oral styles; oos, outer oral styles; ps, primary spinoscalids.

**Fig. 6.** *Condyloderes* sp. 1, adult female from the Gulf of Castellammare (Sicily). DIC photomicrographs. **A**, ventral view; **B**, close up of the trunk showing an oocyte; **C**, close up of segments 9-11. go, gonopores; ltas, lateral terminal accessory spine; mts, midterminal spine; oo, oocyte.

Fig. 7. Number of species and genera found in the 16 locations investigated during the present study.

Fig. 8. Italian Kinorhyncha; drawings of the described Allomalorhagida. Neocentrophyidae: 1, Paracentrophyes quadridentatus; Pycnophyidae: 2, Kinorhynchus giganteus; 3, Pycnophyes carinatus; 4, Pycnophyes communis; 5, Pycnophyes echinoderoides; 6, Pycnophyes flaveolatus; 7, Pycnophyes ponticus; 8, Pycnophyes robustus; 9, Pycnophyes rugosus; 10, Pycnophyes zelinkaei. (1-4,6-10 modified from Adrianov and Malakhov, 1999; 5 modified from Zelinka, 1928).

Fig. 9. Italian Kinorhyncha; drawings of the described Cyclorhagida. Antygomonidae: 1, Antygomonas caeciliae; 2, Antygomonas incomitata; Centroderidae: 3, Centroderes spinosus; Echinoderidae: 4, Echinoderes agigens; 5, Echinoderes capitatus; 6, Echinoderes citrinus; 7, Echinoderes dujardinii; 8, Echinoderes ferrugineus; 9, Echinoderes gerardi; 10, Echinoderes setiger; 11, Echinoderes subfuscus; 12, Meristoderes macracanthus; Semnoderidae: 13, Semnoderes armiger. (1 modified from Dal Zotto, 2015; 2 modified from Sørensen et al., 2010b, 2008; 3 modified from Sørensen and Pardos, 2008; 4,6,8,10 modified from Adrianov and Malakhov, 1999; 5 modified from Nebelsick, 1992; 7 modified from Higgins, 1985; 9 modified from Higgins, 1978; 11 modified from Zelinka, 1928; 12 modified from Herranz et al., 2012; 13 modified from Sørensen et al., 2009).

**Table 1** – Sixteen new or newly sampled Italian locations. The geographic coordinates, water depth, sediment type and date of sampling are reported. Numbers in brackets indicate the biogeographic zone in which the Italian seas are partitioned (Figure 1), according to the checklist of Italian marine species (Relini and La Posta, 2005).

LOCATION (BIOGEOGRAPHIC ZONE)	COORDINATES	<b>D</b> EPTH (m)	SEDIMENT TYPE	DATE								
Ligurian Sea												
1. Meloria Shoals (1)	43°32'50.06'' N, 10°12'58.80'' E	2-7	Coarse sand	10/2008								
<b>2.</b> Leghorn 1 (1)	43°37'49.31'' N, 10°08'24.18'' E	105-110	Fine sand-coarse silt	07/1996								
3. Leghorn 2 (1)	43° 37' 58,50'' N, 9° 59' 31,6'' E	97-130	Fine sand-coarse silt	08/2012 - 09/2015								
4. Lo Scollione, Capraia (1)	43°02'01.46'' N, 09°43'52.64'' E	30	Medium sand	05/2007								
		enian Sea										
<b>5.</b> P.ta del Marchese, Pianosa (2)	42°36'00.34'' N, 10°05'46.76'' E	6-7	Medium-fine sand	06/2004								
<b>6.</b> Formiche di Grosseto (2)	42°34'43.12'' N, 10°52'56.77'' E	37	Medium sand	06/2009								
7. Gulf of Follonica (2)	42°53'41.50'' N, 10°46'51.20'' E	3-8	Fine sand-coarse silt	06/2010								
8. Budelli Island, Sardinia (2)	41°16'45.62'' N, 09°21'21.74'' E	7	Medium-fine sand	09/2009								
9. Nereo Cave, Sardinia (2)	40°34'10.50'' N, 08°10'02.90'' E	20	Medium sand	07/2005								
10. Le Bombarde, Sardinia(2)	40°35'02.00'' N, 08°15'37.90'' E	1,5-3	Medium sand	07/2005								
11. Castellammare, Sicily (3)	38°02'58.00'' N, 12°52'97.00'' E	35-50	Fine sand-coarse silt	06/2006 - 12/2007								
12. Trappeto, Sicily (3)	38°04'51.79'' N, 13°01'19.45'' E	30-40	Fine sand-coarse silt	06/2006 - 01/2008								
		an Sea										
13. Porto Cesareo (6)	40°13'09.10'' N, 17°55'35.50'' E	2	Medium-fine sand	06/2005								
		atic Sea										
<b>14.</b> Chioggia (9)	45°05'74.01'' N, 12°35'82.59'' E	20-29	Coarse silt	06/2011 – 07/2015								
15. Vallona Lagoon (9)	45°02'05.32'' N 12°23'25,72'' E	1-3	Coarse silt	06/2011 – 07/2015								
<b>16.</b> Cesenatico (9)	44°13'14.00'' N, 12°28'61.00'' E	11-12	Coarse silt	10/2008								

