

Fabriciidae (Annelida, Sabellida) from a naturally acidified coastal system (Italy) with description of two new species

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*Polychaete worms are known to thrive in extreme environmental conditions, however little is known about how polychaete species will respond to major climatic stressors, such as ocean acidification. Here, we examined the distribution of Fabriciidae (Annelida, Sabellida) species along a gradient of ocean acidification, caused by carbon dioxide (CO₂) vent emissions in a shallow, coastal system off the island of Ischia (Tyrrhenian Sea, Italy). A total of 265 specimens of Fabriciidae, representing six species from five genera, were collected along the gradient. Most of the species were found across the entire CO₂ gradient, suggesting polychaetes may have a high tolerance for ocean acidification in the future. Two of the species were new to science, and two of the genera were previously unrecorded in the Mediterranean. A full description of the new species *Brifacia aragonensis* sp. nov. and *Parafabricia mazzellae* sp. nov. is given, both of which were most abundant in the most acidified areas (pH 6.6–7.2). The geographical distribution and ecology of the new taxa, as well as of the other fabriciid species collected, is discussed. Taxonomic keys to identify the Fabriciidae species currently recorded in the Mediterranean Sea are also provided.*

Keywords: polychaetes, Fabriciidae, ocean acidification, Mediterranean Sea

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INTRODUCTION

Polychaete worms are common in benthic marine ecosystems worldwide. Despite their widespread distribution and wide range of adaptations to extreme environments, assessments of their diversity are incomplete, especially for inconspicuous forms. While there is a relatively large literature on polychaetes as bioindicators of pollution and various disturbance factors (Giangrande *et al.*, 2005), little is still known about how polychaetes will respond to major climatic stressors, and in particular to ocean acidification (OA). OA is caused by the absorption of atmospheric CO₂ into the ocean, which causes a shift in seawater carbonate chemistry and a reduction in pH. OA is predicted to decrease seawater pH 0.3–0.4 units below the present values by the end of the century (Feely *et al.*, 2008), which is likely to affect a wide-range of marine species (Doney *et al.*, 2009; Kroeker *et al.*, 2013). At present, most research suggests OA will negatively impact various biological processes, such as calcification, growth and reproduction, as highlighted by recent reviews and meta-analyses, mainly based on laboratory and mesocosm experiments (Kroeker *et al.*, 2010; Harvey *et al.*, 2013). While the relatively few studies conducted in naturally acidified ecosystems, such as those caused by volcanic CO₂ vents, have revealed an overall reduction in diversity with ocean acidification, these studies also suggest that some species are capable of thriving in

relatively low pH conditions (Kroeker *et al.*, 2011), including several polychaete species (Cigliano *et al.*, 2010; Kroeker *et al.*, 2011; Ricevuto *et al.*, 2012). Here, we examine the distribution and ecology of polychaetes within the family Fabriciidae along a gradient of OA caused by natural CO₂ venting in a coastal system off the coast of Ischia (Tyrrhenian Sea, Italy) due to their high abundance encountered in these low pH environments and potential tolerance of OA.

Fabriciidae (Annelida, Sabellida) are small-sized worms (from 0.5 to 2 mm) that are distributed worldwide. The taxon has over 70 nominal species, mostly inhabiting shallow waters or intertidal hard substrates. A single species, *Raficiba barryi* (Fitzhugh, 2001) is known to live up to 100 m depth, while *Monroika africana* (Monro, 1939) and *Manayunkia* spp. thrive in freshwater habitats (Zenkevitsch, 1925; Monro, 1939; Fitzhugh, 1992). Previously regarded as a subfamily of Sabellidae, with the name Fabriciinae Rioja (1923) (Fitzhugh, 1989), Fabriciidae has only recently been erected as a family showing higher phylogenetic affinity to Serpulidae (sister group) than to the rest of Sabellidae, based on DNA sequence data (Kupriyanova & Rouse, 2008; Capa *et al.*, 2011). The monophyly of this group is supported by a series of morphological apomorphies (derived characters or innovations), such as the absence of ventral lips, modification of abdominal uncini to an elongate manubrium, and presence of branchial hearts (Fitzhugh, 1989). Additional apomorphies were identified by Rouse (1995a, b) and by Huang *et al.* (2011) based on reproductive characters such as spermiogenesis only in the thorax, spermiogenesis in large clusters with a central cytophore, single dorsal sperm duct, sperm nuclear projection, thickening of the sperm nuclear membrane and the sperm

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extra-axonemal sheath. The relationships among genera were further examined using morphological data in a series of papers by Fitzhugh (1990a, b, c, 1991, 1993, 1994, 1995, 1996, 1998, 1999, 2001, 2002). More recently a phylogenetic analysis has been conducted using only molecular data (Kupriyanova & Rouse, 2008), as well as an analysis with the molecular data integrated with morphological information (Huang *et al.*, 2011).

