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The Pandora's box: Morphological diversity within the genus *Amphiglena* Claparède, 1864 (Sabellidae, Annelida) in the Mediterranean Sea, with description of nine new species

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Abstract

We report the description of nine new taxa of sabellid polychaetes belonging to the genus *Amphiglena,* of which diversity in the Mediterranean Sea has been widely underestimated. Examined material derived from both new collections along the Italian coast, including four CO_2 vents/hydrothermal systems, and from a re-examination of older material previously attributed to *A. mediterranera* (Leydig, 1851) which was so far the only species of the genus reported for the Mediterranean area. The analysis revealed the presence of different taxa also consistent with a previous molecular analysis conducted on material from the Gulf of Naples and the Salento coast (Ionian Sea). This led to an increase in the number of species in the genus and to highlight the occurrence in the Mediterranean Sea of a high diversity within the genus. A key to the Mediterranean Sea species of *Amphiglena* is also provided. Some taxa, however, remain for the moment undescribed due to the poor preservation of the old material, and the lack of the type material for this taxon. A major revision of all the Mediterranean material previously attributed to *A. mediterranea* from both morphological and molecular points of view is needed.

Key words: Polychaeta, Sabellidae, *Amphiglena*, morphology, Mediterranean Sea, CO_2 vent /hydrothermal systems

Introduction

Amphiglena Claparède, 1864 belongs to the Family Sabellidae and includes a small number of species, whose monophyly was assessed by Rouse & Gambi (1997; 1998) and Capa & Rouse (2007). Phylogenetic relationships were also investigated using both morphological data (Rouse & Gambi 1997; Capa & Rouse 2007), and more recently by molecular sequence data (Calosi *et al.* 2013; Tilic *et al*. 2019). According to the last phylogenetic analysis by Tilic *et al*. (2020), *Amphiglena* genus is included in the clade Myxicolinae which contains two tribes, named Amphiglenini and Myxicolini.

In the last decade, the examination of material coming from several geographic areas led to an increase in the number of accepted species to 14. All the *Amphiglena* species were described from shallow waters hard substrates (often covered by macrophytes) in temperate and tropical latitudes around the world, although half of the species were collected and described in Australian waters, due to the careful examination of material by specialists (Rouse 1993; Rouse & Gambi 1997; Capa & Rouse 2007; Tilic *et al*. 2019).

Currently in the Mediterranean only the species *A. mediterranea* (Leidy, 1851) is reported. This taxon is the type species of the genus *Amphiglena*, described from Nice (Western Mediterranean, France) as *Amphicora mediterranea* Leydig, 1851, and widely reported in the Mediterrenean Sea. Therefore, *A. mediterranea* was proposed to have a pan-Mediterranean distribution (Sardà 1991). Calosi *et al.* (2013), analyzed the COI of few specimens of *A. mediterranea* collected inside a CO_2 vent' systems (Ischia island, Italy) (where due to the CO_2 surplus the water

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are naturally acidified), and compared it with other populations collected in nearby other areas not influenced by the vents (where the water has normal seawater pH values), revealing for the first time the existence of different genotypes. More recently, Tilic *et al*. (2019), based also on the GenBank data from the Calosi *et al.* (2013) analysis, confirmed the existing genetic diversity within putative *A. mediterranea*, considering the Mediterranean *Amphiglena* species present along the Italian coast as a complex of at least six cryptic taxa. Cryptic speciation in annelids is not uncommon (Nygren 2014), but actually it is important to couple this molecular diversity to the morphology, if present and detectable.

In the Mediterranean *A. mediterranea* is currently one of the most widely reported species in different shallow environments from pristine rocky bottoms to more disturbed ones, as well as seagrass meadows (Giangrande 1988; Giangrande *et al*. 2004). The genus *Amphiglena*, always identified as *A. mediterranea*, represents one of the few taxa, and often the most abundant among polychaetes, thriving in volcanic CO_2 vents, which represent naturally acidified sites (Calosi *et al*. 2013; Ricevuto *et al*. 2014; Gambi *et al*. 2016; Vizzini *et al*. 2017; Auriemma *et al.* 2019). It seems likely therefore that these may represent different species linked to different environments present along the Mediterranean coast.

In this paper, several specimens from several sites along the Italian coast, and previously identified as *A. mediterranea*, were re-examined morphologically. The material includes specimens recently collected in areas along the Italian coast, some of which characterized by CO_2 vents/hydrothermal systems (Ischia-Castello, Pozzuoli-Secca delle Fumose, Vulcano-Baia di Levante and Panarea-Bottaro Islet), as well as individuals present in the collection of one of the authors (AG).

Materials and methods

The most recently examined material was collected from twelve sites along the Italian coast in the Ligurian and Tyrrhenian Seas (Western Mediterranean), and in the Ionian and Adriatic Seas (Eastern Mediterranean) (Fig. 1). In the Ischia Island different sites were sampled: Lacco Ameno, Castello CO_2 vents (north and south sides); Sant'Anna rocks (located 600 m from the Castello CO_2 vents), S. Pietro promontory downhill the Ischia Marine Center (Stazione Zoologica Anton Dohrn, Naples). In the Gulf of Naples samples derived from the Nisida Island and the Secca delle Fumose (hydrothermal system with sulphur emissions), both sites located near the small town off Baia (Gulf of Pozzuoli). In the Southern Tyrrhenian Sea sites included the Aeolian Archipelago (north of Sicily): Vulcano Island - Baia di Levante vents, and Panarea Island - Bottaro crater vents.

Additional material from Brindisi (Adriatic Sea), Leghorn (Northern Tyrrhenian Sea), Portofino (Genoa, Ligurian Sea), and Torre Inserraglio-Santa Caterina (Lecce, Ionian Sea), present in the collection of one of the Authors (AG) deposited at the Museum of Marine Biology Pietro Parenzan of the University of Salento, located at Porto Cesareo (Lecce) (PCZL), was also examined. All this material comes from shallow substrates (1-14 m) with algal cover, but with different environmental conditions.

In order to compare the shape and the morphometry of the uncini, we measured the uncini of all species. Since the overall shape of them was very similar and the only relevant difference was in the length of the handle, we proposed a different, and simplified measure, differing to those proposed by Capa & Murray (2015). As shown by Fig. 3, we measured the length of the handle of thoracic uncini and recorded the ratio between the length of the handle itself (a) and the length of the whole base of the uncinus up to the breast, including the handle (b). Uncini with a ratio from 0.2 to 0.35 were considered short, with a ratio from 0.35 to 0.5 were considered medium sized and uncini with a ratio higher than 0.5 were considered long.

Uncini and chaetae studied were taken from the middle segments of both thorax and abdomen. Width of the body was measured at the fourth thoracic chaetiger.

We also looked at the staining pattern of the segments and of the peristomium, since this technique, first proposed by Hofsummer (1913) is useful to highlight differences among species and has been utilized largely for Sabellidae (e.g., Banse 1972; Tovar-Hernandez 2007). Worms were stained with methyl green stain kept in the solution for 30 seconds, and then rinsed in alcohol, according to the protocol of Hofsummer (1913). Features of the collar and base of the crown were examined using the Shirlastain A staining.

Photographs were taken using a Stereomicroscope SMZ 25 equipped with DS-Ri2 video camera and a video-interactive image analysis system NIS–Elements BR 4.30.02 Nikon Instruments software, while drawing were performed with a camera lucida.

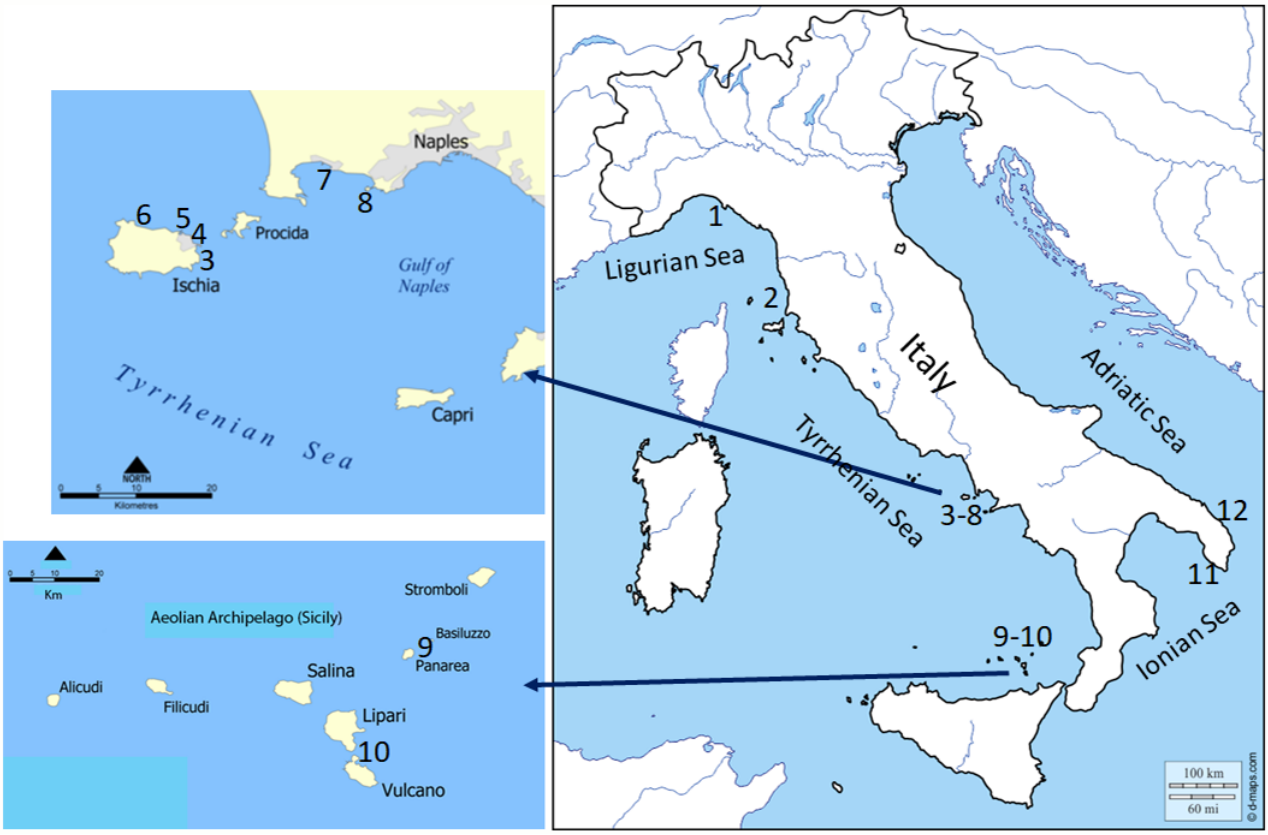

FIGURE 1. Map of the sampling sites where different *Amphiglena* species here described were collected: 1= Portofino (Ligurian Sea); 2= Livorno (Leghorn, Northern Tyrrhenian Sea); 3= Sant'Anna rocks Ischia Island (Tyrrhenian Sea); 4= Castello Aragonese vent's system (Ischia island); 5= San Pietro (Ischia Island); 6= Lacco Ameno (Ischia Island); 7=Secca delle Fumose-Baia vent's system (Pozzuoli, Gulf of Naples); 8= Nisida Island (Gulf of Naples); 9= Panarea Island (Bottaro crater vent's systems) (Aeolian Archipelago, Sicily); 10= Vulcano Island-Levante Bay vent's system (Aeolian Archipelago, Sicily); 11= Brindisi (Southern Adriatic Sea); 12= Santa Caterina (Lecce, Ionian Sea). For the geographic coordinates of each location see the text.

Since the taxa and populations from the Ischia and the Nisida Islands, and from Santa Caterina (Lecce), here morphologically analysed, were the same genetically analysed by Calosi *et al.* (2013) as putative siblings, we used the topological tree of the COI from the Calosi et *al*. (2013), to match each site of the tree with the new species now identified.

Holotypes and paratypes of new species described were deposited at the MNCN (Museo Nacional de Ciencias Naturales, Madrid, Spain). The abbreviation PCZL refers to A. Giangrande's private polychaete collection at the Zoological Laboratory of the Salento University, deposited at the Museum of Marine Biology "Pietro Parenzan" of the University of Salento (Porto Cesareo, Lecce), while MCG refers to M.C. Gambi's private polychaete collection.

Although the description of new species was based on the holotype, drawn and photographs especially of uncini, chaetae and crown features were based on paratypes because during these operations most of the material was destroyed.

For all the taxa up to 50 individuals, when available, were examined to obtain a mean body and crown length, number of thoracic and abdominal chaetigers, and number of chaetae and uncini for chaetigers (Table 1).

Results

Taxonomic account

Family Sabellidae Latreille, 1825

Genus *Amphiglena* Claparède, 1864

Type species: *Amphicora mediterranea* Leydig, 1851, subsequent designation by Bush (1905).

The following diagnosis is based on that by Fitzhugh, 1989, revised by Capa & Rouse (2007).

Diagnosis: Small-sized sabellin species bearing 5 to 8 pairs of radioles; radiolar skeleton with two rows of cells. Palmate membrane and radiolar flanges absent. Dorsal lips with dorsal radiolar appendages; dorsal pinnular appendages absent. Ventral lips absent; parallel lamellae absent. Ventral basal flanges of branchial crown present, as a pair of thin, erect, membranous flanges extending from the base of radioles and posteriorly across the anterior peristomial ring to proximal region of ventral-most radioles. Peristomial ring generally divided by a mid-ventral incision. Anterior margin of anterior peristomial ring low, of equal height all around, but often not visible all around because thinner and fused to the posterior ring. Posterior peristomial ring longer than the anterior ring with anterior margin horizontal or prolonged ventrally on both sides of the mid-ventral incision. Posterior peristomial ring collar absent.

Variable number of thoracic chaetigers. Superior thoracic notochaetae broadly-hooded. Inferior thoracic notochaetae paleate, arranged in single transverse row. A single transverse row of abdominal neurochaetae assumed to be anterior row (posterior row absent); composed of elongate, broadly hooded chaetae. Thoracic uncini with teeth above main fang of equal size; hood absent; breast well developed, expanded; handles of variable length. Companion chaetae with distal end slightly inflated, dentate and with a thin mucro extending from this. Abdominal uncini with main fang surmounted by teeth of equal size; breast well developed, handles very short. Abdominal chaetiger not numerous. Pygidial eyes present.

Amphiglena cf. *mediterranea* (Leydig, 1851)

(Figs 2, 3)

Material examined. 32+18 specimens Italy: from Ischia S. Pietro promontory (Ischia Island, Tyrrhenian Sea), 40°44’47.78”N 13°56’40.93”E; collected on macroalgae (mainly *Halopteris scoparia*) during the years 1975 (Fresi *et al.* 1983) and 2005 (Gambi M.C.) at 1-2 m depth; PCZL S.A.1.4; 36 specimens from Leghorn (Tyrrhenian Sea), 43°28’28.92”N 10°19’42.54”E, collected in 1986 at 3 m depth on rocky substrate covered by coralline algae (Giangrande 1988) PCZL S.A.1.2; 141 specimens from Portofino (Genoa, Ligurian Sea), 44°18’10.70”N 9°12’56.29”E, collected in 1980 from 3 to 5 m depth, on hard substrate with algal cover PCZL S.A.1. All the material fixed in formalin 4% and preserved in ethanol 70%.

Description. Specimens from S. Pietro (Ischia Island, Italy) have 8 thoracic chaetigers and up to 27 abdominal ones (Fig. 2A), a mean body length of about 3.10 mm of which about 0.80 mm is the crown, maximum wide of 0.36 mm (Table 1). The crown is composed of up to 10 radioles (5 pairs) each bearing up to 20 pinnules (10 pairs), arranged in two longitudinal rows alternating along the radiolar length. The gap between pairs remaining quite constant along the radiole. The pinnules show a similar length (about 1/3 of the total radiolar length), with only the first two basal pairs and the last two distal pairs appearing shorter (between 2/3 and 1/2 of the pinnule in medial position). Radiolar tip measuring almost 1/2 of the total radiolar length, with a pointed end; the dorsal lips are around 1/5 of the total radiolar length.

Spermathechae and peristomial eyes not visible, pygidial eyes ellipsoid red. First peristomial ring flattened and visible only dorsally (Fig. 2E). Second peristomial ring slightly higher ventrally, appearing separated and with rounded margins (Fig. 2D, F). High ventral basal flange not connected to the peristomial ring (Fig. 2D, F). Up to six thoracic uncini with handle of medium length (48) and with four teeth (Fig. 3A). Companion chaetae with long mucro (Fig. 3B). Superior thoracic chaetae broadly hooded (Fig. 3D). Three paleate chaetae with short mucro on each thoracic chaetiger (Fig. 3E). Abdominal uncini with short handle (Fig. 3C), abdominal chaetae as in the genus, appearing all similar from the first chaetigers to the last one (Fig. 3F, G).

Staining pattern. Thorax and abdomen intensely coloured and appearing dark blue with the exception of the intersegmental grooves, abdominal segments completely stained (Fig. 2B, C).

Remarks. *Amphiglena mediterranea* is the type species for the genus and is also the most common name used for material collected around the world, and widely reported as a cosmopolitan species (Day 1967; Hart-

mann-Schröder & Rosenfeldt 1989; Hartmann-Schröder 1990; Knight-Jones *et al*. 1991, 2017; Dauvin *et al*. 2003; Dorgham *et al.* 2013; Faulwetter *et al*. 2017), and is still reported in recent ecological and biodiversity studies (Gambi *et al*. 2016; Hutchings & Kupriyanova 2018) as well as in Mediterranean polychaete check-lists (e.g., Castelli *et al.* 2008; Faulwetter *et al*. 2017). However, no type material was deposited from the type locality in Nice (France), and the original description by Leydig (1851) (in German) was not highly informative. Other authors gave a description of this taxon: Claparéde (1864), Saint Joseph (1894) and Rioja (1923), or commented on it (Banse 1957; Fitzhugh 1990). The last detailed re-description was by Rouse & Gambi (1997) from individuals collected off Ischia Island (Gulf of Naples, Italy), however, due to the location, far from the type locality, it was not possible to designate a neotype from this material.

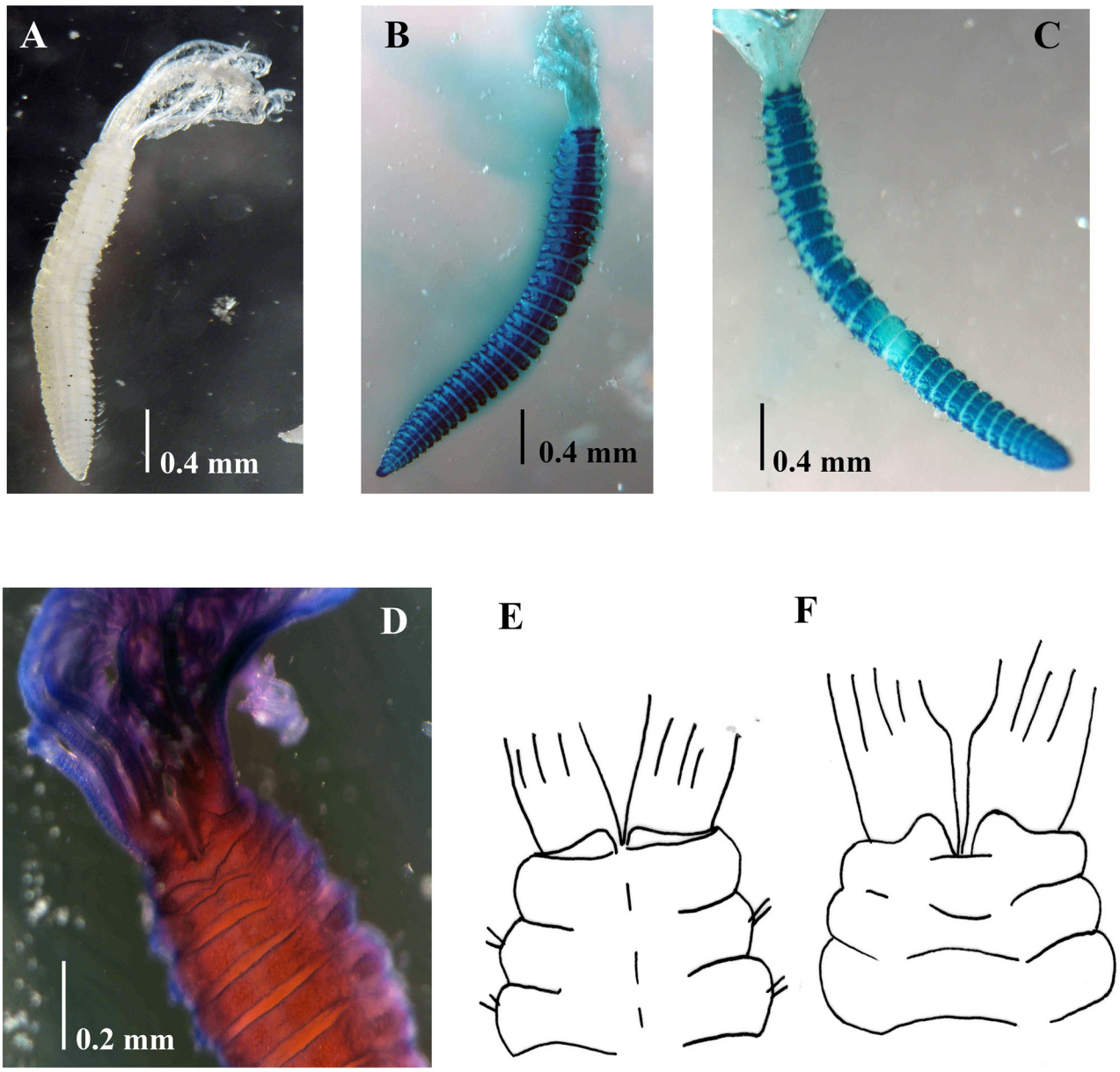

FIGURE 2. *Amphiglena* cf. *mediterranea* **A**. entire worm, specimen from Ischia, Naples; **B**. staining pattern of a specimen from Ischia, Naples; **C**. staining pattern of a specimen from Leghorn; **D**. photo from anterior end ventral view of a specimens from Ischia, material coloured with Shirlastain A; **E**. scheme of the anterior end dorsal view; **F**. scheme of the anterior end ventral view.