**Table 2** – Kinorhynch species list and distribution in the 16 investigated locations and four sea basins.

Taxon Location	Ligurian Sea			n	Tyrrhenian Sea								Ionian Sea	Adriatic Sea		
	1	2	3	4	5	6	7	8	9		11	12	13	14	15	_
ALLOMALORHAGIDA													_			
NEOCENTROPHYIDAE																
Paracentrophyes quadridentatus	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-
Руспорнуграе																
Kinorhynchus giganteus	-	-	+	-	-	-	-	-	-	-	+	+	-	+	-	-
Kinorhynchus sp. 1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Kinorhynchus sp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Pycnophyes carinatus	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Pycnophyes communis	-	-	+	-	-	-	+	-	-	-	+	+	-	+	+	+
Pycnophyes robustus	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Pycnophyes cf. flaveolatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Pycnophyes cf. rugosus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Pycnophyes sp. 1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Pycnophyes sp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Pycnophyes sp. 3	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CYCLORHAGIDA																
ANTYGOMONIDAE																
Antygomonas caeciliae	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Antygomonas incomitata	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
CENTRODERIDAE																
Centroderes spinosus	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Condyloderes sp. 1	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Condyloderes sp. 2	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
ECHINODERIDAE																
Echinoderes agigens	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Echinoderes capitatus	-	-	+	-	-	-	-	-	-	-	+	+	-	+	-	-
Echinoderes ferrugineus	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Echinoderes gerardi	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Echinoderes sp. 1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Echinoderes sp. 2	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Echinoderes sp. 3	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Echinoderes sp. 4	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Echinoderes sp. 5	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Echinoderes sp. 6	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
Meristoderes macracanthus	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
SEMNODERIDAE																
Semnoderes armiger	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-
Total number of species	2	1	10	1	2	1	1	2	1	1	10	11	1	8	4	2

<sup>+,</sup> species present; - species absent

**Table 3** – Species found during our research listed according to depth ranges.

<b>D</b> ЕРТН ( <b>m</b> )	LOCATION	SPECIES
1-9	1, 5, 7, 8, 10, 13, 15	Antygomonas caeciliae; Antygomonas incomitata; Echinoderes sp. 5; Echinoderes sp. 6; Meristoderes macracanthus; Kinorhynchus sp. 2; Pycnophyes communis; Pycnophyes cf. flaveolatus; Pycnophyes sp. 2
10-29	9, 14, 16	Centroderes spinosus; Echinoderes agigens; Echinoderes capitatus; Echinoderes sp. 1; Meristoderes macracanthus; Kinorhynchus giganteus; Kinorhynchus sp. 1; Pycnophyes communis; Pycnophyes sp. 1
30-50	4, 6, 11, 12	Condyloderes sp. 1; Condyloderes sp. 2; Semnoderes armiger; Echinoderes capitatus; Echinoderes ferrugineus; Echinoderes gerardi; Echinoderes sp. 4; Echinoderes sp. 6; Paracentrophyes quadridentatus; Kinorhynchus giganteus; Pycnophyes carinatus; Pycnophyes communis; Pycnophyes robustus
90-130	2, 3	Condyloderes sp. 2; Semnoderes armiger; Echinoderes capitatus; Echinoderes sp. 2; Echinoderes sp. 3; Paracentrophyes quadridentatus; Kinorhynchus giganteus; Pycnophyes communis; Pycnophyes cf. rugosus; Pycnophyes sp. 3