All the Fabriciidae studied to date are gonochoric, with females storing sperm that were previously released by males into the water (Rouse, 1996). They are also brooders, with direct-developing larvae (Rouse & Fitzhugh, 1994). The limited dispersal capabilities may favour some species to reach high population densities, as recorded for *Fabricia stellaris stellaris* (Müller, 1774) (*F. sabella sensu* Fauvel, 1923) (Lewis, 1968). Although *F. stellaris stellaris* remains the most common and widespread taxon among Fabriciidae at present, it is likely that some specimens collected in the past, from the Mediterranean and other geographical zones, have been erroneously attributed to this species due to the difficulty in identifying taxa within this family and the paucity of specialists on this group.

A large number of Fabriciidae are found in the boreal hemisphere (60%), while others, mainly belonging to the genera *Augeneriella* (71% of the species) and *Fabriciola* (38% of the species), are distributed in the tropics (Giangrande & Licciano, 2004). Of the 17 currently recognized genera, eight are monotypic (Fitzhugh, 2010). At present, nine species belonging to six genera are reported in the Mediterranean. However, Fabriciidae are rarely included in ecological surveys, and the ecology of the species is relatively unstudied. This is most likely due to their small size, comparable to meio-faunal taxa, which makes morphological examination difficult.

The volcanic CO₂ vents near Ischia have been widely used as a natural laboratory to study the effects of ocean acidification on the local species and communities (Hall-Spencer *et al.*, 2008; Kroeker *et al.*, 2011, 2012; Porzio *et al.*, 2011; Gambi & Ricevuto, 2012). CO₂ emissions at this site provide a unique opportunity to assess the effects of acidification on marine organisms, populations and communities in their natural setting. Initial surveys of the rocky reef communities along a gradient of pH (from the most acidified areas to ambient seawater conditions) revealed a relatively high abundance of polychaetes among other invertebrates (Kroeker *et al.*, 2011). Over half of the polychaetes collected from the vent ecosystem belonged to the order Sabellida (Sabellidae, Fabriciidae and Serpulidae), and one species, *Amphiglena mediterranea* (Leydig, 1851), was the most abundant species in the most acidic areas (pH range 6.6–7.2) (Kroeker *et al.*, 2011, Gambi *et al.*, unpublished data). Due to their unusually high abundance in this unique habitat, we focus our analyses on distribution and ecology of Fabriciidae collected along a gradient of OA. Our analyses reveal two species new to science and two genera previously unrecorded in the Mediterranean Sea, which we include in an updated identification key of known species of Fabriciidae in the Mediterranean Sea.

MATERIALS AND METHODS

Study site

The study area is the Castello Aragonese, a small islet located on the north-east side of the volcanic island of Ischia

(Tyrrhenian Sea, Italy) (Figure 1). The Castello Aragonese is connected to the main island of Ischia by a road and a bridge. On the south and north sides of the islet there are shallow areas (1–3 m depth) characterized by intense venting of gas, composed primarily (~95%) of CO₂ (Tedesco, 1996; Hall-Spencer *et al.*, 2008), no sulphur present. Temperature, salinity, and oxygen concentration are similar to the shallow coastal waters of the Central Tyrrhenian Sea (Lorenti *et al.*, 2005). In the areas of most intense venting on both the south and north sides of the islet, the seawater next to the rocky reef substrate is highly acidified compared to the normal seawater conditions (mean pH_T = 6.6–7.2; Hoffmann *et al.*, 2011, Kroeker *et al.*, 2011). From these extreme low pH zones, as defined by Kroeker *et al.* (2011), there are gradients of acidification along the rocky reefs spanning approximately 150 m of the coastline on the northern and southern sides of the Castello Aragonese. Approximately 150 m from the areas of highest venting, the pH_T is comparable to normal conditions (mean pH_T 8.1–8.0; Kroeker *et al.*, 2011). Along these gradients (in both the north and south), three zones were selected: 'extreme low pH' (pH mean range 6.6–7.2); 'low pH' (7.6–7.8); and 'ambient pH' (normal pH conditions, 8.1–8.0). For pH measurements, we used *in situ* pH meters (using Honeywell Durafet® pH sensors) measuring hourly *in situ* pH values (see Hoffmann *et al.*, 2011; Kroeker *et al.*, 2011 for more details and carbonate chemistry). Benthic invertebrates were collected in November 2008. Samples were collected from 20 × 20 cm (400 cm²) rocky reef plots at 1–1.5 m deep on continuous, rocky substrate ranging from 50° to 85° in orientation from horizontal. In order to take into consideration the spatial variability of each pH-zone identified (~50 m long), four plots were sampled in each area: the first two plots at the beginning of the pH-zone and the other two plots towards its final part (see Kroeker *et al.*, 2011 for more details on sampling design). We sampled a total of 24 plots (12 on the south side and 12 on the north side). Small mobile invertebrates (<5 cm) were collected using a vacuum device powered by a SCUBA cylinder (air-lift sampler in Buia *et al.*, 2004) that was placed over the plot