A comparison of the descriptions of *A. mediterranea* over time suggests that a large morphological variability exists concerning size, number of chaetigers, and number of radioles that need further exploration (Tilic *et al*. 2019).

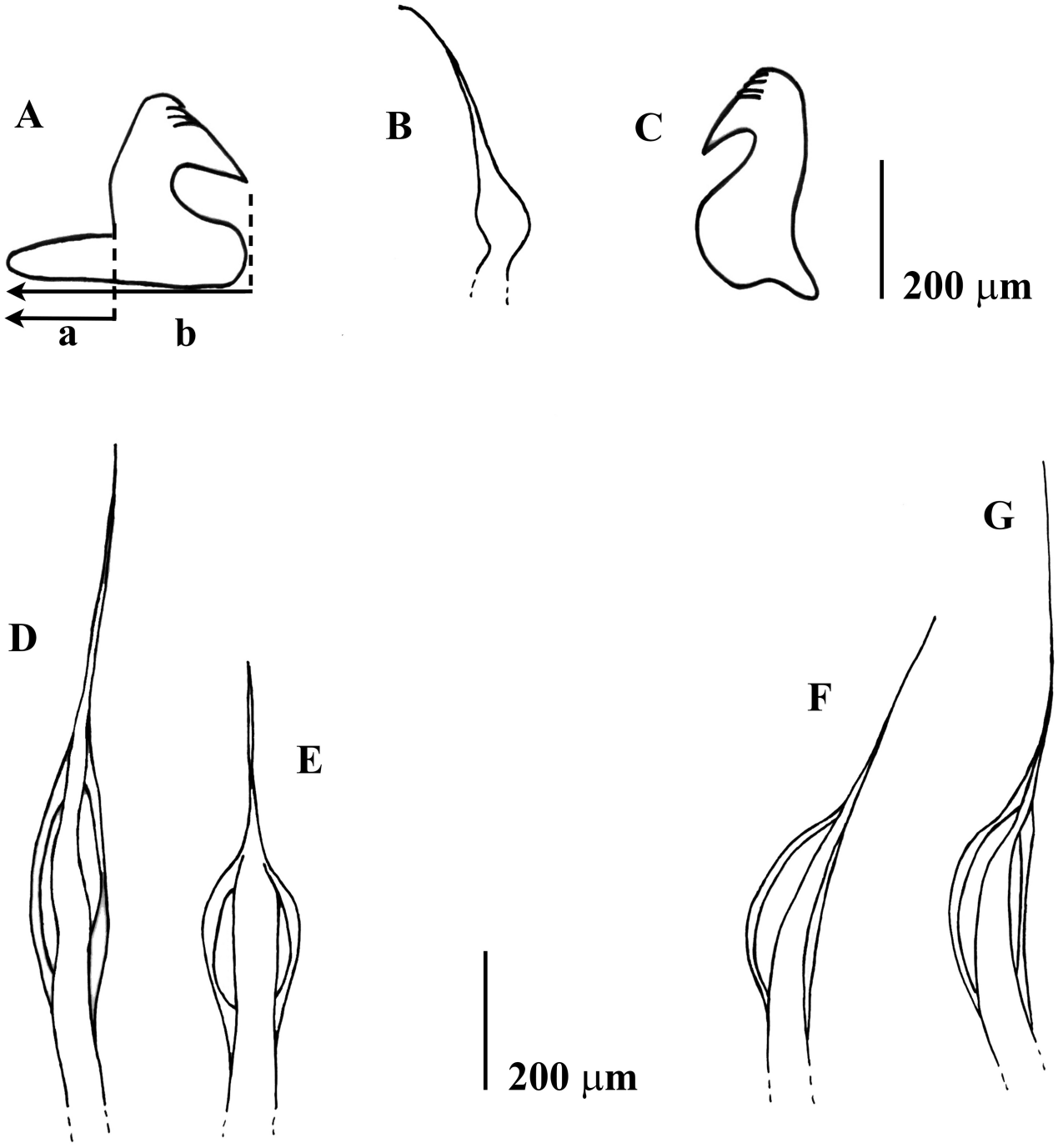

FIGURE 3. *Amphiglena* cf *mediterranea* **A**. thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from a first abdominal segment; **G.** abdominal chaeta from a middle abdominal segment.

The taxon here re-described and collected from Ischia S. Pietro is the most similar and well corresponding to the re-description of *A. mediterranea* by Rouse & Gambi (1997), for material collected in Ischia in 1994 (Castello Islet), on hard substrate with *Cladophora* sp. and *Halopteris scoparia* algal cover, at 1-2 m depth. It corresponds well with regards to the shape and length of handles of thoracic uncini, and in the number of radioles and compact appearance (Fig. 2A), even though the small basal flanges in the description of these authors were connected to the peristomial ring. Similar features are also present in specimens from Portofino having a crown/body ratio of 0.3. By contrast, specimens from Leghorn examined during this study are slightly smaller and with a crown/body ratio of 0.49 (Table 1). In the specimens examined from all these sites located along the Tyrrhenian coast, also the ventral

peristomial ring is similar to that described in the material of Rouse & Gambi (1997), even if in the material from San Pietro (Ischia) the peristomial ring appears ventrally more opened and rounded (Fig. 2F) compared to that of material from Leghorn. Therefore, all these forms showed a very similar external appearance including the staining pattern with the abdomen fully coloured (Fig. 2B, C), this latter feature was not provided in the description by Rouse & Gambi (1997).

Amphiglena aenariensis **sp. nov.**

(Figs 4, 5)

Material examined. Holotype: (MNCN 16.01/18721): Italy: Ischia Castello Islet 25.11.2011 station S3 (south side acidified station of the CO_2 vent system), 40°43'51.99"N 13°57'47.63"E; 2 m depth.

Paratypes: MNCN 16.01/ 18722: 6 specimens from the same locality and date as the holotype: 16 specimens all collected in the same locality and date as the holotype; PCZL S.A.2.1.: 22 specimens from the same site, 19.6.2012, in station N3 (north side of the CO_2 vent's system, 1.5 m depth), 40°43'54.98"N 13°57'48.34"E PCZL S.A.2.2. Material fixed in formalin 4% and preserved in ethanol 70%; some material fixed in ethanol 95%.

Description. Holotype complete, with eight thoracic and 24 abdominal chaetigers. Body length 2.3 mm and crown length 1.5 mm; width 0.4 mm. Body brown coloured on ventral side, quite flattened abdomen (Fig. 4A). Crown bearing five pairs of radioles with 12 pairs of pinnules arranged in two longitudinal rows slightly alternating along the radiolar length. Pinnules appear slightly swollen and with a similar length shorter than the radiolar tip, measuring about 1/5 of the total radiolar length (Fig. 4C). Most distal pairs of radioles having pinnules decreasing in length with longer pinnules in median position along the radiole. The radiole's distal end is 1/4 of the total radiolar length and appears elongate, slender but with a blunt, slightly swollen finger-like end. Radiolar skeleton with two rows of cells. Dorsal lips short, almost 1/7 of the total radiolar length. Anterior peristomial ring not visible, posterior peristomial ring slightly higher ventrally and showing a mid-ventral incision with well separated margins where the ventral basal flanges are fused. Ventral basal flanges low, connected as prominent ridges from the base of ventral-most radioles. (Fig. 4D, E). Peristomial eyes slight brown and difficult to see. Pygidial eyes brown as double clusters on lateral margins of pygidium. Thorax longer than wide. First thoracic chaetiger bearing only 3 chaetae similar in shape to superior chaetae of the following chaetigers. From the second to the eighth thoracic chaetiger, 6 uncini in each torus, with rounded and flattened breast, with approximately four rows of long teeth above main fang, and short handles (0.23 ratio) (Fig. 5A). Companion chaetae present, with straight shaft and long mucro (Fig. 5B). Four thoracic chaetae, one superior thoracic hooded chaeta (Fig. 5D), 3 inferior paleate chaetae, with mucro longer half of the tip of the superior chaetae (Fig. 5E, F). Six abdominal uncini on each torus with 3 teeth of similar-size and short handle (Fig. 5C). First abdominal chaetigers bearing three broadly-hooded neurochaetae similar in shape to the inferior thoracic paleate chaetae, but longer (Fig. 5G), and becoming longer and more geniculate in the median abdominal segments (Fig. 5F). Spermathechae brown/red coloured.

Staining pattern. In both thorax and abdomen stain only ventral shields. Peristomial rings and first thorax segment well coloured. Abdomen paler, showing a double square design in each chaetiger (Fig. 4B).

Variability. Individuals can have up to 29 abdominal chaetigers, up to 8 thoracic uncini and 6 abdominal ones (Table 1). Some specimens were observed to have 5 radioles in one lobe and 6 in the other, and in some specimens the presence of a bifid radiole was also detected. Spermathechae were very pale in the holotype, and darker in some paratypes.

Remarks. This species is distinguished from the specimens here reported as *A.* cf. *mediterranea* in the staining pattern, especially in the abdomen, and in the more connected ventral basal flanges. Moreover, specimens appeared more compact and shorter with a flattened abdomen and a higher body/crown ratio (Table 1). In addition, specimens belonging to this taxon are characterized by having a very short-handle of thoracic uncini, the shortest observed in all the taxa here reported. The features of the peristomial rings of *A. aenariensis* **sp. nov.** resemble *A. jimenezi* Capa & Lopez, 2004, described from Panama, from which it is distinguished by the length of the radiolar appendage, but especially for the short-handles of thoracic uncini. The short-handle of thoracic uncini distinguishes this species from most of the other known species of *Amphiglena.* Among the non Mediterranean species, only *A. nishi* Capa & Rouse, 2007 has a similar handle length, however, it differs from *A. aenariae* **sp. nov.** in the higher number of thoracic uncini, in the relative length of the crown and in the higher number of radioles having shorter pinnules,

moreover *A. nishi* has more developed ventral basal flanges. Lastly, thoracic uncini of *A. aenariensis* are the largest in the genus when compared to the worm size, together with very long abdominal neurochaetae.

Etymology. Named from the collection site which is part of the Bay of Cartaromana, where the ancient Roman submerged town Aenaria, was discovered. Aenaria is also the name used to designate the Island of Ischia during the ancient Roman colonization.

Distribution and Ecology. This species is one of the most abundant polychaetes in the CO_2 vents at the Castello (Ricevuto *et al.* 2014; Gambi *et al.* 2016). The collection site, the Castello Aragonese at Ischia, is a rocky islet, the remains of a volcanic structure that, along its south and north sides, is characterized by gas emissions from the seafloor, composed by more than 95% by CO_2. This CO_2 surplus causes a natural acidification of the surrounding waters (down to < 6.4 pH units in the most intense bubbling on the southern side) (Kroeker *et al*. 2011; Hofmann *et al.* 2011). However, both salinity and temperature remain similar to the ambient waters, and the site is therefore intensively used as a natural laboratory to study the effect of ocean acidification for the benthic biota since 2006 (Hall-Spencer *et al.* 2008, see Foo *et al*. 2018 for a review of studies at this site).

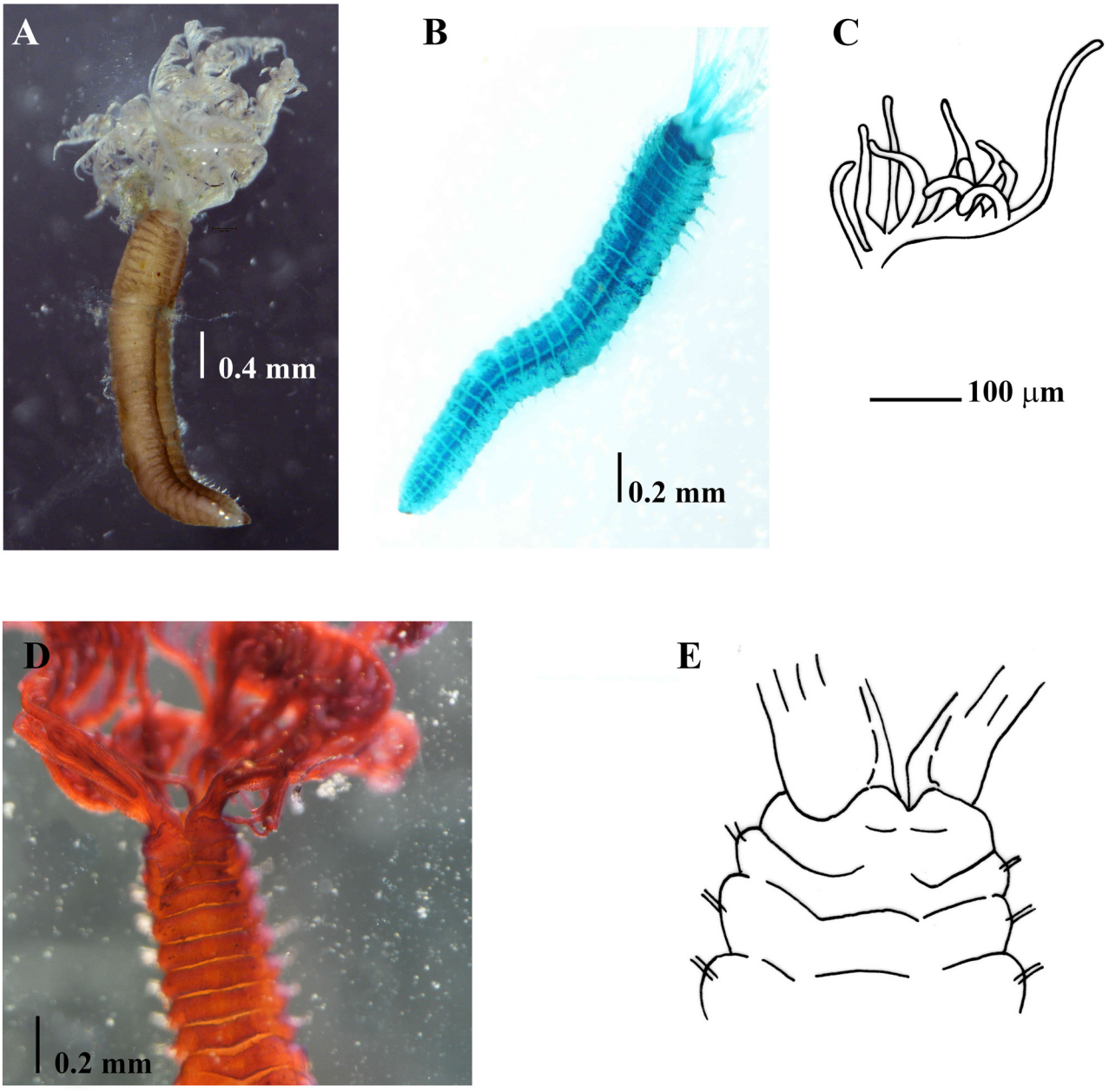

FIGURE 4. *Amphiglena aenariensis* **sp**. **nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

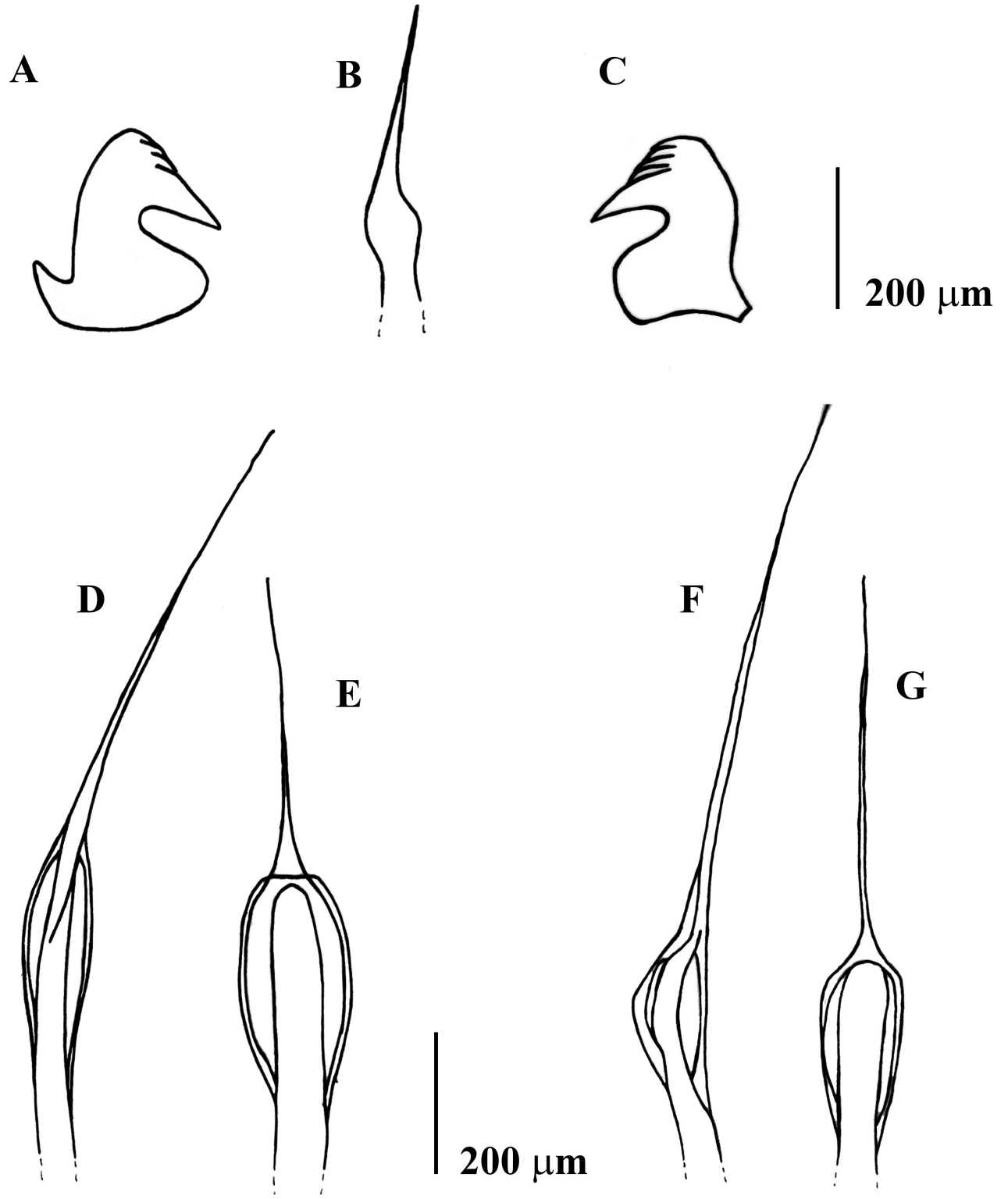

FIGURE 5. *Amphiglena aenariensis* **sp**. **nov. A**. thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from a middle abdominal segment; **G.** abdominal chaeta from the anterior abdominal segment.