Table 4 - Species found during our research grouped according to the sediment type.

SEDIMENT TYPE	LOCATION	SPECIES						
Coarse sand	1	Antygomonas caeciliae; Antygomonas incomitata						
Medium/fine sand	4, 5, 6, 8, 9, 10, 13	Antygomonas caeciliae; Antygomonas incomitata; Echinoderes sp. 4; Echinoderes sp. 5; Echinoderes sp. 6; Meristoderes macracanthus						
Very fine sand/ coarse silt	2, 3, 7, 11, 12, 14, 15, 16	Centroderes spinosus; Condyloderes sp. 1; Condyloderes sp. 2; Semnoderes armiger; Echinoderes agigens; Echinoderes capitatus; Echinoderes ferrugineus; Echinoderes gerardi; Echinoderes sp. 1; Echinoderes sp. 2; Echinoderes sp. 3; Paracentrophyes quadridentatus; Kinorhynchus giganteus; Kinorhynchus sp. 1; Kinorhynchus sp. 2; Pycnophyes carinatus; Pycnophyes communis; Pycnophyes robustus Pycnophyes cf. flaveolatus; Pycnophyes cf. rugosus; Pycnophyes sp. 1; Pycnophyes sp. 2; Pycnophyes sp. 3						

**Table 5** – An updated checklist of the Italian kinorhynch species. Distribution is according to the 9 marine biogeographic zones recognized by 2008 National checklist of marine species (see Relini and La Posta, 2005). References point to the author(s) credited for the record(s).