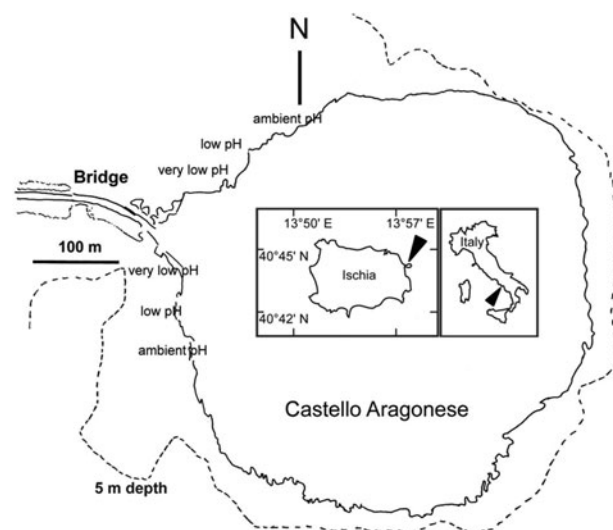


Fig. 1. Map of the study site at the Castello Aragonese (Ischia Island, Tyrrhenian Sea) with location of the pH gradient on the south and north sides of the islet.

for approximately 30 s. The remaining benthos (including all the macroalgae) was scraped off the substrate and collected for processing. The samples were placed in 4% buffered formalin for 24 h and then transferred to a 70% ethanol solution for storage. A dissecting microscope was used to separate the invertebrates from the algae.

The Fabriciidae were separated from the other families and examined under the stereo and compound microscopes. Drawings were made with a camera lucida mounted on the microscope, while photographs were also taken with an Axio Cam ERc5S camera mounted on a microscope Zeiss Primo Star. Due to the relatively modest number of species (six) and individuals collected over the gradient, as well as a patchy distribution for most of the species, data are not suitable for statistical analysis, nor was it possible to apply any statistical test to the abundance or diversity data. Abundance of each species was summed within the pH-zones to highlight the distribution patterns along the pH gradient.

Holotypes and paratypes of the two new species described were deposited at the MNCN (Museo Nacional de Ciencias Naturales, Madrid, Spain). The abbreviation PCZL refers to Giangrande's private polychaete Collection at the Zoological Laboratory of the Salento University, while MCG refers to Gambi's private polychaete Collection at the Stazione Zoologica Anton Dohrn (Ischia, Napoli).

RESULTS

A total of 265 specimens of Fabriciidae, belonging to six species and five genera, were collected. Two of the species, *Brifacia aragonensis* sp. nov. and *Parafabricia mazzellae* sp. nov., are new to science and belong to genera previously unreported in the Mediterranean Sea.

Distribution pattern

Most of the species were present across the entire pH gradient (Figure 2), with the exception of *Novafabricia infratorquata* (Fitzhugh, 1983) for which only eight specimens were collected in the low pH zone in the southern site. *Fabricia stellaris stellaris* was present across all pH zones, including the extreme low pH zones (20 specimens). In addition, the two new taxa, *Brifacia aragonensis* sp. nov. and *Parafabricia mazzellae* sp. nov., were present in all pH zones and most abundant in the extreme low pH zones (32 and 93 specimens, respectively). The abundance pattern of these taxa in extreme low pH was more pronounced at the southern site. *Parafabricia mazzellae* was the most abundant taxa in the extreme low pH. *Novafabricia posidoniae* Licciano & Giangrande, 2004, and *Rubifabriciola tonerella* (Banse, 1959), were relatively scarce, although a few specimens of both taxa were collected also in extreme low pH conditions.