***Amphiglena pithecusensis* sp. nov.**
(Figs 6, 7)

Material examined. Holotype (MNCN 16.01/18901): Italy: Lacco Ameno (Ischia), 40°45'24.38"N 13°53'6.26"E, June 2012, 2–3 m depth, on hard bottoms covered by macroalgae, mainly the brown alga *Halopteris scoparia*.

Paratypes: MNCN 16.01/18902: 5 specimens from the same locality and date as the holotype,13 specimens from the same site of the holotype; PCZL S.A. 3.1.; 5 specimens from S. Anna rocks (Ischia-Cartaromana Bay), 40°43'34.58"N 13°57'35.98"E, 26 August 2014. PCZL S.A. 3.2. Most material fixed in formalin 4% (including holotype and paratypes) and preserved in 70% ethanol, some material fixed in ethanol 95%.

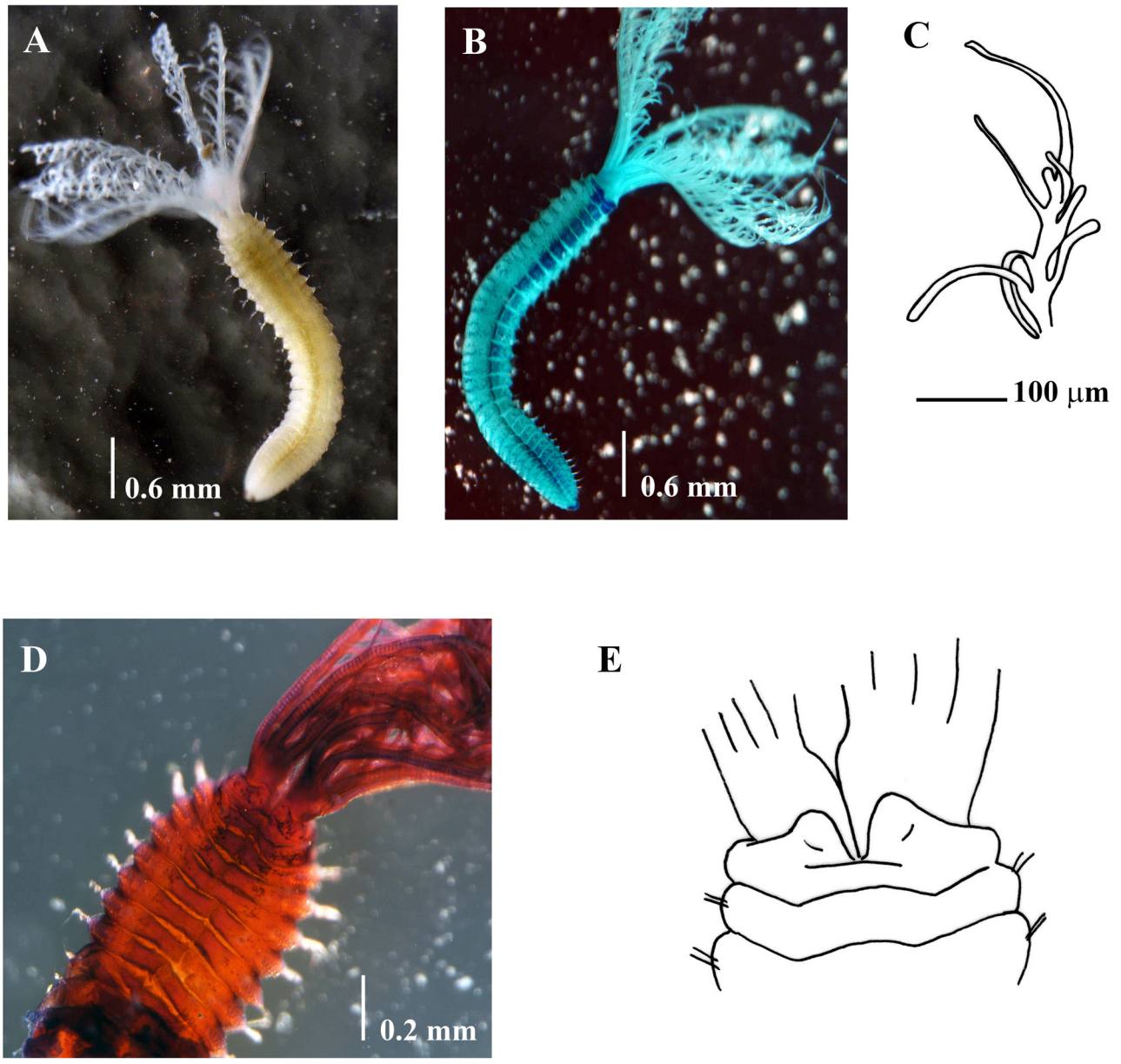

FIGURE 6. *Amphiglena pithecusensis* **sp. nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

Description. Holotype complete, with eight thoracic and 24 abdominal chaetigers. Body length 2.5 mm (branchial crown 1.5 mm); width 0.4 mm (Table 1). Body light brown coloured on ventral side and with a flattened abdomen (Fig. 6A). Crown with five pairs of radioles with 12 pairs of pinnules arranged in two longitudinal rows slightly alternating along the radiolar length. Gap between pinnules pairs decreasing along the radiole, with the first basal pair appearing slightly more separated from the others. Pinnules all of similar length, measuring about 1/4 of the to-

tal radiolar length, except first basal pair and the two distal pairs slightly shorter. Tip of radioles elongated, reaching almost 1/3 of the total radiolar length, thinning toward its blunt end (Fig. 6C). Anterior peristomial ring not visible. Posterior peristomial ring higher ventrally with a well separated ventral incision and margins with an inflated appearance (Fig. 6D, E), connected to ventral basal flanges extending as prominent ridges from the peristomial ring to the base of ventral-most radiole. Peristomial eyes brown. Dorsal lips measuring 1/5 of the radiolar length. Pygidial eyes present as small clusters of red spots on lateral margins of pygidium. Thorax longer than wide. First thoracic chaetiger bearing only 2 chaetae similar in shape to superior chaetae of the following chaetigers. From the second to the eighth thoracic chaetiger, 7 uncini in each torus, with a short handle approximately 1/4 of the total uncinus length (0.31), with approximately four rows of teeth above main fang, and with rounded and flattened breast (Fig. 7A). Companion chaetae present, with straight shaft and short mucro (Fig. 7B). Two superior broadly hooded chaetae (Fig. 7D); 3 inferior paleate chaetae with short mucro as long as the paleate region (Fig. 7E). Three abdominal uncini, with similar-sized small teeth above the main fang, with short-handle and appearing higher than long (Fig. 7C). Two abdominal broadly hooded chaetae, similar, but longer, to the thoracic paleate chaetae both in anterior and median abdominal segments (Fig. 7F, G). Spermathechae visible brown/red coloured.

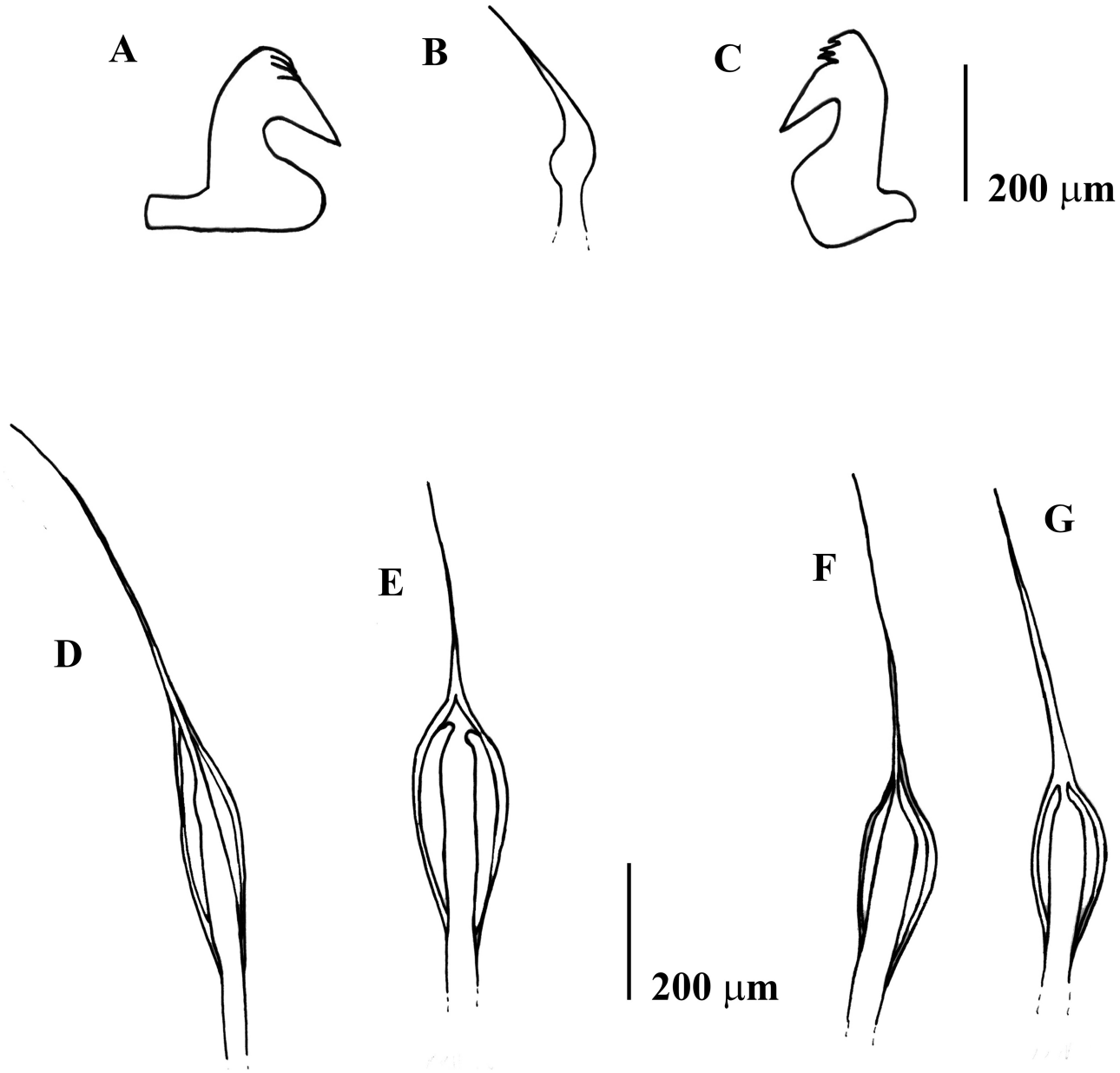

FIGURE 7. *Amphiglena pithecusensis* **sp**. **nov. A**. thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from a middle abdominal segment; **G.** abdominal chaeta from anterior abdominal segment.

Staining pattern. In both thorax and abdomen stain only the ventral shields, with a very narrow pattern especially in the abdomen remaining poorly stained (Fig. 6B).

Variation. Individuals with 8 thoracic chaetigers and up to 24 abdominal segments. Mean body length of 2.1 mm and mean crown length of 1.3 mm (Table 1). Crown asymmetrical in some specimens composed of 11 radioles (5 pairs +1).

Remarks. Specimens similar in external morphology to *A. aenariensis* **sp. nov.** previously described, but distinguished from that species by having slightly longer handles of the thoracic uncini, although not as long as in *A*. cf *mediterranea*. Moreover, hooks are proportionately smaller. Other differences are in a different ratio between body and crown (Table 1), in the narrow paleate chaetae in the thorax and abdominal chaetae of the mid body chaetigers which in *A. aenariensis* appear more geniculate and longer. Differences also in the ventral shape of the posterior peristomial ring with slight inflated margin, and in the shorter dorsal lips, and the ventral shield stain in this taxon appears less intense and narrower especially in the abdomen. Finally, the space between the pinnules in the radioles appears wider than in *A. aenariensis* **sp. nov.**.

Etymology. Named from type locality. Lacco Ameno represents the first site of settlement of the ancient Greek populations (Eubei) at Ischia in the 8th century BC, who called the island Pithekoussai, or Pithecusae in ancient Latin, due to the production of the local clay pottery.

Distribution and Ecology. This species belongs to a group of similar taxa that are abundant around the coast off the Ischia Island. This species is present all around Ischia on hard bottoms with different algal cover, as well as on *Posidonia oceanica* seagrass beds under "normal pH condition", except at the San Pietro site, where a form similar to *A. mediterranea* is present.

Amphiglena nisidensis sp. nov.

(Figs 8, 9)

Material examined. Holotype (MNCN 16.01/18903): Italy: Nisida Island (Naples), 40°47'31.52"N 14° 9'47.29"E, 14 November 2011; 2 m depth. Collected on hard bottom with algal cover, mainly *Halopteris scoparia.*

Paratypes: MNCN 16.01/18904: 6 specimens from the same locality and date as the holotype; 22 specimens from the same locality and date as the holotype PCZL S.A. 4.1. Most material fixed in formalin 4% (including holotype and paratypes) and preserved in ethanol 70%; some material fixed in ethanol 95%.

Description. Holotype complete with eight thoracic and 28 abdominal chaetigers. Body length 2.5 mm, with crown 1.8 mm long, and 0.4 mm wide. Body light brown coloured on ventral side and with flattened abdomen (Fig. 8A). Crown with five pairs of radioles with 12 pairs of pinnules of medium length each, arranged in two longitudinal rows with a small space gap between the pinnules of the same pair, and with space increasing at the base of the radiole. The pinnules appear longer in median position where they measure 1/4 of the total radiolar length, and becoming considerably shorter towards the end of the radiole. Tip of radiole long, slender and pointed (Fig. 8C). Dorsal lips measuring 1/5 of the total radiolar length. Anterior peristomial ring not visible. Posterior peristomial ring higher ventrally with a mid-ventral incision where low ventral basal flanges attach, extending as prominent ridges from base of ventral-most radioles (Fig. 8D, E).

Small brown peristomial eyes present. Pygidial eyes present as clusters of red spots on lateral margins of pygidium. Thorax longer than wide. First thoracic chaetiger with only 3 chaetae similar in shape to the superior chaetae of the following chaetigers. From the second to the eighth thoracic chaetiger, 5 uncini on each torus. Thoracic uncini with rounded and flattened breast, with four rows of teeth above main fang, and with handles long approximately 1/3 of the total uncinus length (0.35) so considered between medium and short length (Fig. 9A). Companion chaetae present, with straight shaft and long mucro (Fig. 9B). Thoracic chaetae from second to eighth chaetigers, number 4, of which one superior broadly-hooded chaeta (Fig. 9D) and three paleate chaetae in inferior row with mucro long as the hood (Fig. 9E). Abdominal uncini in number of 3, with similar-sized small teeth above the main fang, higher than the thoracic one and with short handle (Fig. 9C). Three abdominal neurochaetae thin and broadly hooded, similar to paleate of the thorax but longer in the first abdominal segments (Fig. 9G) and becoming longer and more geniculate starting from mid abdomen (Fig. 9H). Spermathechae present brown/red coloured.

Staining pattern. In both thorax and abdomen stain only ventral shields. Peristomium, thorax and abdomen showing an intense colouration in the central part, but with a pattern covering most of the segment width (Fig. 8B).

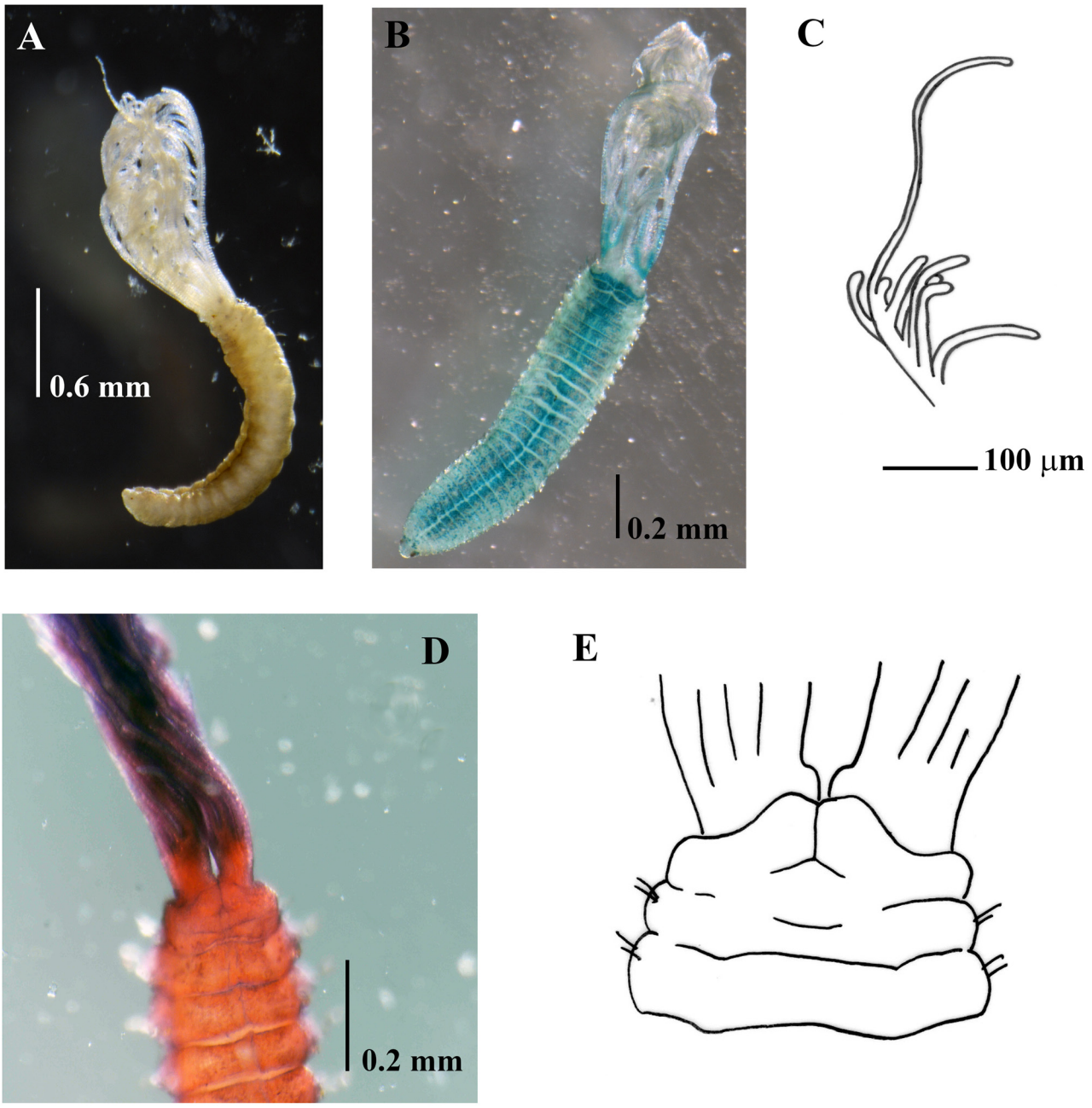

FIGURE 8. *Amphiglena nisidensis* **sp**. **nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

Variation. Individuals have eight thoracic chaetigers and up to 31 abdominal chaetigers. Mean body length of 1.65 mm and mean crown length of 1.5 mm. (Table 1).

Remarks. The material from Nisida is very similar morphologically to *A. pithecusensis* **sp. nov.** previously described. The specimens from Nisida, however, appeared highly flattened in the abdomen and with wider bands of stained tissue as wide as the thoracic segment. Moreover, specimens have a larger ratio between body and crown (Table 1), with a longer and slender tip of radioles and pinnules arranged in pairs, not alternating along the radiolar length. Thoracic uncini are also similar in the length of the handles but with a slender main fang. The main difference is, however in the shape of the ventral margin of the peristomial ring showing an incision without separated margins, and with a lower basal flange. Ventral peristomial ring appears similar to *A. bondi* Capa & Rouse, 2007, from which it is distinguished by the short handle of thoracic uncini, and by the number of radioles.