Biogeographic zone										
Taxon		2	3	4	5	6	7	8	9	References
CLASS ALLOMALORHAGIDA		_		-		Ŭ	-	Ŭ	_	2000000
FAMILY NEOCENTROPHYIDAE										
Paracentrophyes quadridentatus (Zelinka,										
1928)	Χ		Χ							Zelinka, 1928; Sánchez, 2015; present study
FAMILY PYCNOPHYIDAE										
Kinorhynchus giganteus (Zelinka, 1928)	Χ		Χ						Χ	Zelinka, 1928; present study
Kinorhynchus sp. 1									Χ	Present study
Kinorhynchus sp. 2									Χ	Present study
Pycnophyes carinatus Zelinka, 1928			Χ						Χ	Zelinka, 1928; present study
	Х	Х	Х						Χ	Zelinka, 1928; F. Semprucci and M.V.
Pycnophyes communis Zelinka, 1928	<u> </u>									Sørensen, pers. comm.; present study
Pycnophyes echinoderoides Zelinka, 1928			X							Zelinka, 1928
Pycnophyes flaveolatus Zelinka, 1928			X						Χ	Zelinka, 1928; Sánchez, 2015; present study
Pycnophyes ponticus (Reinhardt, 1881)			X							Zelinka, 1928; Sánchez, 2015
Pycnophyes robustus Zelinka, 1928			X						Χ	Zelinka, 1928; Sánchez, 2015; present study
Pycnophyes rugosus Zelinka, 1928	Х		Χ							Zelinka, 1928; Sánchez, 2015; present study
Pycnophyes zelinkaei			Χ							Sánchez, 2015
Pycnophyes sp. 1									Х	Present study
Pycnophyes sp. 2									Х	Present study
Pycnophyes sp. 3	Х									Present study
CLASS CYCLORHAGIDA										
FAMILY ANTYGOMONIDAE										
Antygomonas caeciliae Dal Zotto, 2015	Χ	Χ								Dal Zotto and Todaro, 2009; Dal Zotto, 2015
	\ \	\				\ \			\ \	Nebelsick, 1990; Sørensen et al., 2009; Dal
Antygomonas incomitata Nebelsick, 1990	X	Χ				Χ			Х	Zotto, 2015; present study
FAMILY CENTRODERIDAE			\ <u>'</u>						\ <u>'</u>	
Centroderes spinosus (Reinhardt, 1881)			X						Χ	, , , , , , , , , , , , , , , , , , , ,
Condyloderes sp. 1			X							Present study
Condyloderes sp. 2	Х		Χ							Present study
FAMILY ECHINODERIDAE										
Echinoderes agigens Bâcescu, 1968										Present study
Echinoderes capitatus (Zelinka, 1928)	Х		X						Х	Zelinka, 1928; present study
Echinoderes citrinus Zelinka, 1928			Χ							Zelinka, 1928
										Metschnikoff, 1865; Panceri, 1878; Schepotieff, 1907; Zelinka, 1928; Mari and
Echinoderes dujardinii Claparède, 1863			Χ				Χ		Х	Morselli, 1987
Echinoderes ferrugineus Zelinka, 1928			Χ						Χ	Zelinka, 1928; present study
Echinoderes gerardi Higgins, 1978			Х							Higgins, 1978; present study
Echinoderes setiger Greeff, 1869	ı								Χ	Zelinka, 1928; present study
Echinoderes subfuscus Zelinka, 1928									Χ	Zelinka, 1928
Echinoderes sp. 1	ı								Χ	Present study
Echinoderes sp. 1  Echinoderes sp. 2										Present study
Echinoderes sp. 2  Echinoderes sp. 3										Present study
Echinoderes sp. 3  Echinoderes sp. 4	Х	Χ								Present study
Echinoderes sp. 5	1	Х								Present study
Echinoderes sp. 6	X	Х								Present study
Lemmoneres sp. 0										

<i>Meristoderes macracanthus</i> Herranz et al., 2012		Χ					Herranz et al., 2012; present study
FAMILY SEMNODERIDAE							
Semnoderes armiger Zelinka, 1928	Х		Х			Х	Zelinka, 1928; Mari and Morselli, 1987; present study