SYSTEMATICS

Family FABRICIIDAE Rioja, 1923

Genus *Fabricia* Blainville, 1828

Fabricia stellaris stellaris (Müller, 1774). Type locality: Atlantic Ocean, Denmark.

Synonyms: *Tubularia stellaris* Müller, 1774; *F. stellaris* Blainville, 1828; *T. fabricia* Müller, 1776; *Othonia fabricii* Johnston, 1835; *Amphicora sabella* Ehrenberg, 1836; *F. quadri-punctata* Frey & Leuckart, 1847; *F. affinis* Leuckart, 1849; *F. sabella* Grube, 1850; *F. amphicora* Quatrefages, 1865; *A. fabricia* Malmgren 1867; *F. fabricii* Bush, 1905; *F. leidy* Verril, 1873; *F. dubia* Wesenberg-Lund, 1941; *Haplobranchus atlanticus* Treadwell, 1932; *Fabriciola bochmanni* Friedrich, 1939.

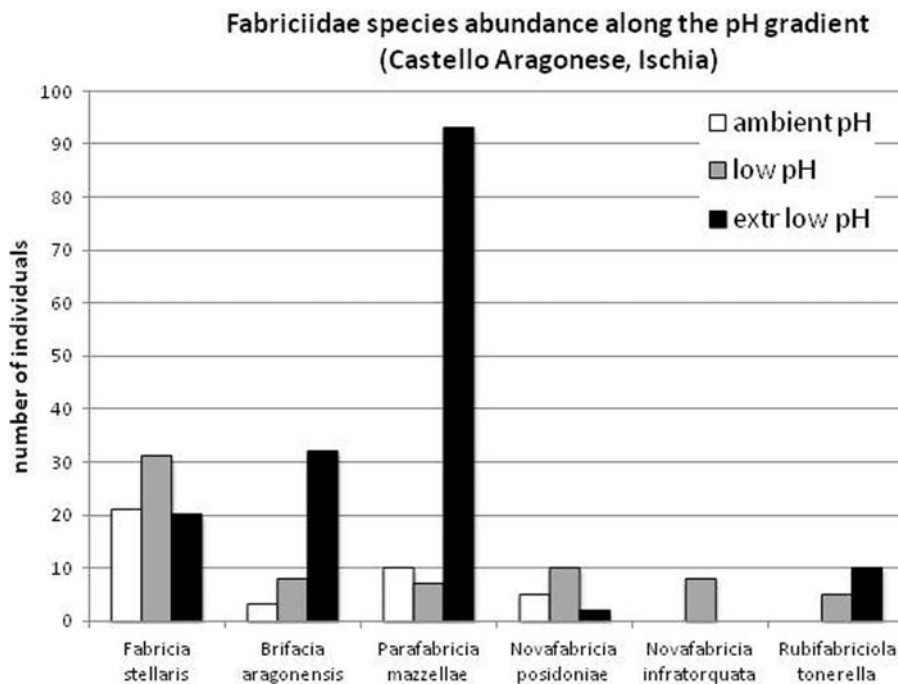


Fig. 2. Fabriciidae species abundance along the pH gradient at the Castello Aragonese (Ischia).

MATERIAL EXAMINED

72 specimens; ambient pH stations: N1a: 14 individuals; N1b: 2, N1c: 4; S1a: 1; low pH stations: N2a: 14, N2b: 2; N2c: 1; S2a: 14; extreme low pH stations: N3a: 1, N3b: 5; N3c: 1; N3d: 7; S3a: 1; S3c: 5. N, north; S, south; 1, ambient pH; 2, low pH; 3 extra low pH; a, b, c and d, sample replicates.