Etymology. Named from type locality, the Island of Nisida, located between the Gulf of Naples and the Gulf of Pozzuoli.

Distribution and Ecology. Distributed on hard bottoms with algal cover. This species belongs to the same group of similar taxa that includes specimens from Ischia vent and non-vent's areas, except the San Pietro site.

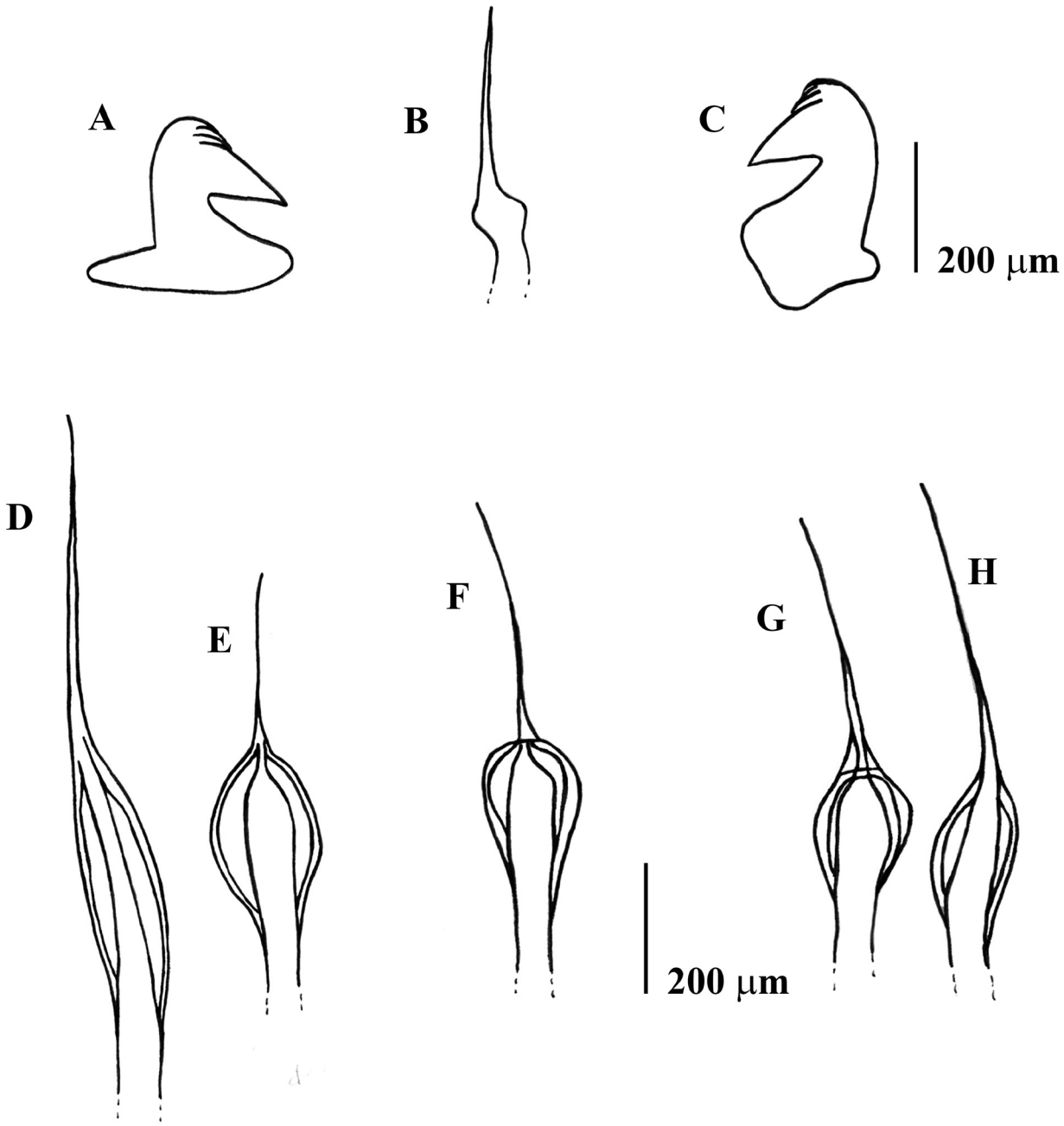

FIGURE 9. *Amphiglena nisidensis* **sp**. **nov. A**. thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from anterior abdominal segment; **G.** abdominal chaeta from a middle abdominal segment.

***Amphiglena phlegreensis* sp. nov.**
(Figs 10, 11)

Material examined. Holotype (MNCN 16.01/18905): Italy 30 November 2015, 14 m depth, Secca delle Fumose (Gulf of Pozzuoli), 40◦ 490 23 00 N 14◦ 050 500 E, among algae, mainly *Dictyota* sp.

Paratypes: MNCN 16.01/18906: 8 specimens from the same locality and date as the holotype; 21 specimens collected in the same locality and date as the holotype. PCZL S.A. 5.1. Most material fixed in ethanol 70%; some specimens in ethanol 95%.

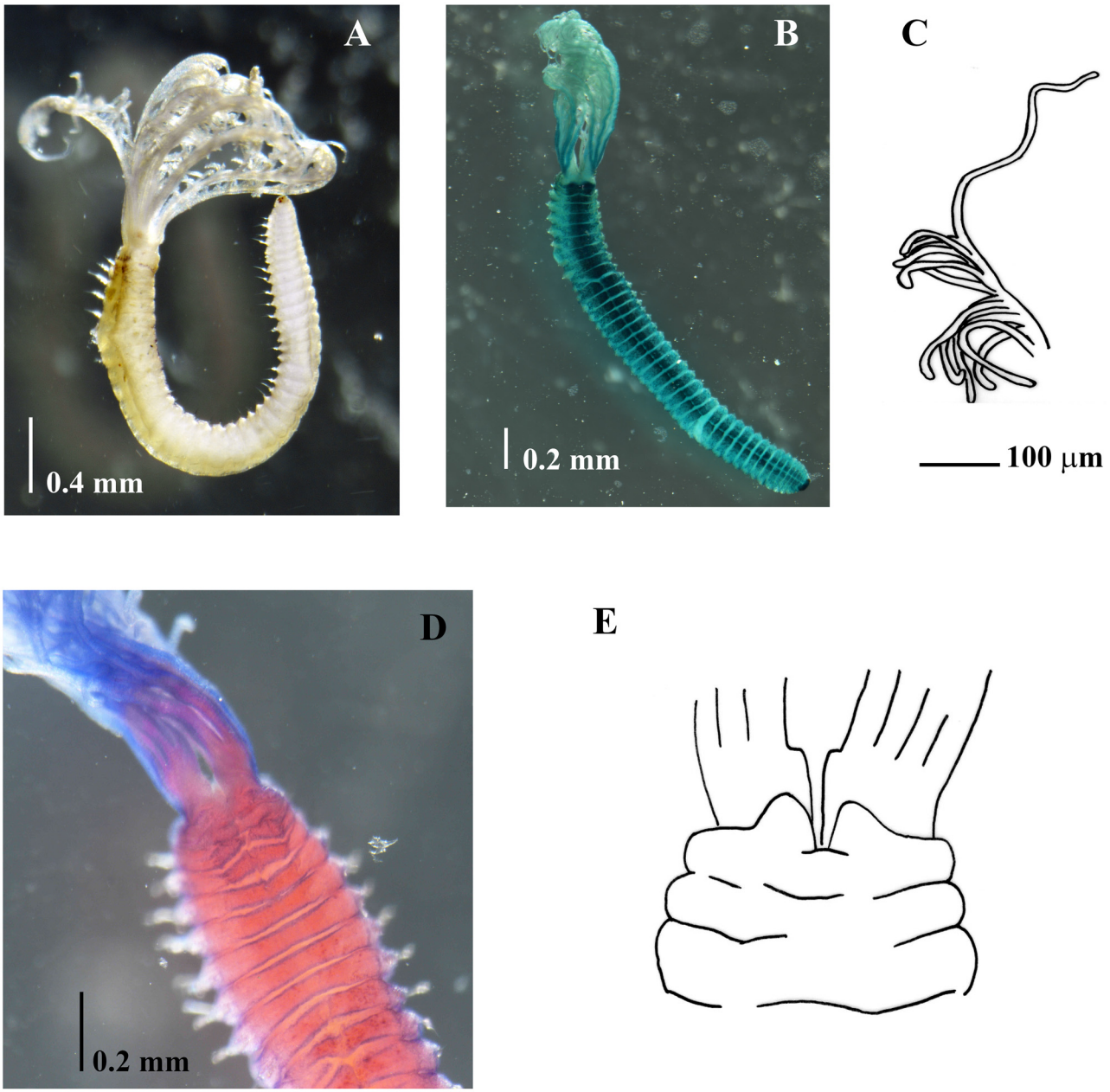

FIGURE 10. *Amphiglena phlegreensis* **sp. nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

Description. Holotype complete, with eight thoracic and about 32 abdominal chaetigers. Body length 3 mm, branchial crown 2 mm; maximum body width 0.4 mm (Fig. 10A). Brown colouration present especially in the thorax. Crown with five pairs of radioles each with 17 pairs of long pinnules arranged in two alternating rows along the radiole, especially in the distal half of radiole. Pinnule show a similar length (around 1/6 of the total radiolar length) and are slightly shorter in the last two distal pairs and in the first two basal pairs which appear more separated from the other pairs. Tip of radioles extremely elongated reaching 1/3 of the total radiolar length, slender but with a blunt end (Fig. 10C). Radiolar skeleton with two rows of cells. Anterior peristomial ring not visible. Posterior peristomial ring low and higher ventrally with a well separated ventral incision connected to well-developed ventral basal flanges, extending as a prominent ridge from the base of ventral-most radioles (Fig. 10D, E). Peristomial eyes brown red. Dorsal lips with a rounded dorsal radiolar appendages measuring 1/4 of radiolar length. Pygidial eyes as brown clusters on lateral margins of pygidium. Thorax longer than wide. The first thoracic chaetiger bearing only two chaetae similar to the superior chaetae of the following chaetigers. From the second to the eighth thoracic chaetiger, 5 uncini in each torus. Thoracic uncini with well-developed breast, large as the distance to main fang,

with approximately four rows of similar teeth above main fang, and a handle measuring approximately 1/3 of the total uncinus length, and considered as medium-short handle (0.35) (Fig. 11A). Companion chaetae present, with straight shaft and long mucro (Fig. 11B). Second to eighth thoracic chaetigers with 5 thoracic chaetae of which two superior broadly hooded chaetae (Fig. 10D) and three inferior paleate chaetae with a mucro as long as the hood (Fig. 10E). Abdominal uncini, in number of 4 on each torus, with 3-4 similar-sized small teeth above main fang and short handle, appearing higher than longer (Fig. 11C). Three broadly-hooded abdominal neurochaetae, very similar to the thoracic paleate chaetae in the first abdominal segments (Fig. 11G) and becoming narrower and more geniculate in the median abdominal segments (Fig. 11F). Spermathechae whitish.

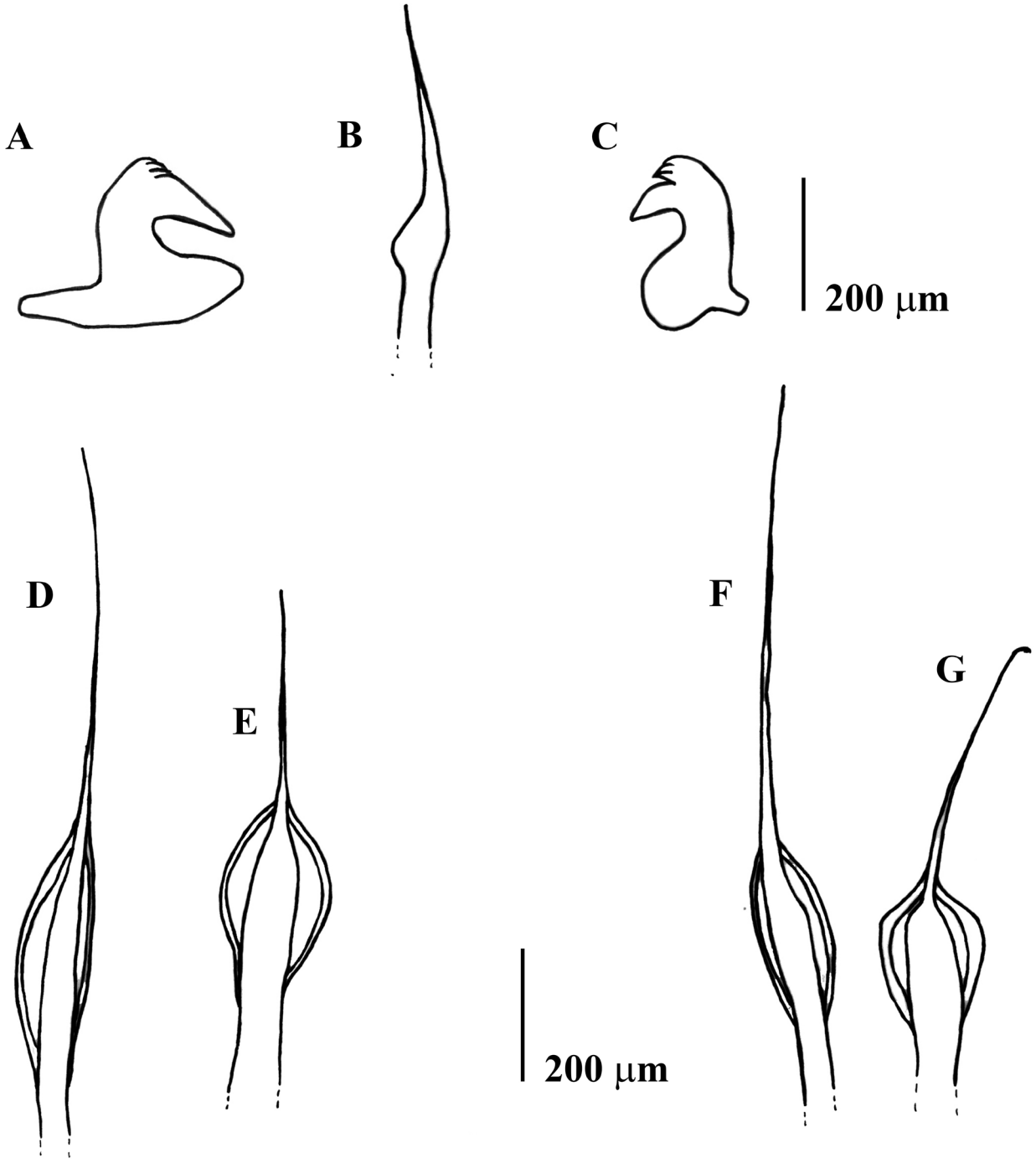

FIGURE 11. *Amphiglena phlegreensis* **sp. nov. A.** thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from a middle abdominal segment; **G.** abdominal chaeta anterior abdominal segment.

Staining pattern. In both thorax and abdomen stain only ventral shields. Peristomium, thorax and abdomen intensely coloured, with larger rectangular pattern in the abdominal segments (Fig. 10B).

Variation. Individuals with 8 thoracic chaetigers and up to 32 abdominal chaetigers. Mean body length of 2.2 mm and mean crown length of 1.4 mm (Table 1).

Remarks. Excluding *A.* cf. *mediterranea,* this taxon is very similar to the group of species here described from the Gulf of Naples, however it has a greater number of pinnules on the radioles. The peristomial ring is more similar to *A. pithecusensis* **sp. nov.**, but thoracic uncini are more similar to *A. nisidensis* **sp. nov.**, from which it differs in having longer handles. Additionally, it has very long abdominal chaetae, similarly to *A. aenariensis* **sp. nov**.

Etymology. Named from type locality, since the Secca delle Fumose is within the Gulf of Pozzuoli, part of the Phlegrean Fields, a large and still active volcanic area in the Gulf of Pozzuoli (Naples). The name derives from the ancient Greek word *phlego*=to burn.

Distribution and Ecology. The species is known only from this location (Secca delle Fumose). This site is a rocky area which is formed by rocky columnar structures (named *pilae*) representing the columns of the ancient Roma harbour of Baia, submerged due to the tectonic subsidence of the area (bradeysism). This site is a hydrothermal vent system, where CO_2, sulphur (H_2S) emissions, and also hot water fluids, emerge from the bottom. A description of the area and its benthic community was provided by Donnarumma *et al*. (2019).

Amphiglena messapica sp. nov.

(Figs 12, 13)

Material examined. Holotype (MNCN 16.01/18907): Italy: Santa Caterina-Torre Inserraglio (Apulian Coast, Ionian Sea), 40° 8’22.13”N 17°58’47.31”E, collected in 2002 from hard bottom at 1 m depth, covered by *Halopteris* sp. and *Dictyota* sp.

Paratypes: MNCN 16.01/18908: 7 specimens from the same locality and date as the holotype; 118 specimens all collected in the same locality and date as the holotype PCZL S.A. 6.1. Most material fixed in formalin 4% (including holotype and paratypes) and preserved in ethanol 70%; some material fixed in ethanol 95%.

Description. Holotype complete, with eight thoracic and 28 abdominal chaetigers. Body length 3 mm, branchial crown 1 mm; maximum body width 0.35 mm (Fig. 12A). Crown with five pairs of radioles with 11 pairs of pinnules arranged in two longitudinal rows not alternating along the radiolar length. Gap between pinnule pairs constant along the radiole. Pinnules quite short and with a similar length (1/4 of the total radiolar length), being shorter only in the first two basal pairs and in the last two distal pairs. Radiolar tip reaching between 1/3 and 1/4 of the total radiolar length and with a blunt tip (Fig. 12C). Radiolar skeleton with two rows of cells. Dorsal radiolar appendages long 1/3 of total radiolar length. Anterior peristomial ring not visible. Posterior peristomial ring short but oblique, appearing as having pointed margin (Fig. 12D, E). Ventral basal flanges high extending as prominent ridge from base of the ventralmost radioles across the anterior peristomial ring and highly connected with the peristomial ring (Fig. 12D, E). Peristomial eyes not visible. Pygidial eyes brown spots on lateral margins of pygidium. Thorax longer than wide. The first thoracic chaetiger bearing only 3 chaetae similar in shape to the superior thoracic chaetae. From the second to the eighth thoracic chaetiger, 6 uncini on each torus, with well-developed breast, large distance to main fang, and approximately four rows of long teeth above main fang, and medium handles, measuring more than 1/3 of the total uncinus length (0.43) so that the uncinus appears longer than higher (Fig. 13A). Companion chaetae present, with straight shaft and long mucro (Fig. 13B). From the second to the eighth thoracic chaetiger, 2 superior broadly hooded chaetae (Fig. 13D) and 4 paleate chaetae in inferior group with mucro long as the hood (Fig. 13E). Four abdominal uncini with similar-sized small teeth above main fang, longer than thoracic ones and with short handle (Fig. 13C). Spermathechae not visible. Up to 3 abdominal hooded neurochaetae with hood similar to the inferior chaetae in the first abdominal segments (Fig. 13F) becoming longer and more geniculate in the median abdominal segments (Fig. 13G).

Staining pattern. In both thorax and abdomen stain only ventral shields with a large rectangular pattern in the thorax, and a double clear squared pattern on each abdominal segment (Fig. 12B).

Variation. Individuals always with eight thoracic chaetigers and up to 28 abdominal chaetigers. Mean body length of 2.3 mm and mean crown length of 0.9 mm (Table 1).