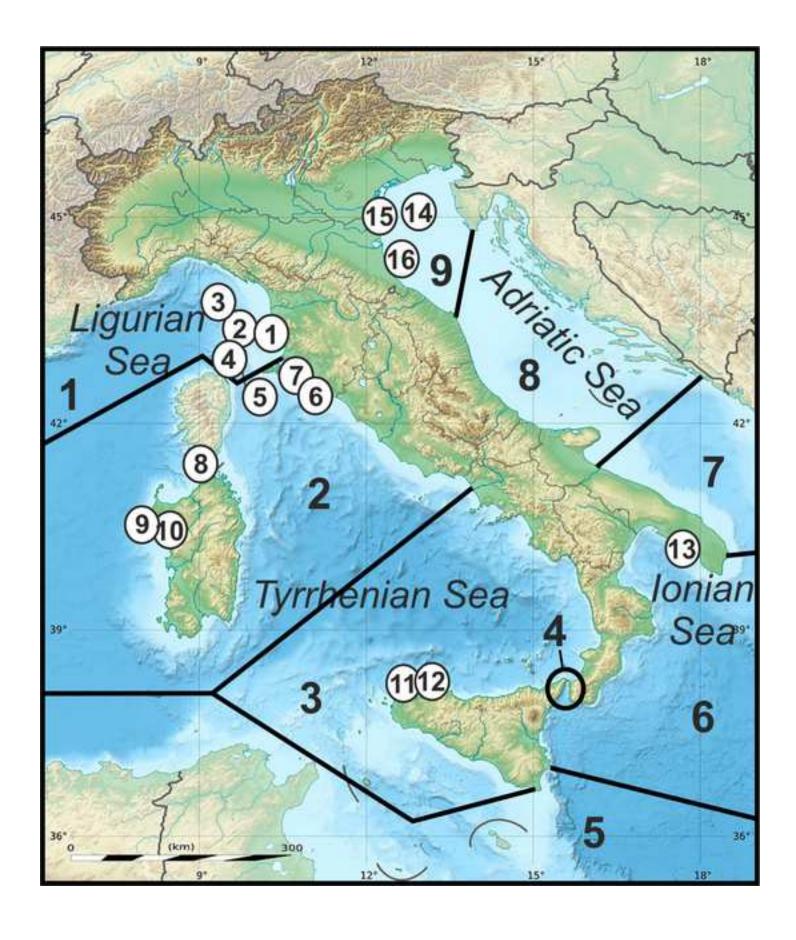
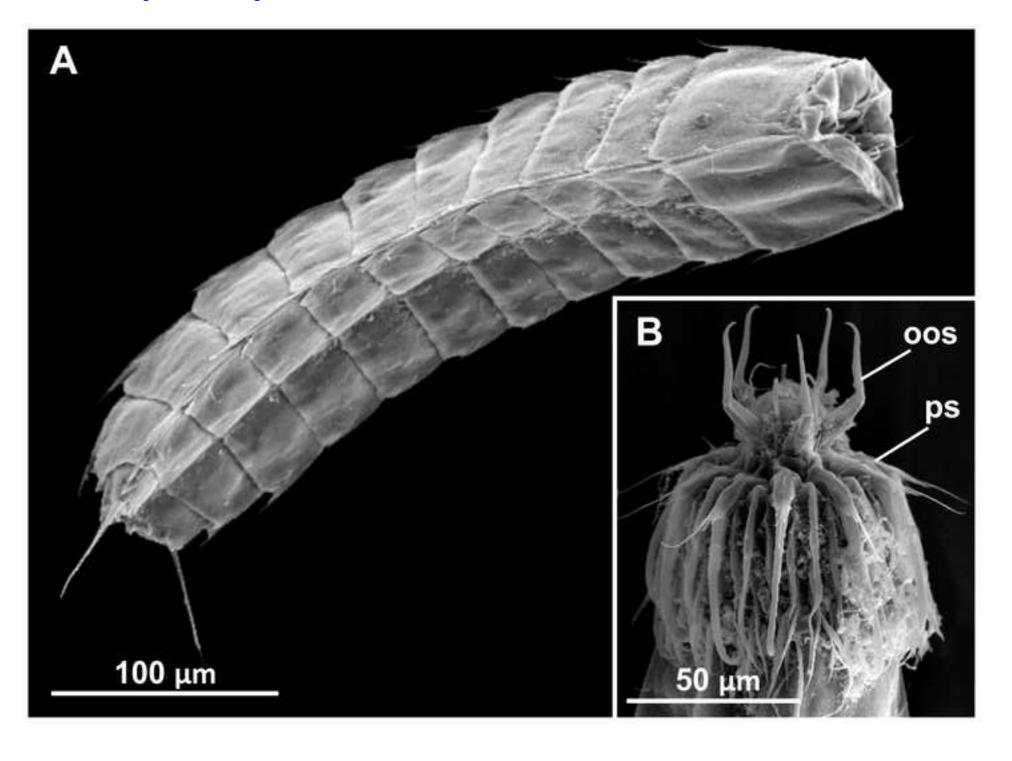