DESCRIPTION OF MATERIAL COLLECTED IN THE CASTELLO ARAGONESE AREA (TYRRHENIAN SEA)

Small specimens about 1.5 mm length and 0.15 mm wide (1 mm body length), with eight thoracic and three abdominal chaetigers (Figures 3A, 4A). Three radioles per lobe. Six to eight pairs of pinnule per radiole, terminating at the same height, branchial hearts present, ventral filamentous appendages absent. Dorsal lips triangular and well developed (Figures 3B, 4B). Anterior peristomial ring wider than long, developed ventrally as a rounded conical lobe. Single pair of peristomial eyes, black and rounded. Superior thoracic noto chaetae elongate, narrowly hooded, 4–6 per fascicle. Inferior thoracic noto chaetae shorter and narrowly hooded in chaetigers 2–8, 2–3 per fascicle. Pseudospatulate chaetae (Figure 3C) present at chaetigers 3–7 in number of two per fascicle. Thoracic uncini acicular, 7–9 per fascicle. Abdominal modified neurochaete elongate, narrowly hooded, 2–3 per fascicle. Dentate region of abdominal uncini with 9–10 rows of teeth, 3–4 teeth per row. Handle about two times longer than dentate region (Figure 3D, E).

REMARKS

This genus is monotypical with most of the species previously attributed to the genus *Fabricia* transferred to the genus *Novafabricia* Fitzhugh, 1990 (Fitzhugh, 1990a). The two genera differ mostly in the distribution of pseudospatulate chaetae of the thoracic chaetigers, that in *Novafabricia* can

be present in chaetigers 3–5 or 3–6, while in *Fabricia* are distributed in chaetigers 3–7, moreover the dorsal lips in *Novafabricia* are reduced to low, narrow ridges or absent, while in *Fabricia* they are well developed (1.5 was the ratio between length of dorsal lips and length of first thoracic chaetiger).

This is the first documentation of the dorsal lips from material of *F. stellaris stellaris* collected in the Mediterranean Sea. They seem less developed than in specimens collected both from the North Sea and the Atlantic Ocean, as shown in Banse (1956). No further pictures of dorsal lips in the genus *Fabricia* are available after this description. Indeed, figures of this feature are very rare in literature and are not present also in the revision of *Fabricia* (Fitzhugh, 1990b). Within the Mediterranean basin, another taxon is present with dorsal lips similar to Mediterranean specimens of *F. stellaris stellaris*: *F. stellaris adriatica* (Banse, 1956). This taxon was considered a valid species by Fitzhugh (1990b), however, he did not discuss its systematic position due to the poor preservation of the specimens. An examination of material from the North Adriatic Sea showed that this taxon differs from *F. stellaris stellaris* due to a shorter branchial crown with only three pairs of pinnules (Figure 4C), pseudospatulate chaetae with shorter tips, and abdominal uncini with only six teeth in profile (Figure 3E, F). Based on these differences, we suggest it should be considered a separate taxon.

As far as reproductive features of the Mediterranean specimens of *F. stellaris stellaris*, we were unable to observe eggs in the available specimens, while spermatogenesis occurs in chaetigers 3–8.

HABITAT

The species lives in sediment tubes in intertidal or shallow sub-tidal areas, in vermetid reefs, within macroalgae, seagrass meadows, mussel beds and barnacles, but especially in sheltered areas with high sedimentation and high organic matter content.

DISTRIBUTION

Boreal hemisphere until the Arctic region. Mediterranean distribution: Medes Islands (Spain), Sea of Marmara (Turkey), Adriatic Sea, Tyrrhenian Sea, Ionian Sea. Records for the Antarctic and Tropical Australia waters are likely incorrect (Fitzhugh K., personal communication)

Genus *Brifacia* Fitzhugh, 1998

Brifacia aragonensis sp. nov.

MATERIAL EXAMINED

43 specimens, stations: ambient pH: N1d: 2 individuals; S1c: 1; low pH: N2c: 1; N2d: 4; S2b: 2; S2c: 1; extreme low pH: N3a: 3; S3a: 2; S3b: 7; S3d: 20. Holotype from sample S3d (extreme low pH) MNCN 16.01/14697; paratypes from sample S3d (extreme low pH) deposited at MNCN 15332. Remaining specimens deposited in the PCZL collection at the University of Salento (Lecce, Italy), and in the MCG collection at the Stazione Zoologica Anton Dohrn (Ischia, Napoli).