Remarks. Although being a compact form like the previous described species, this taxon is larger and with a

longer appearance, especially in the abdominal chaetigers. The handles of the thoracic uncini, although having a similar length as *A.* cf. *mediterranea*, appear more pointed. *Amphiglena messapica* **sp. nov.** differs from this taxon especially in the shape of the peristomial ring which is ventrally higher and with more developed basal flanges, connected to the peristomial ring, so that it appears as pointed. The staining pattern is similar to *A. aenariensis* **sp. nov.**, and to all the related taxa of the group from the Gulf of Naples. This species is distinguished from this group of taxa for the length of the handles of the thoracic uncini, and for the body/crown ratio, this last feature being similar to *A.* cf. *mediterranea* (Table 1). Among the non Mediterranean taxa, *A. gracilis* Capa & Rouse (2007) has the most similar ventral peristomial appearance, however, this species does not have the peristomial edge so connected to the ventral basal flanges, moreover, *A. gracilis* is a very thin species with only four pair of radioles, has a shorter handle of thoracic uncini, and a longer dorsal radiolar appendages. Lastly *A. messapica* **sp. nov**. is among the few Mediterranean species not showing an alternate arrangement of the pinnnule rows along the radiole.

Etymology. The species was named from the "Messapi", an ancient tribe inhabiting in pre-Roman time the Southern Apulia (Salento), where the species was collected.

Distribution and ecology. The species is distributed on shallow and sheltered hard shallow substrates.

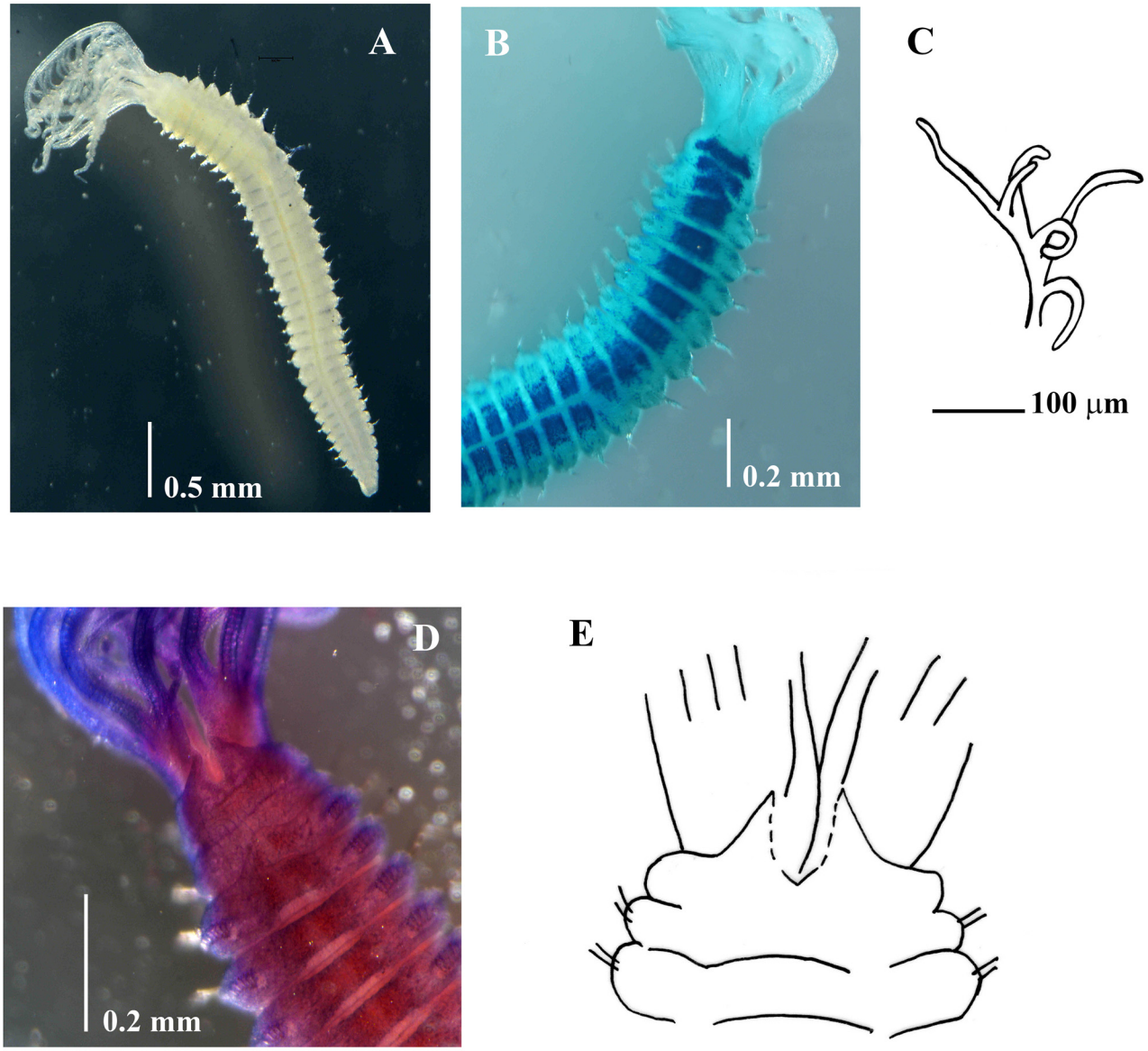

FIGURE 12. *Amphiglena messapica* **sp. nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

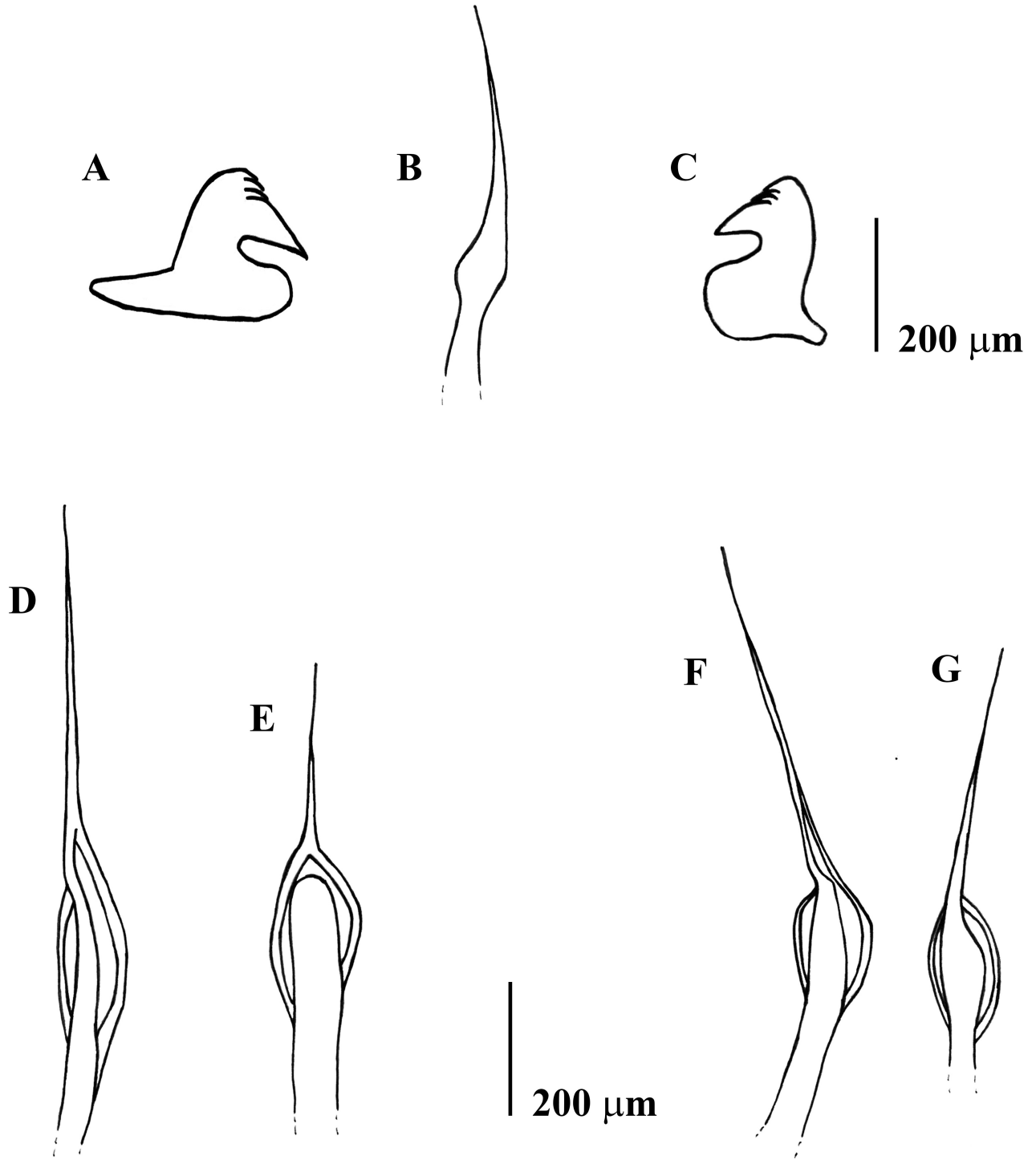

FIGURE 13. *Amphiglena messapica* **sp. nov. A.** thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from a middle abdominal segment; **G.** abdominal chaeta from anterior abdominal segment.

***Amphiglena gravinae* sp. nov.**
(Figs 14, 15)

Material examined. Holotype: (MNCN 16.01/18909): Italy: Adriatic Sea, Brindisi, 40°38'53.39"N 18° 0'42.01"E, in 1985, 5 m depth, on corallinae algae.

Paratypes: MNCN 16.01/18910: 5 specimens from the same locality and date as the holotype; 296 specimens, all collected in the same locality and date as the holotype. PCZL S.A. 7.1. Material fixed in formalin 4% and preserved in ethanol 70%.

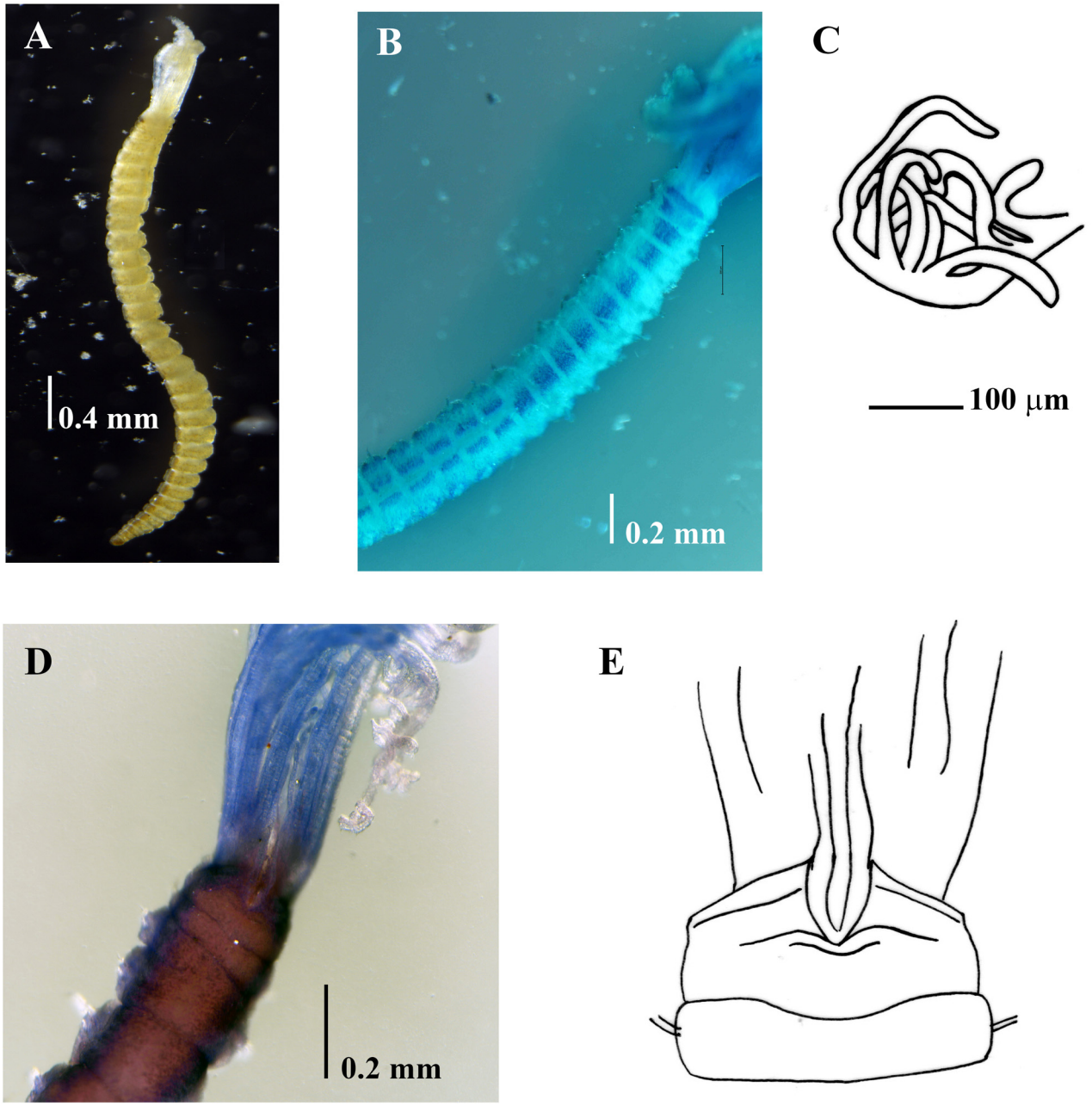

FIGURE 14. *Amphiglena gravinae* **sp. nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole**;** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

Description. Holotype complete with 8 thoracic and 22 abdominal chaetigers. Total body length 4 mm, crown 0.6 mm; maximum body width 0.3 mm (Fig. 14A). Crown holding five pairs of radioles with 11 pairs of short pinnules arranged in two alternating rows of similar length (between 1/5 and 1/4 of the total radiolar length. The gap between pairs remains quite constant along the radiole. First basal pair and last distal pair with pinnules shorter than that in the medial radioles. Elongated radiolar tip, reaching more than 1/3 of the total radiolar length and of uniform width, and with a blunt tip (Fig. 14C). Radiolar skeleton with two rows of cells. Dorsal lips with long and pointed dorsal radiolar appendages (1/3 of crown length). Anterior peristomial ring with similar height all around, visible also on ventral side. Posterior peristomial ring elongate and connected to high ventral basal flanges, which extends as prominent ridge from base of ventralmost radioles (Fig. 15D, E). Peristomial eyes not visible, pygidial eyes present, as very small spots on lateral margins of pygidium. Thorax longer than wide. The first thoracic chaetiger bear-

ing only 3 chaetae similar in shape to the thoracic superior chaetae. From the second to the eighth thoracic chaetiger, 6 uncini on each torus with well-developed breast, and approximately four rows of similar teeth above main fang, large space to main fang, handle elongated, substantially longer than 1/3 of the total uncinus length and considered long handles (0.52) (Fig. 15A). Companion chaetae present, with straight shaft and long mucro (Fig. 15B). Second to eighth thoracic chaetigers with 4 chaetae, one superior broadly hooded chaeta (Fig. 15D), and three inferior paleate chaetae with a short mucro, long slightly less than the hood (Fig. 15E). Four abdominal uncini on each torus with similar-sized small teeth above the main fang and medium handle (Fig. 15C). Four abdominal broadly hooded neurochaetae, but with a very large hood similar to the thoracic paleate chaetae, even if longer. The shape of the chaetae is similar along the abdominal segments even if in the last segments they become longer and with a more geniculate appearance (Fig. 15G, F). Pair of light spermathechae present at the base of dorsal lips.

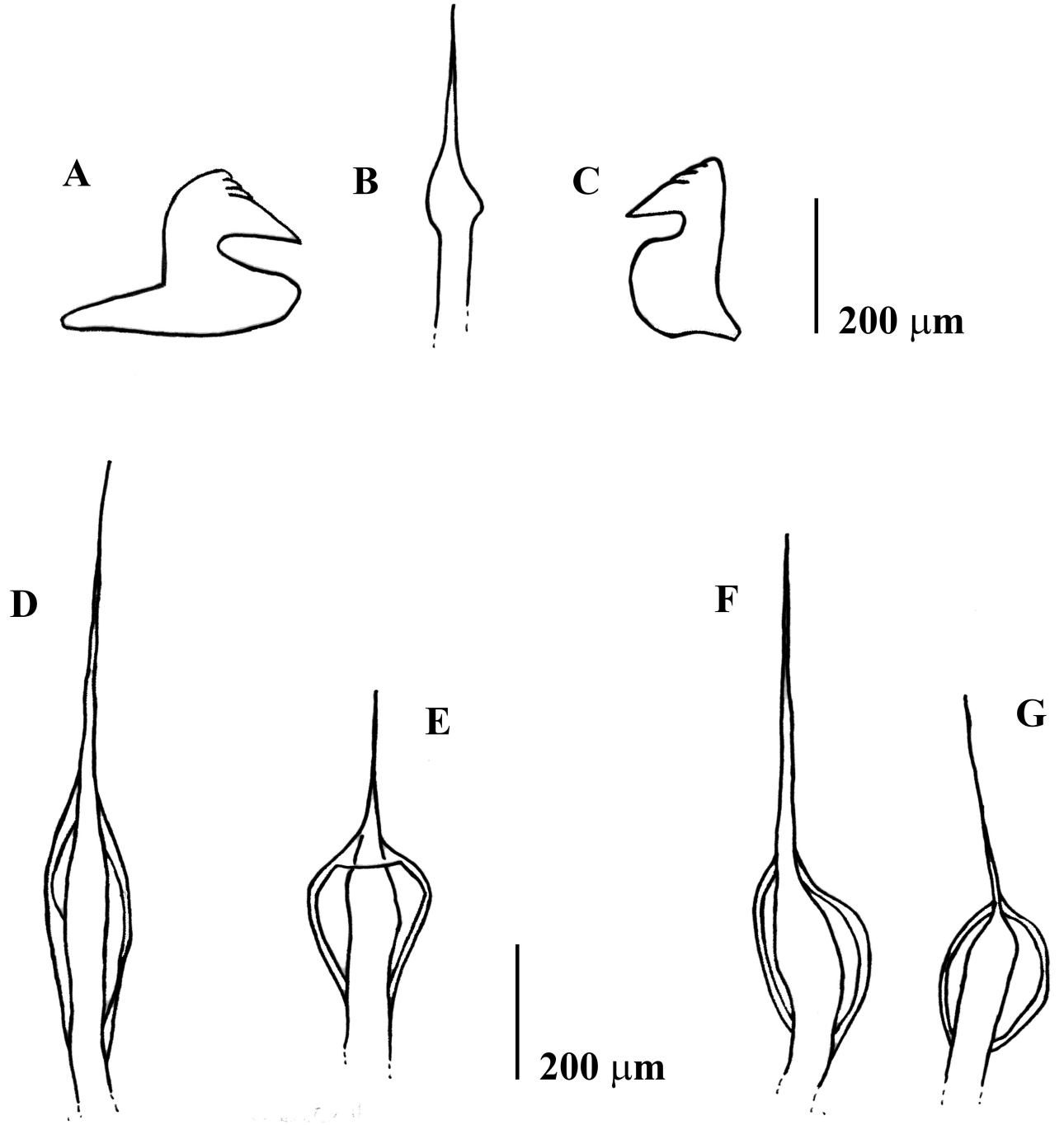

FIGURE 15. *Amphiglena gravinae* **sp. nov. A.** thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from a middle abdominal segment; **G.** abdominal chaeta from anterior abdominal segment.

Staining pattern. In both thorax and abdomen stain only the ventral shields, with a pale colouration, abdomen with a double rectangle in each segment (Fig. 14B).

Variation. Individuals always with 8 thoracic chaetigers and up to 30 abdominal chaetigers. Up to 7 thoracic uncini. Mean body length of 2.6 mm and mean crown length of 0.9 mm (Table 1).

Remarks. This new taxon has a more elongate appearance than other species in the genus, including the peristomial rings, and the base of the crown appearing sometimes bearing a high basal web, and making difficult to see the separation between the peristomial ring and the base of the crown. In this feature it is very similar to *A. panareensis* **sp. nov**., which is the other elongate Mediterranean species, described in the present paper. The two species, however show a different development of both first and second peristomial rings. Among the non Mediterranean species, high developed ventral flanges are present also in *A. lenae* Capa & Rouse, 2007, but this is a thin and more compact species, with smaller radiolar appendages.

The staining patter is similar to *A. messapica* **sp. nov.**, even if it appears narrower and more elongated following the elongated shape of the segments. It is distinguished from this species by the shape of the peristomial rings and for the abdominal chaetae, maintaining a paleate appearance long all the abdomen. This feature is present also in *A. nisidensis* **sp. nov.**, and in *A. vulcanoensis* **sp. nov**. where, however, chaetae are less broadly-hooded.