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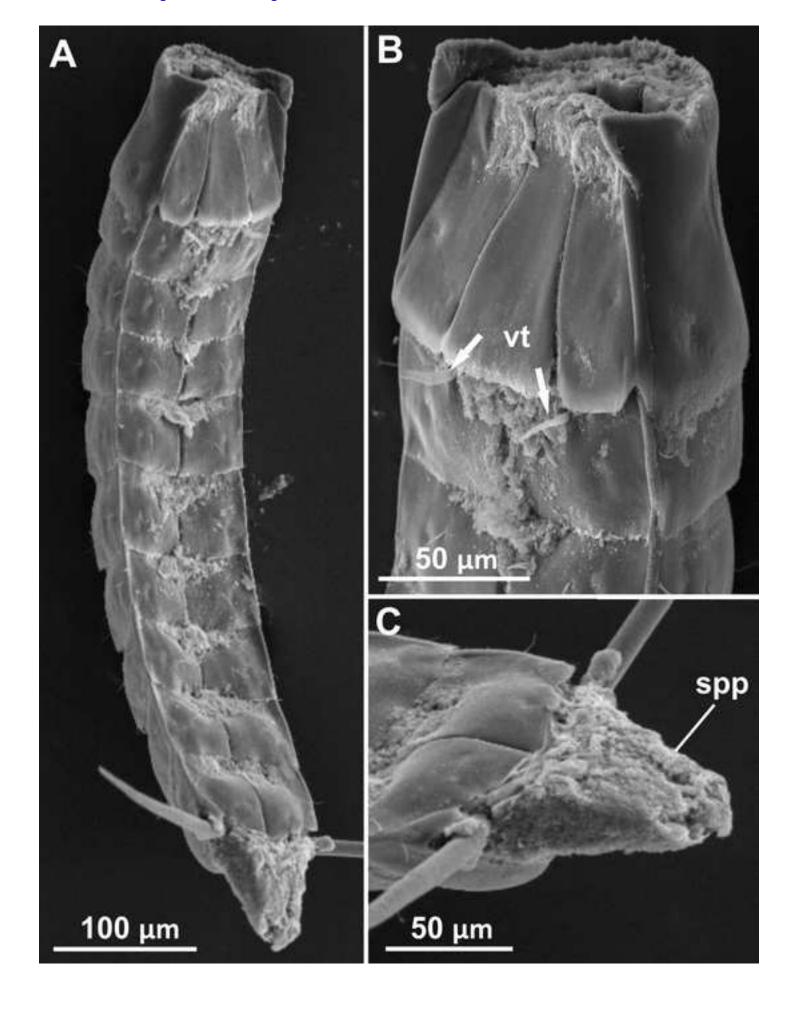


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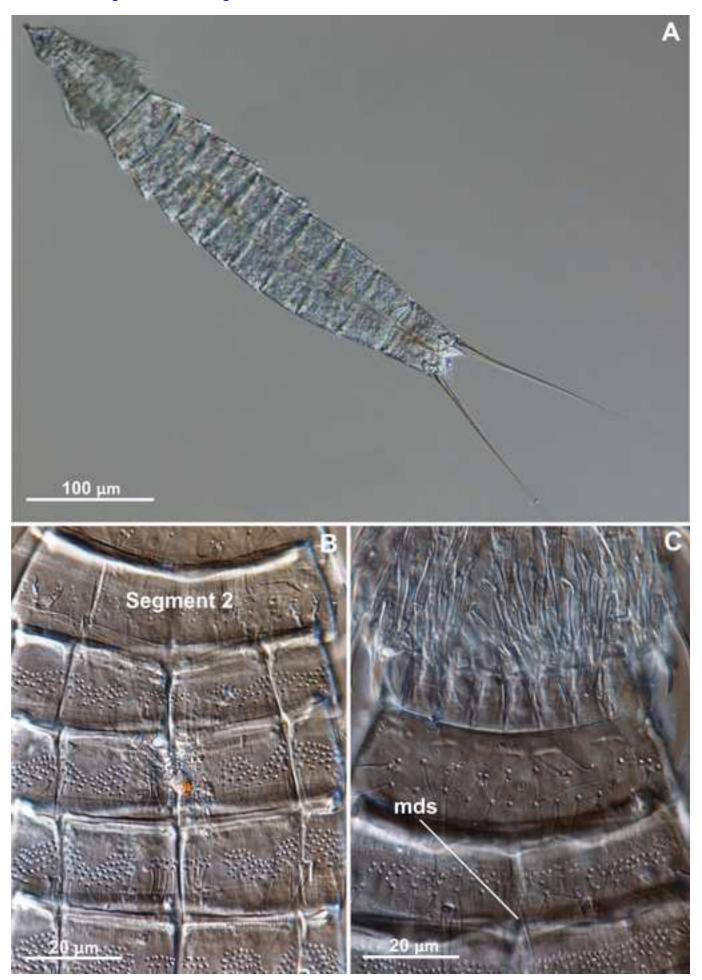