Lastly, *A. gravinae* has the thoracic uncini with the longest handle compared to all the Mediterranean species here described, although very similar in shape to *A. messapica* **sp. nov**.

Etymology. The species is named after the Dr. Maria Flavia Gravina who collected the material, in honor of her valuable contribution to the knowledge of the taxonomy and ecology of polychaetes, as well as for our estimation, long-lasting friendship and collaboration.

Distribution and Ecology. The species was collected in a polluted and stressed area, characterized by the presence of high sedimentation rate, and in the vicinity of a power plant.

Amphiglena vulcanoensis sp. nov.

(Figs 16, 17)

Material examined. Holotype: (MNCN 16.01/18911): Italy: Vulcano Island-Baia di Levante vent system, 8 May 2013, 38°25'10.10"N 14°57'43.38"E; 1.5 m depth (Vizzini *et al.* 2017).

Paratypes: MNCN 16.01/18912: 3 specimens from same locality and date as the holotype; 49 specimens, all collected in the same locality and date as the holotype PCZL S.A. 8.1. Material fixed ethanol 95% (including holotype and paratypes).

Description. Holotype complete, with eight thoracic and 32 abdominal chaetigers. Body length 4.2 mm, branchial crown 1.5 mm; maximum body width 0.5 mm, abdomen largely flattened. Yellow colouration presents especially in the thorax, highlighting the mid-dorsally faecal groove, and ventral shields (Fig. 16A). Crown with 7 pairs of radioles with 16 pairs of pinnules arranged in two longitudinal rows alternating along the radiolar length. The gap between pinnule pairs remaining constant along the radiole, with only the first basal pair more separated from the successive one. The pinnules show a variable length appearing very short and with a characteristic swell on the end, not observed in other species. The tip of radioles short and blunt (Fig. 16C). Dorsal lips with very long and rounded dorsal radiolar appendages, (1/3 of radiolar length). Radiolar skeleton with two rows of cells. Anterior peristomial ring not visible. Posterior peristomial ring low with two small ventral projections with low margin, ventral basal flanges thick and extending as prominent ridge from the base of the ventral-most radioles. Base of crown distinctly thick (Fig. 16D, E). Peristomial eye not visible. Pygidial eyes present as cluster of brown spots on lateral margins of pygidium. Thorax longer than wide. First thoracic chaetiger bearing only 3 chaetae similar in shape to the superior chaetae of the other thoracic segments. From the second to the eighth thoracic chaetiger, 7 uncini in each torus, with approximately five rows of similar-sized small teeth above the main fang and well-developed breast, going beyond the main fang and with large distance above main fang. Medium-short handles long around 1/3 of the total uncinus length (0.35); the uncini are also larger in height than the other species (Fig. 17A). Companion chaetae present, with straight shaft and short mucro (Fig. 17B). Second to eighth thoracic chaetigers with 5 chaetae of which one superior chaeta broadly hooded and four inferior paleate chaetae of two different typologies, one having a mucro long as the hood, the second showing a very short mucro and a reduced length (Fig. 17D, E, H). Four abdominal uncini on each torus with 4 teeth, uncini higher than longer and with a medium handle (Fig. 17C). Four broadly hooded abdominal

neurochaetae similar to the inferior paleate chaetae both in the first and median abdominal segments, but the last becoming longer (Fig. 17F, G). Spermathechae light brown/coloured.

Staining pattern. In both thorax and abdomen stain only ventral shields, intensely coloured on the peristomial ring, and abdomen with a narrow stained string (Fig. 16B).

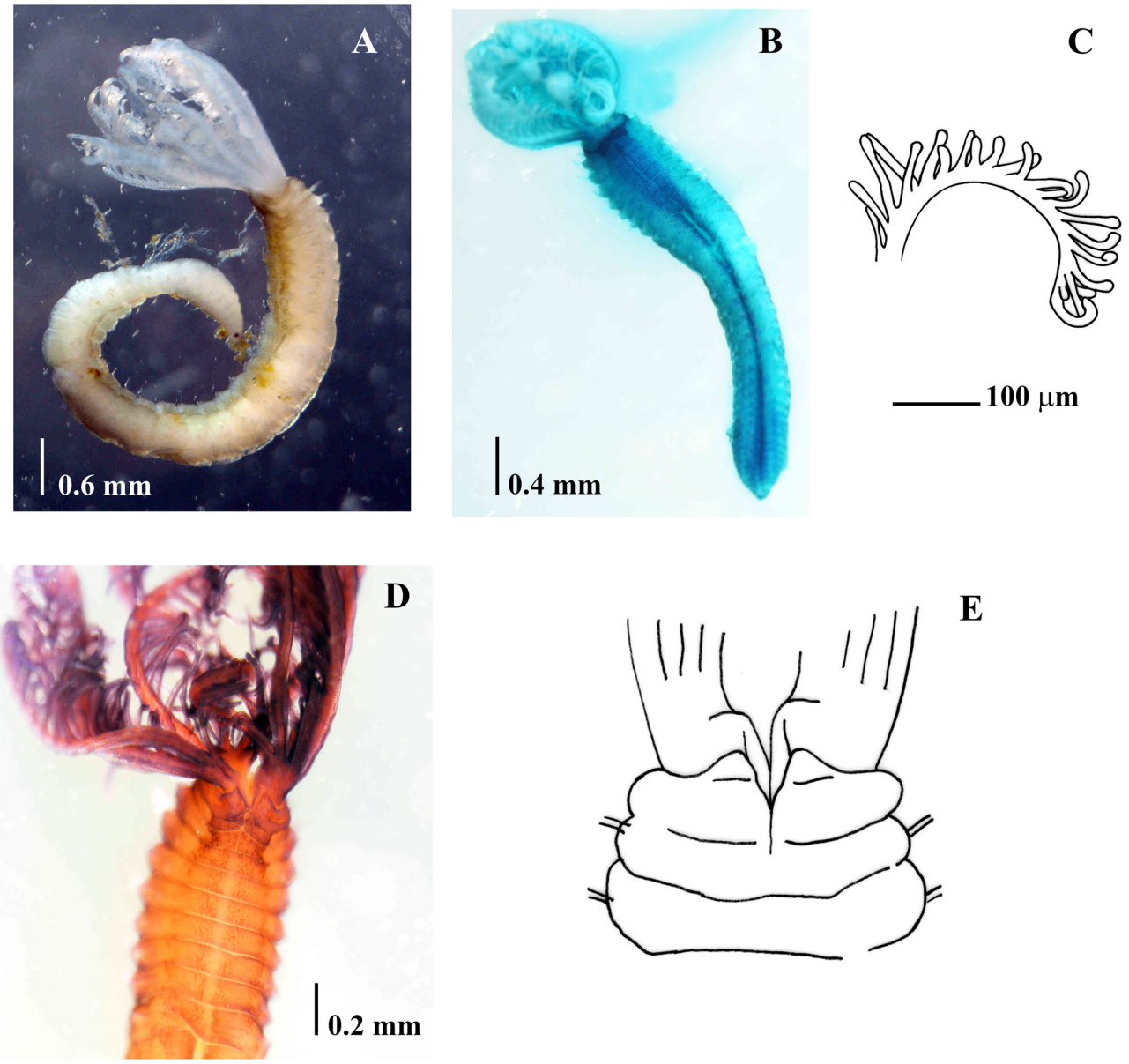

FIGURE 16. *Amphiglena vulcanoensis* **sp. nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

Variation. Individuals always with 8 thoracic chaetigers and up to 32 abdominal chaetigers. Mean body length of 3.7 mm and mean crown length of 1.3 mm. Up to 10 thoracic uncini on each torus and 7 abdominal uncini (Table 1).

Remarks. This is a species larger than the other congeneric species, with a compact appearance and with the largest number of radioles of all Mediterranean species, and non Mediterranean species. In this aspect, it resembles the Australian species *A. magna* Capa & Rouse, 2007 from which it is distinguished by the length of handles of thoracic uncini, but also by the ventral peristomial shape. This feature is similar to *A. terebro* Rouse, 1993, which is a smaller species with fewer radioles and 12 thoracic chaetigers.

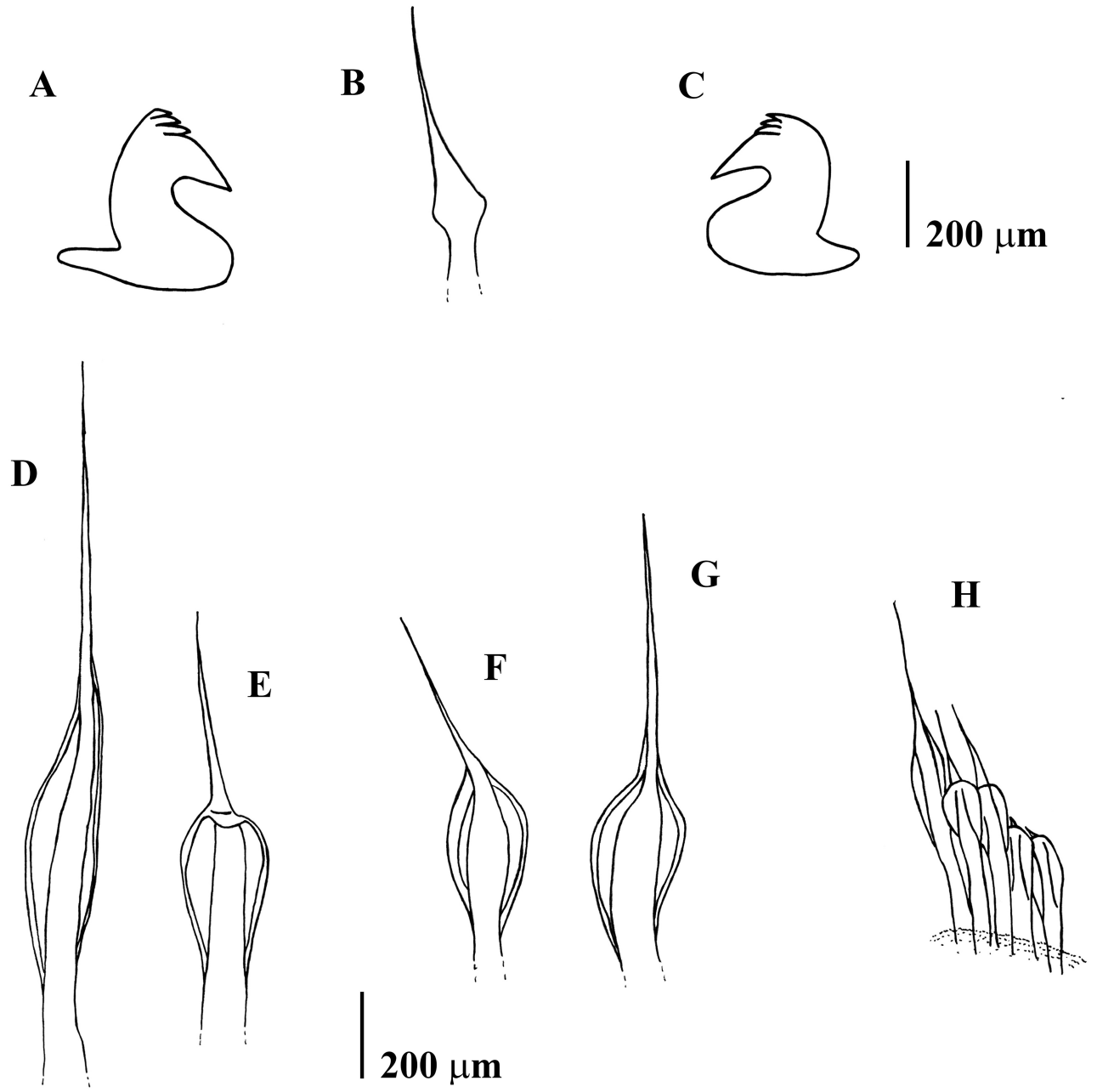

FIGURE 17. *Amphiglena vulcanoensis* **sp. nov. A.** thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from anterior abdominal segment; **G.** abdominal chaeta from a middle abdominal segment; **H.** thoracic chaetae arrangement.

Etymology. The species is named from type locality, the Island of Vulcano (Aeolian Archipelago, north Sicily).

Distribution and Ecology. The species up to date has been collected only in Baia di Levante hydrothermal vent' system of Vulcano island. This vent's system is unique since it is not only acidified due to CO_2 emissions from the primary vent source (approx. 300 m from the collection area), but has also some sulphur (H_2S) and is enriched by metal ions (see Vizzini *et al*. 2017, and references herein for a description of the system and its benthic community). *Amphiglena vulcanoensis* **sp. nov.** was collected with a few other polychaetes, among which *Platynereis* cf. *massiliensis* Moquin-Tandon, 1869 was the dominant species (Waege *et al*. 2017, Vizzini *et al*. 2017).

***Amphiglena aeoliensis* sp. nov.**
(Figs 18, 19)

Material examined. Holotype (MNCN 16.01/18913): Italy: Panarea Island (Aeolian Archipelago), near the Bottaro crater, 27 September 2016, 8 m depth; station B2, 38°38'14.49"N 15° 6'34.47"E).

Paratypes: MNCN 16.01/18914: 8 specimens from the same locality and date as the holotype; PCZL: 19 specimens B2 site, 27 September 2016; PCZLS.A. 9.1. 79 specimens B3 site, 27 September 2016; PCZL S.A. 9.2. 39 specimens B3 site, September 2018PCZL S.A. 9.3. The remaining material is in the MCG collection. Most material fixed in ethanol 70% (including the holotype and paratypes), and some in ethanol 95%.

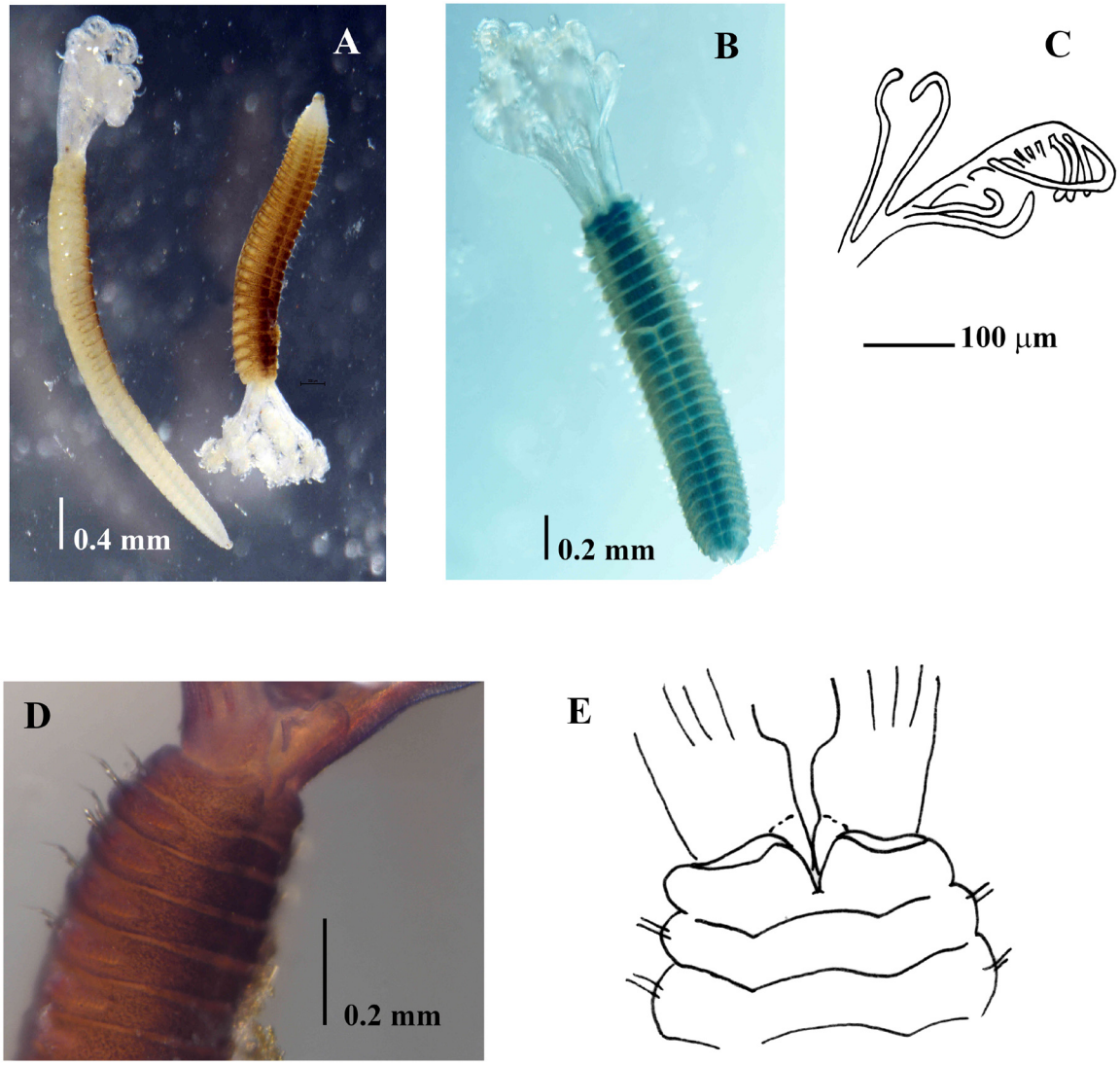

FIGURE 18. *Amphiglena aeoliensis* **sp. nov. A**. entire worms, photo from two paratypes; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view

Description. Holotype complete, with eight thoracic and 28 abdominal chaetigers. Body length 2.8 mm, branchial crown 1 mm long, maximum body width 0.43 mm. Natural dark brown colouration presents especially in the thorax and highlighting the mid-dorsal faecal groove, and ventral shields. (Fig. 18A). Crown with five pairs of radioles with 14 pairs of pinnules arranged in two longitudinal rows alternating along the radiolar length. Gap between pairs decreasing along the radiole from the base to the distal end, with the first two basal pairs more separated from the others. Pinnules slender and elongated, showing a similar length (1/4 of the total radiolar length) with the distal pairs and the first two basal pairs slightly shorter. Tip of radioles elongated as long as pinnule length (measuring 1/4 of the total radiolar length) and with a blunt end (Fig. 18C). Radiolar skeleton with two rows of cells. Dorsal lips with pointed dorsal radiolar appendages clearly shorter and wider than the pinnule, being 1/7 of the total radiolar

length. Anterior peristomial ring even in height all around and visible also ventrally. Posterior peristomial ring low. Ventral basal flanges high, extending as prominent ridge from base of ventralmost radioles, across anterior peristomial ring but appearing not connected (Fig. 18D, E). Peristomial eyes not visible. Pygidial eyes present, as clusters of brown spots on lateral margins of pygidium. Thorax longer than wide. First thoracic chaetiger bearing only 3 chaetae similar in shape to the thoracic superior chaetae. From the second to the eighth thoracic chaetiger, 6 uncini in each torus with well developed breast, large distance to main fang, with approximately four rows of long teeth above main fang, and short handles long 1/3 of the total uncinus' length (0.30) (Fig. 19A). Companion chaetae with straight shaft and short mucro (Fig. 19B). Second to eighth thoracic chaetigers with 4 chaetae, of which one superior broadly hooded chaeta (Fig. 19D) and three paleate chaetae on each thoracic chaetiger, with short mucro (Fig. 19E). Five abdominal uncini with similar-sized small teeth above the main fang, higher than thoracic ones and with short handle; the uncini are also larger in height than the other species, as in *A. vulcanoensis* **sp. nov.** (Fig. 19C). Up to 3 abdominal broadly-hooded neurochaetae, becoming narrower and with a more geniculate appearance in the last segments (Fig. 19G, F). A pair of brown/red spermathechae present at the base of dorsal lips.

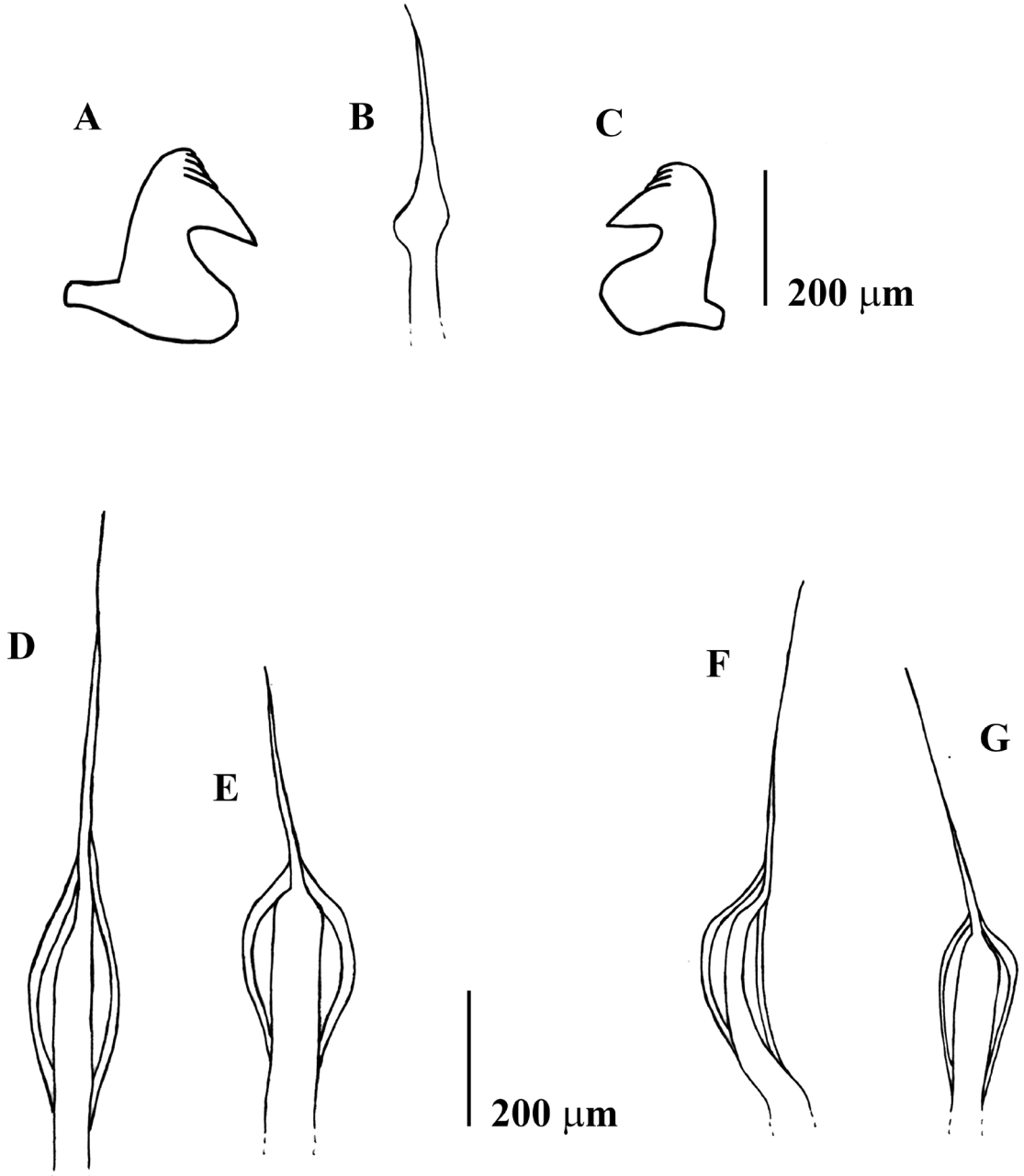

FIGURE 19. *Amphiglena aeoliensis* **sp. nov. A.** thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from a middle abdominal segment; **G.** abdominal chaeta from anterior abdominal segment.

Staining pattern. In both, thorax and abdomen stain only ventral shields overlapping the natural brown colouration. Colouration pattern wide on both thorax and abdomen following the shape of the segments, but not reaching the tori (Fig. 18B).

Variation. Individuals always with 8 thoracic chaetigers and up to 28 abdominal chaetigers. Mean body length of 2.55 mm and mean crown length of 1.08 mm. Peristomial eyes visible only in few specimens. Up to 8 thoracic uncini and 6 abdominal one (Table 1).

Remarks. The new taxon is very characteristic in its colouration, although variable in intensity among specimens, as well as for its very compact appearance. This dark brown colour pattern is quite unusual among *Amphiglena* species.

Peristomial rings and basal ventral flanges are quite similar to *A.* cf. *mediterranea*, from which it is distinguished for the first peristomial ring visible also ventrally, for the staining pattern, for a more compact appearance with wider and lower segments, but especially for the short handle of thoracic uncini. Among the non Mediterranean species, the new taxon is similar to *A. nishi* Capa & Rouse, 2007 described from Japan, especially in the shape of peristomial rings and ventral basal flanges. This species is however a smaller taxon with only 4 radioles.

Etymology. The species is named from the type locality, the Aeolian Archipelago (north Sicily) where the species was collected.

Distribution and ecology. This species is one of the most abundant taxa of the benthic community associated to the brown macroalga *Cystoseira brachycarpa*, dominating the rocks around the Bottaro crater and the hydrothermal system around it (see Auriemma *et al*. 2019, for a description of the collection area). Its local distribution in the area and its relationship with another new congeneric species are discussed below.

Amphiglena panareensis sp. nov.

(Figs 20; 21).

Material examined. Holotype (MNCN 16.01/18915): Italy: Panarea Island (Aeolian Archipelago), Bottaro crater, 27 September 2016, 8 m depth; station B3, 38°38’14.49”N 15° 6’34.47”E.

Paratypes: MNCN 16.01/18916: 9 specimens from the same locality and date as the holotype; 53 specimens B3 site, 27 September 2016 PCZL S.A. 10.1; 7 B1 site, 27 September 2016 PCZ S.A. 10.2.; 63 specimens, B3 site, 23 September 2018; PCZL S.A. 10.3: 76 specimens, B2 site 23 September 2018; 33 specimens, B1 site, 23 September 2018, 10 m depth, PCZL S.A. 10.4. Remaining specimens in MCG collection. Most of the material fixed in ethanol 70% (including the holotype and paratypes); some material fixed in ethanol 95%.

Description. Holotype complete, with eight thoracic and 40 abdominal chaetigers. Body length 5.5 mm, branchial crown 1.5 mm, maximum body width 0.26 mm. Natural brown colouration present especially in the thorax, highlighting the mid-dorsally faecal groove, and ventral shields (Fig. 20A). Crown holding five pairs of radioles with 15 pairs of long pinnules in two symmetrical not alternating rows. Gap between pairs decreasing along the radiole from the base to the distal end, with the first two basal pairs more separated from the others. Pinnules of similar length (1/4 of the total radiolar length) and with the last two distal pairs and the first two basal pairs slightly shorter. Radiolar bare tips long1/3 of the total radiolar length, with a blunt end (Fig. 20C). Radiolar skeleton with two rows of cells. Dorsal lips almost identical in length to pinnules, being 1/4 of the total radiolar length. Anterior peristomial ring visible, high and with similar height all around. Anterior peristomial ring low. Both peristomial rings appeared connected to highly developed ventral basal flanges, which extend as prominent ridges from base of ventralmost radioles (Fig. 20D, E). Peristomial eyes not visible. Pygidial eyes present as brown cluster spots on lateral margins of pygidium. Thorax longer than wide. First thoracic chaetiger bearing only 3 chaetae similar in shape to the superior thoracic ones. Second to eighth thoracic chaetigers with 4 uncini per torus, having well-developed breast, large space to main fang, approximately four rows of long teeth above main fang, and a medium handle (0.40) (Fig. 21A). Companion chaetae with straight shaft and long mucro (Fig. 21B). Second to eighth thoracic chaetigers with 4 chaetae, of which one is a superior broadly hooded chaeta (Fig. 21D), and three paleate chaetae in inferior row on each thoracic chaetiger, with long mucro (longer than the hood) (Fig. 21E). Four abdominal uncini on each torus higher than longer and with similar-sized small teeth above the main fang, with a medium handle, and also in this species with a large height as observed in *A. vulcanoensis* **sp. nov.** and *A. aeoliensis* **sp. nov.**, (Fig. 21C). Two abdominal broadly hooded neurochaetae similar to the thoracic paleate in the first abdominal chaetigers, becoming more geniculate in the middle segments (Fig. 21G, F). Spermathechae light brown/red coloured, not conspicuous.

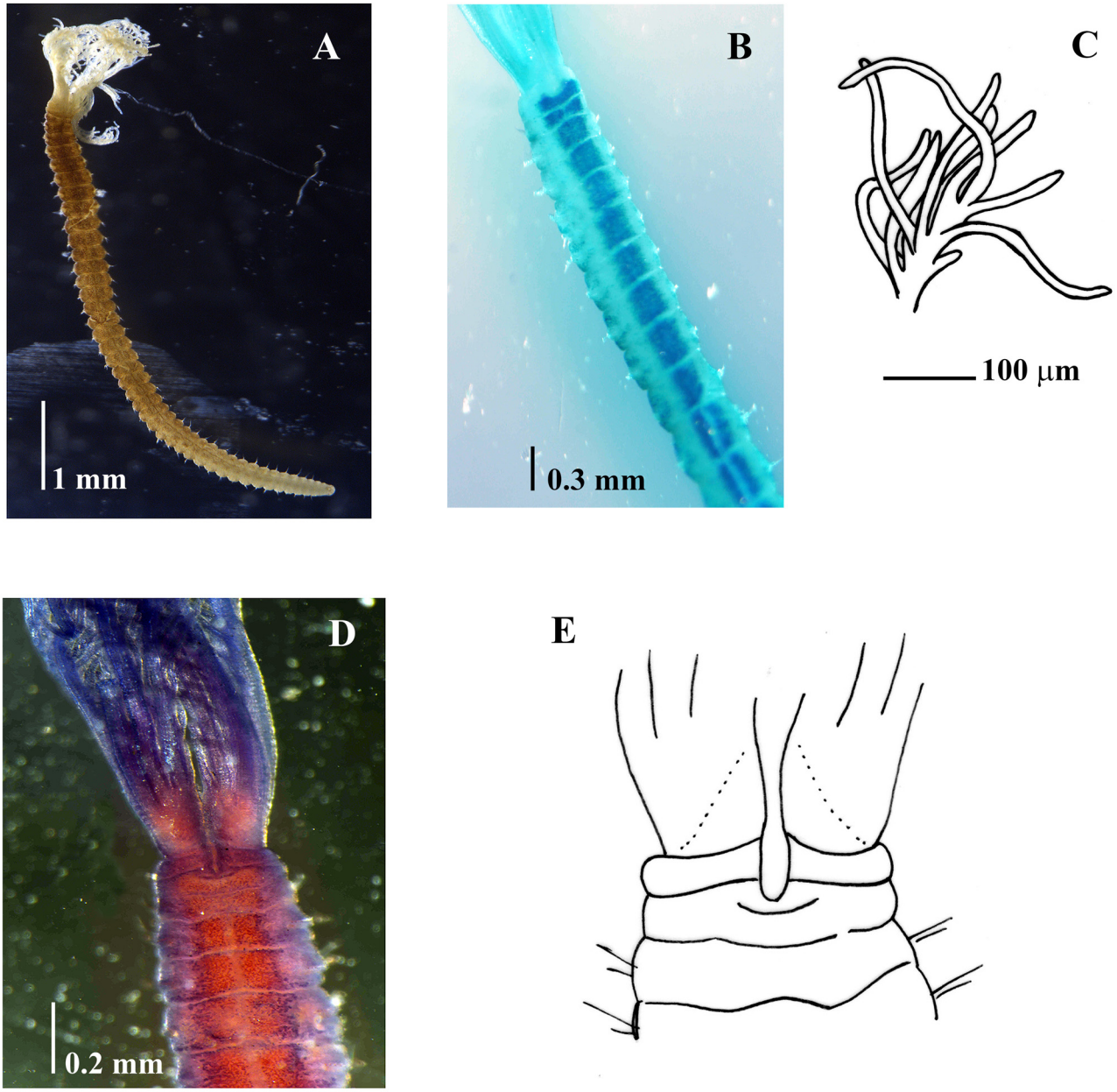

FIGURE 20. *Amphiglena panareensis* **sp. nov. A**. entire worm, photo from the holotype; **B.** staining pattern; **C.** tip of the radiole; **D.** photo from anterior end, ventral view, material coloured with Shirlastain A; **E.** scheme of the anterior end ventral view.

Staining pattern. In both thorax and abdomen stain only ventral shields, showing a square shape in the thorax and a longer double thin rectangular shape in the abdomen (Fig. 20B).

Variation. Individuals always with 8 thoracic chaetigers and up to 42 abdominal chaetigers. Mean body length of 4 mm and mean crown length of 1.3 mm. Peristomial eyes visible in some specimens. Up to 6 thoracic uncini on each torus and 6 thoracic uncini one on each torus (Table 1).

Remarks. The main feature distinguishes this new taxon is its elongate appearance, that also includes the base of the crown. Moreover, members of this species have the highest and more developed ventral basal flanges, extending as prominent ridges, observed in the genus. The elongate base of the crown appears similar to a web and in some specimens makes difficult to see the base of the peristomial ring. This is similar to *A. gravinae* **sp. nov.**, described from the Adriatic Sea, from which it is distinguished especially for the different development of both the peristomial rings, but also for the length of handle of abdominal uncini, for the highest number of thoracic paleate chaetae, and for the shape of abdominal chaetae. Moreover, *A. panareensis* **sp. nov.** is longer than *A. gravinae* **sp. nov.** In addition, this is the second Mediterranean taxon showing pinnules arranged in pairs not alternating along

the radiole. Among the non Mediterranean taxa, high developed ventral flanges are present also in *A. bondi* Capa & Rouse, 2007, however, this is a thin and more compact species, with a longer branchial crown bearing 6 radioles with similar length. Lastly, the brown colouration appears clearer and more homogeneous than that observed in specimens of *A. aeoliensis* **sp. nov**. described in the present paper from the same area.

Etymology. The species is named from type locality, the island of Panarea (Aeolian Archipelago) (see Auriemma *et al.* 2019 for a description of the collection area).

Distribution and Ecology. This species represents, together with *A. aeoliensis* **sp. nov.**, one of the most abundant taxa of the benthic community associated to the macroalga *Cystoseira brachycarpa*, the brown habitat-former alga dominating the rocks of the Bottaro crater and the hydrothermal system around it (Auriemma *et al.* 2019). Its local distribution in the area and its relationship with *A. aeoliensis* **sp. nov.** are discussed below.

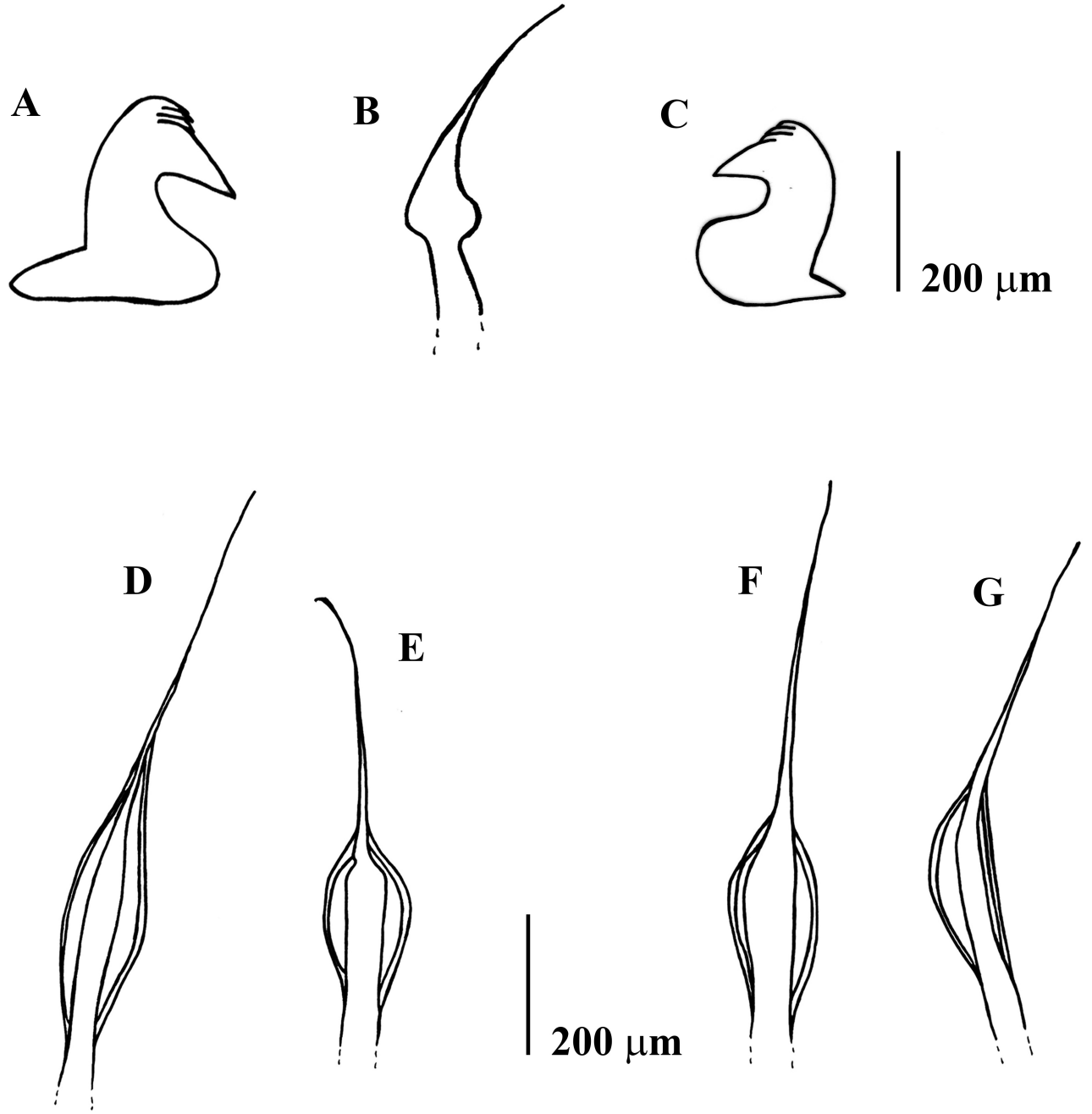

FIGURE 21. *Amphiglena panareensis* **sp. nov. A.** thoracic uncinus; **B.** companion chaeta; **C.** abdominal uncinus; **D.** superior thoracic chaeta; **E.** inferior thoracic chaeta; **F.** abdominal chaeta from anterior abdominal segment; **G.** abdominal chaeta from a middle abdominal segment.

TABLE 1. Comparative view of the main characters of the described *Amphiglena* species.