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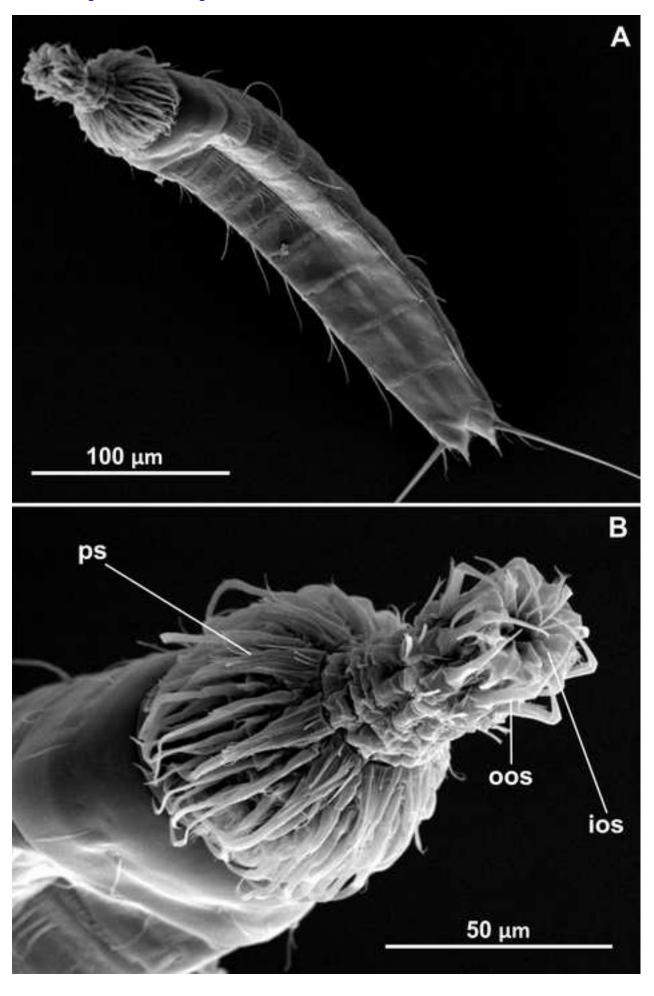