	A. cf *mediterranea* (S. Pietro-Ischia)	*A.* cf *mediterranea* (Leghorn)	*A.* cf. *mediterranea* (Portofino)	*A. aenariensis* **sp. nov.**
number of radioles	5	5	5	5/6
number of pinnules	10 pairs alternating	10 pairs alternating	10 pairs alternating	12 pairs alternating
dorsal lips (ratio to crown length)	short (1/5)	short (1/5)	short (1/5)	short (1/7)
basal flanges	high not connected	high not connected	high not connected	low, connected
spermathechae	not visible	not visible	not visible	brown/red
number of thoracic uncini per chaetiger	up to 6	up to 6	up to 6	up to 8
thoracic uncini handle (ratio a/b)	medium (0.48)	medium (0.48)	medium-(0.48)	short (0.23)
number of abdominal uncini per chaetiger	up to 6	up to 6	up to 6	up to 6
abdominal uncini handle	short	short	short	short
number of abdominal chaetigers	up to 27	up to 25	up to 21	up to 29
mean body length (mm)	2.82 ±0.75	2±0.48	2.65±0.7	1.87± 0.35
mean crown length (mm)	0.73± 0.25	0.98±0.12	0.8± 0.25	1.07±0.29
crown/body ratio	0.25	0.49	0.3	0.57
max width (mm)	0.36	0.3	0.3	0.4
staining pattern	entire	entire	entire	partial
peristomial eyes	not visible	not visible	not visible	brown
pygidial eyes	ellipsoid red	not visible	not visible	brown

.....continued on the next page

TABLE 1. (Continued)

	A. pithecusensis **sp. nov.**	*A. nisidensis* **sp. nov.**	*A. phlegreensis* **sp. nov.**	*A. messapica* **sp. nov.**
number of radioles	4/5	5	5 to 6	5
number of pinnules	12 pairs alternating	12 pairs alternating	16 pairs alternating	11 pairs not alternating
dorsal lips (ratio to crown length)	short (1/5)	short (1/5)	medium length(1/4)	long (1/3)
basal flanges	low, connected	low, connected	high, connected	high, connected
spermathechae	brown/red	brown	light	not visible
number of thoracic uncini per chaetiger	up to 7	up to 5	up to 8	up to 7
thoracic uncini handle (ratio a/b)	short (0.31)	medium-short (0.35)	medium-short (0.35)	medium (0.43)
number of abdominal uncini per chaetiger	up to 3	up to 3	up to 4	up to 4
abdominal uncini handle	short	short	short	short
number of abdominal chaetigers	up to 24	up to 31	up to 32	up to 28
mean body length (mm)	1.9±0.4	1.66±0.61	2.21±0.49	3.1±1.64
mean crown length (mm)	1.3 ± 0.27	1.1±0.31	1.38±0.27	1.5±0.73
crown/body ratio	0.62	0.66	0.62	0.48
max width (mm)	0.4	0.4	0.4	0.3
staining pattern	partial	partial	partial	partial
peristomial eyes	brown	brown	brown red	not visible
pygidial eyes	red	red	brown	brown

.....continued on the next page

TABLE 1. (Continued)

	A. gravinae **sp. nov.**	*A. vulcanoensis* **sp. nov.**	*A. aeoliensis* **sp. nov.**	*A. panareensis* **sp. nov.**
number of radioles	5	6 to 7	5	5 to 6
number of pinnules	11 pairs alternating	16 pairs not alternating	14 pairs alternating	15 pairs not alternating
dorsal lips (ratio to crown length)	long (1/3)	long (1/3)	short (1/7)	short (1/4)
basal flanges	very high and connected	low, connected	high not connected	very high and connected
spermathechae	white	light brown	brown/red	light brown
number of thoracic uncini per chaetiger	up to 7	up to 10	up to 8	up to 6
thoracic uncini handle (ratio a/b)	long (0.52)	medium-short (0.35)	short (0.30)	medium (0.40)
number of abdominal uncini per chaetiger	up to 5	up to 7	up to 6	up to 4
abdominal uncini handle	medium	medium	short	medium
number of abdominal chaetigers	up to 30	up to 32	up to 28	up to 42
mean body length (mm)	2.64±0.95	3.67±0.68	2.55±0.48	4±0.76
mean crown length (mm)	0.87±0.21	1.3±0.26	1.07±0.24	1.33±0.33
crown/body ratio	0.33	0.35	0.39	0.33
max width (mm)	0.3	0.5	0.43	0.26
staining pattern	partial	Partial	partial	partial
peristomial eyes	not visible	not visible	not visible	not visible
pygidial eyes	very small	brown	brown	brown

Keys to the Mediterranean taxa of *Amphiglena*

1 a) Shape of the body elongate, staining pattern of each abdominal segment longer than wide 2
b) Shape of the body compact, staining pattern of each abdominal segment wider than long 3
2. a) Anterior peristomial ring similar in height to the posterior ring. Abdominal chaetae broadly hooded similar to thoracic paleate chaetae in all the abdominal segments and not showing the geniculate appearance . . . . . *A. gravinae* **sp. nov.**
b) Anterior peristomial ring very thin, posterior very high. Abdominal chaetae appearing quite geniculate starting from the middle segments . . . . . *A. panareensis* **sp. nov.**
3. a) Staining pattern present in all the thorax and abdomen except than intersegmental grooves, abdomen fully coloured . . . . . *A.* cf. *mediterranea*
b) Staining pattern only in ventral shields 4
4. a) Large species with more than 5 radioles . . . . . *A. vulcanoensis* **sp. nov.**
b) Small species with a maximum of 5 radioles 5
5. a) Staining pattern more developed in the thorax, thoracic uncini with short or medium-short handles, long crown compared to the body (body/crown ratio over 0.50) 6
b) Staining pattern well developed also in the abdomen, thoracic uncini with medium handle, body/crown ratio below 0.50 . . . . . *A. messapica* **sp. nov.**
6 a) Thoracic uncini with short handle 7
b) Thoracic uncini with medium length handle 8
7 a) Basal flanges low and connected to the ventral peristomial ring . . . . . *A. aenariensis* **sp. nov.**
b) Basal flanges high and not connected, natural dark colouration especially in the thorax . . . . . *A. aeoliensis* **sp. nov.**
8 a) 16 pairs of pinnules along the radiole, abdominal chaetae of the middle abdominal segments with a very long blade . . . . . *A. phlegreensis* **sp. nov.**
b) 12 pairs of pinnules along the radiole, ... 9
9. a) Staining pattern large both in thorax and abdomen, handle of thoracic uncini with a truncate end . . . . . *A. nisidensis* **sp. nov.**
b) Staining pattern narrow both in thorax and abdomen, handle of thoracic uncini with a pointed end . . *A. pithecusensis* **sp. nov.**

Morphological and available molecular data comparison

Molecular diversity within the the species complex of *A. mediterranea* was already discussed by Calosi *et al.* (2013) in specimens from Ischia, the Gulf of Naples and Santa Caterina (Ionian Sea, Lecce), addressing high divergence (Fig. 22), although these authors did not discuss the possible existence of cryptic/sibling species, because the study was more focused in the identification of genetic differentiation between populations living under acidified conditions and those under control/normal conditions. However, in the study of Calosi *et al.* (2013), no genetic separation was recorded between populations collected in acidified and non-acidified sites.

A more recent molecular analyses by Tilic *et al.* (2019), using GenBank deposited sequences, including those in Calosi *et al.* (2013) and some additional material, pointed out the presence of at least six different taxa, and classifying *A. mediterranea* as a complex of cryptic species. Cryptic morphology was also stated for non Mediterranean species (Tilic *et al.* 2019) with *A. seaverae* Tilic, Feerst & Rouse, 2019, morphologically indistinguishable from *A. lindae* Rouse & Gambi, 1997, but distinguished only by molecular tools.

No morphological information on the majority of the analyzed sequences is currently available, and the present paper is a first contribution in trying to resolve the Mediterranean diversity of this taxon, and that needs a major revision and designating a neotype of *A. mediterranea* material from type locality of (Nice, France). Future research will be addressing the morphological diversity and the finding of more morphological features which can enable taxonomists to easily distinguish among different taxa, and also in the formal naming of cryptic species, since this can be of crucial importance for biodiversity assessment and conservations efforts (Delić *et al.* 2017). Cryptic species constitute an important part of biodiversity and their finding is not uncommon in annelids, where it has been shown repeatedly that DNA sequencing can reveal species that are morphologically similar (Halt *et al*. 2009; Pleijel *et al.* 2009; Capa *et al*. 2010, 2013; Nygren 2014; Álvarez-Campos *et al.* 2017).

In the present paper we have provided some morphological differences existing among the material already examined from a molecular point of view by Calosi *et al*. (2013) and later by Tilic *et al.* (2019) and which gave the COI tree topology showed in Fig. 22. The morphological examination allowed to give a name to the taxa which were identified by molecular tools. Within the tree topology, the new species *A. aenariensis* (Ischia Castello), *A. pithecusensis* (Ischia Lacco Ameno and S. Anna) and *A. nisidensis* (Nisida Island), which showed a very similar morphology, including the staining pattern, form a clade separated from *A.* cf. *mediterranea* (Ischia S. Pietro), which is the taxon genetically closer to the new species here described as *A. messapica* from S. Caterina (Lecce). Therefore,

morphological pattern can be well overlapped by the molecular one. In this regard, it must also be stressed that the taxon collected in Nisida, which is paraphyletic in the molecular tree of Calosi *et al.* (2013), probably really contains two cryptic taxa not morphologically separable.

Some other Mediterranean populations of *Amphiglena* here described from different sites, previously identified as *A. mediterranea,* were recognized as new species, but only from morphological point of view because molecular data for these taxa, are not available at present.

Morphological differentiation is very subtle in these small worms, and relatively few characters/features are available. In the present paper, new diagnostic characters were utilized. The general aspect, longer or more compacted, that cannot be related only to the fixation (see *A. aeoliensis* and *A. panareensis* that were collected in the same samples and fixed at the same time), is used for the first time as a discriminant feature, as well as the staining pattern, which can be useful in a rapid separation of main groups of taxa. For example, *Amphiglena* cf. *mediterranea* in which the abdomen results completely coloured, could be separated from all the other species here described where only the ventral shield resulted coloured in the abdomen; while the colouration is also useful to highlight the elongation of abdominal segments in the elongated species.

The main useful features remain, however, the length of the handles of thoracic uncini, with most of the Mediterranean species having a short or medium/short-handles compared to the other described species around the world, as well as the shape of the posterior peristomial ring and the development of basal flanges that can be more or less connected to the peristomial ring.

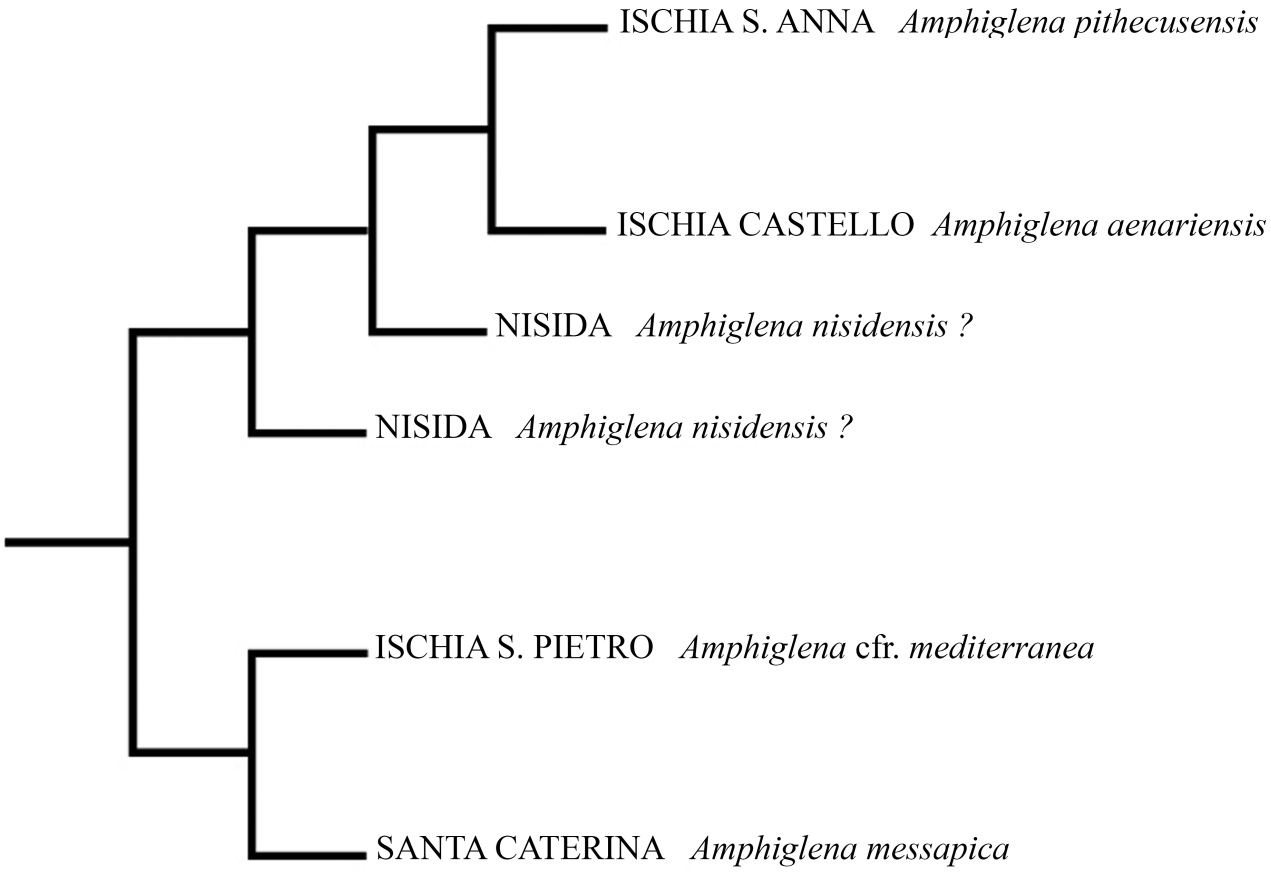

FIGURE 22. Molecular tree derived from the studies of Calosi *et al*. (2013) and Tilic *et al.* (2019) (modified), based on COI analysis, over-imposed with the names of the new described taxa after our morphological examination.

Ecological considerations

All the new species here described have been collected at shallow depths (1-14 m depth) and associated with macroalgae on hard bottoms (mainly photophilous algae in the upper littoral horizon, from 1 to 5 m depth). However, some relevant ecological differences in the various sites and habitat should be stressed, with different species found

in different ecological conditions. This is the case for example of *A. messapica* **sp. nov**. found in sheltered zones, or *A. gravinae* **sp. nov**. found in an environment characterized by high sedimentation rate.

The most interesting finding is that 5 of the 9 new described species have been found in CO_2 and hydrothermal vent's systems, were they represented one of the most abundant species among polychaetes occurring in these systems. These are very unique systems of volcanic origin where fluids (gasses and in some case also hot waters) spill out from the bottom, and in all the cases here examined provoke a natural acidification of the surrounding waters. In each of such systems considered, we have found a different species, while at the Panarea vents (Bottaro crater) two of these new taxa coexisted. This is a quite interesting finding and suggests that these peculiar shallow vents systems may host unique species and these sites can be viewed also as natural evolutionary laboratories. In the Castello CO_2 vents at Ischia in particular, one new acoelan worm (Nilsson *et al.* 2011), and two other new species of polychaete Fabriciidae have been already described (Giangrande *et al.* 2014). In addition, it is interesting to underline that specimens from Ischia, corresponding now to *A. aenariensis* **sp. nov**., collected in the CO_2 vents, and specimens from Santa Caterina, corresponding now to *A. messapica* **sp. nov**., but, previously identified as *A. mediterranea*, were used for some laboratory experiments on regeneration, and gave completely different results, with the population from the Ischia vents showing higher robustness to laboratory manipulation than the Santa Caterina one (Giangrande A. & Licciano M., unpublished data).

In the vents off the Bottaro crater at Panarea, the two new *Amphiglena* species, collected in quantitative samples in September 2016 and 2018, along a gradient of ocean acidification (Auriemma *et al.* 2019), going from the rim of the crater (station B3 most acidified) to control area without bubbling (station B1, approx. 35 m form the rim of the crater), show a different distribution (Fig. 23). Members of *A. panareensis* **sp. nov**. are more abundant in the more acidified stations (B3 and B2), while member of *A. aeoliensis* **sp. nov**. are more abundant in the control station B1 (Fig. 23). Thus the two species show somehow a different degree of tolerance to the stress due to OA in this hydrothermal vent system, which deserves future investigations on their biology.

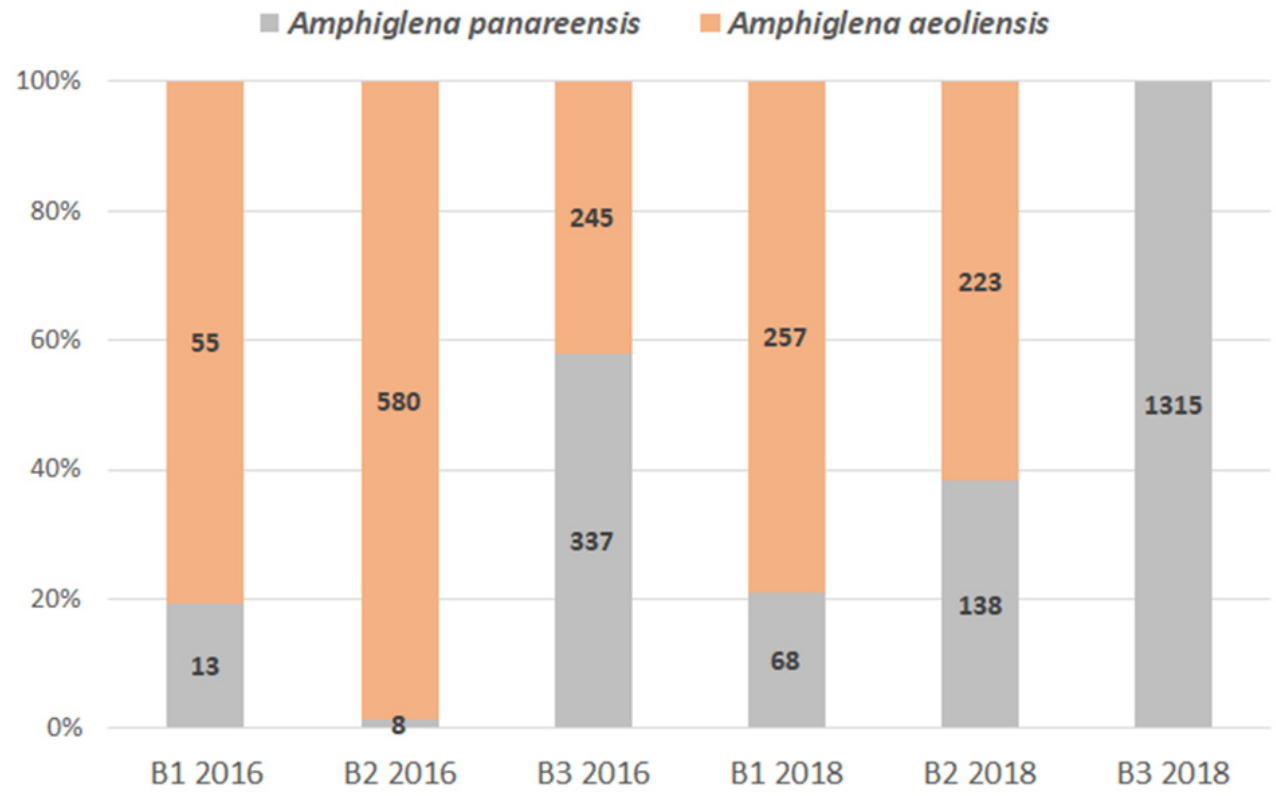

FIGURE 23. Relative dominance and number of individuals (inside columns) of the two new species of *Amphiglena* (*A. panareensis* and *A. aeoliensis*) collected along the ocean acidification gradient at the Bottaro crater vent's system (Panarea Island) (B3 most acidified station near the rim of the crater, B2 intermediate station, B1 control station with normal pH, 35 m out of the crater) in different years (September 2016 and September 2018; see Auriemma *et al*. 2019, for site description). The quantitative samples represent scrapings of the brown alga *Cystoseira brachycarpa* on 20 x 20 cm quadrats on the rocky vegetated bottom (Auriemma *et al.* 2019).

Conclusion

The morphological analysis of most of the Mediterranean material previously identified as *A. mediterranea* as re-described by Rouse & Gambi (1997), revealed the presence of different taxa, leading to the description of nine new species also linked to unique environmental conditions (e.g., shallow hydrothermal systems), and increasing the number of species within this genus to 22. In addition, the analysis here provided showed as the cryptic taxa separated by molecular analysis (Calosi *et al.* 2013; Tilic *et al.* 2019), can be morphologically distinguished.

As a whole, it seems that each area has its own form and that speciation within this genus is an easy and also fast process, leading to the presence of also other cryptic forms (Tilic *et al.* 2019). The high speciation rate within this genus can be linked to the type of development and lifestyle. Species of the genus are in fact, all tubicolous, even if inhabiting transient tubes, and characterized by direct development within the tube (Rouse & Gambi 1998). Therefore, the limited dispersion potential and reduced motility can favor local isolation, and selection of genotypes and species.

A re-description of material of *A. mediterranea* from type locality is still needed in order to clarify the systematic position of what for the moment was here referred to as *A.* cf. *mediterranea*. It is probable that further analyses will increase the number of species within the genus in the Mediterranean area, taking into consideration that other badly preserved material present in the collection of one of the authors (AG) still remains undescribed.

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