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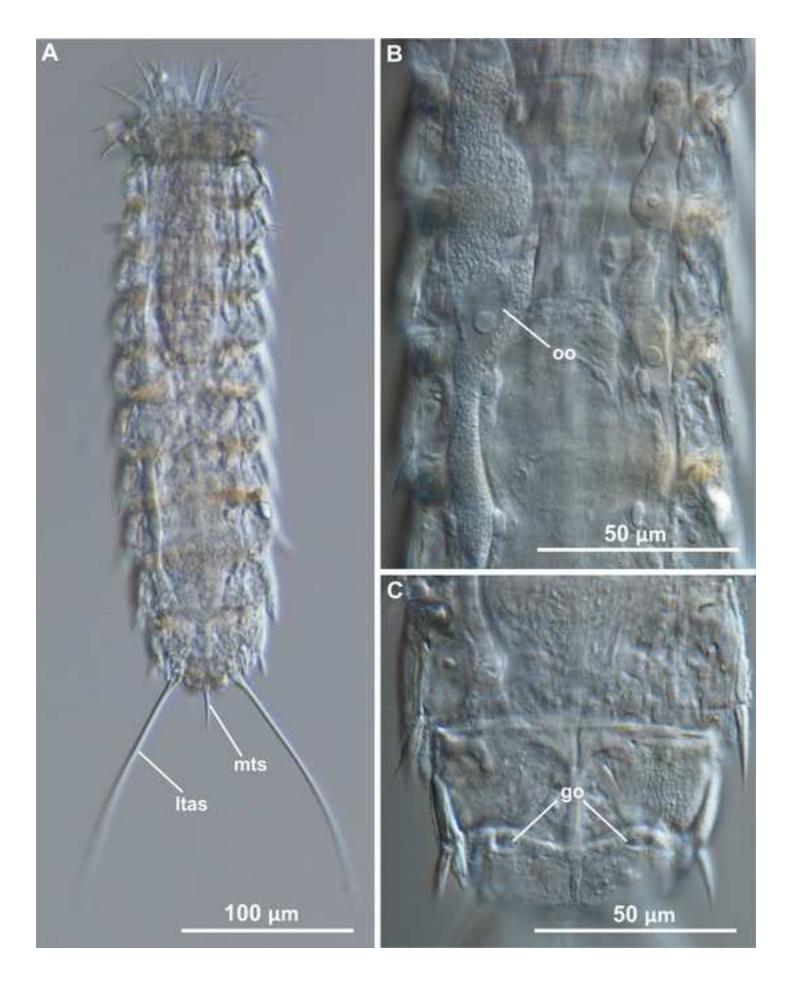


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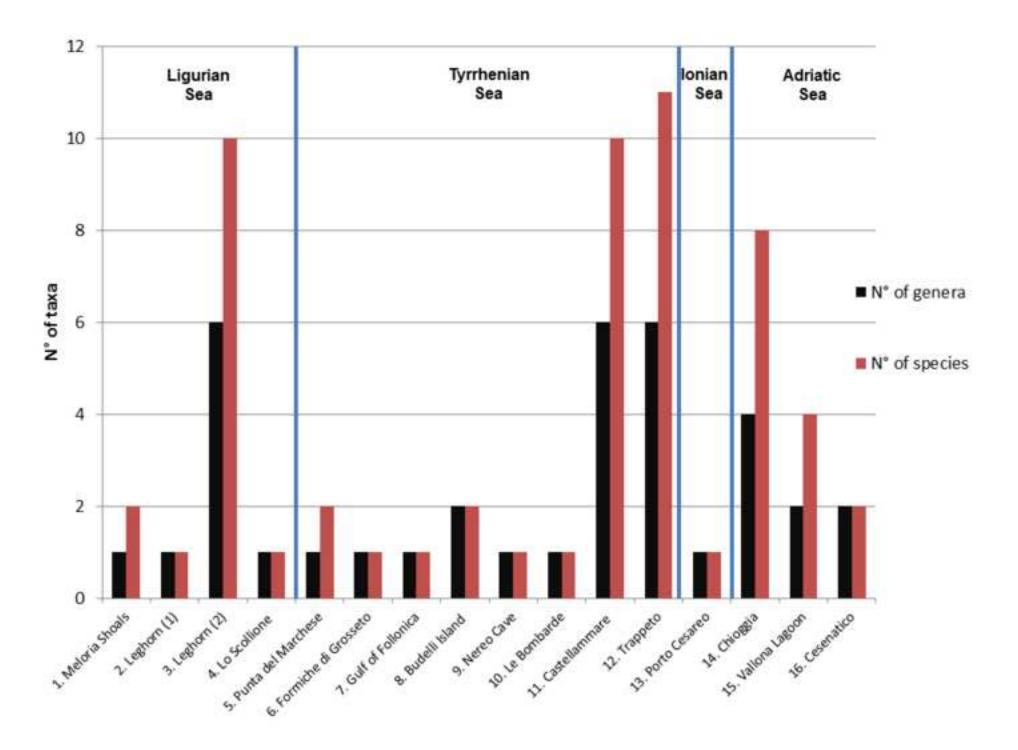


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