DESCRIPTION

Holotype complete with 8 thoracic and 3 abdominal segments. Branchial crown length 0.5 mm, body length 1.1 mm, maximum width 0.3 mm. Body short and massive, slightly tapered anteriorly and posteriorly, with very flattened first

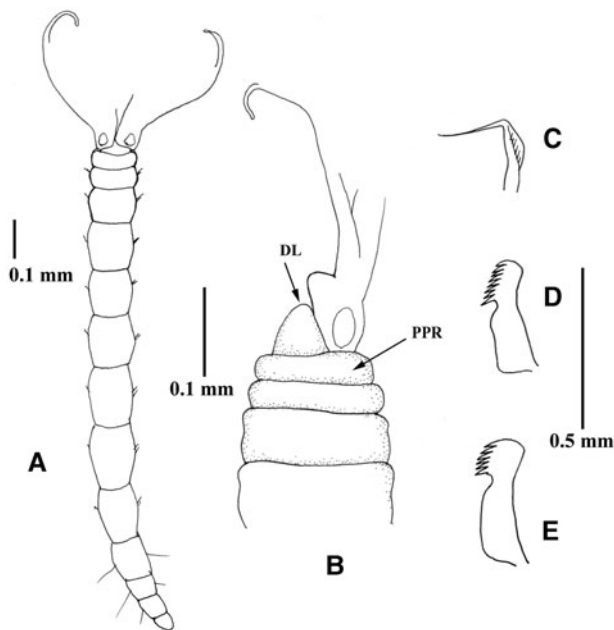


Fig. 3. *Fabricia stellaris stellaris*: (A) entire worm dorsal view; (B) ventral view of the anterior portion of the worm, showing the dorsal lip after cutting half of the crown; (C) pseudospatulate chaeta; (D) abdominal uncinus, profile; (E) abdominal uncinus from the subspecies *F. stellaris adriatica*. PPR, posterior peristomial ring; DL, dorsal lip.

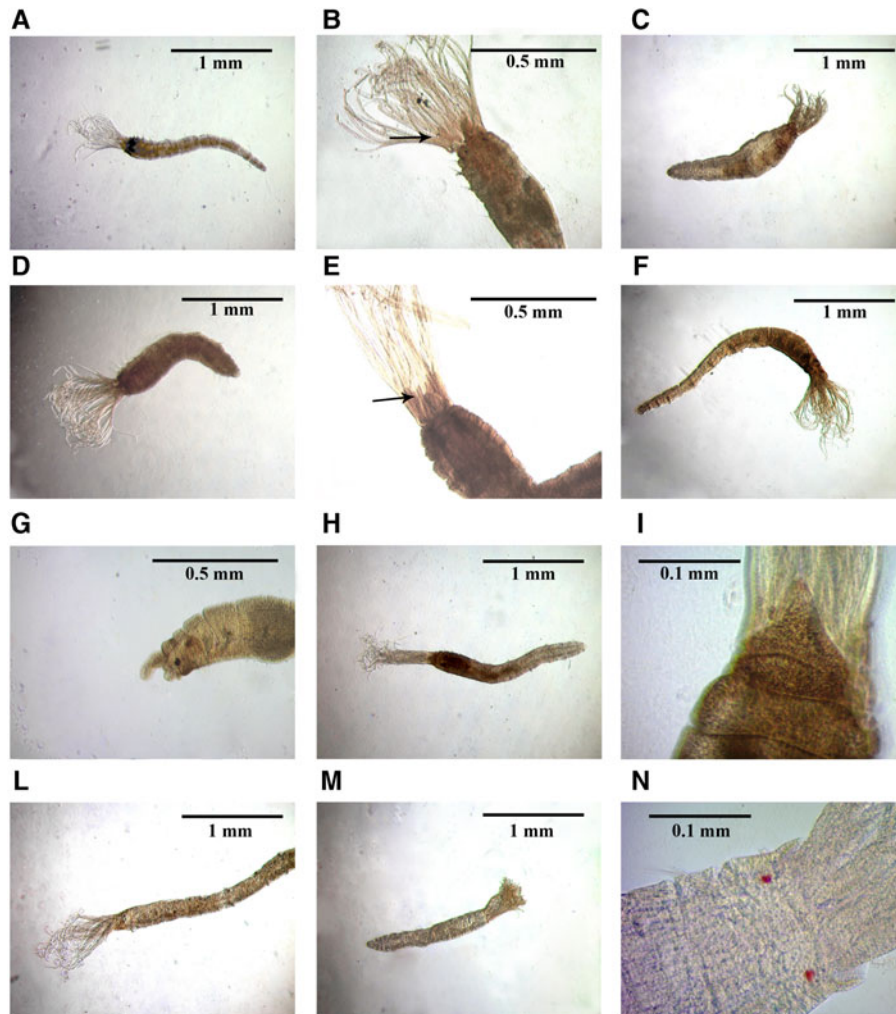


Fig. 4. Photographs of the collected species in the present paper plus *Fabricia stellaris adriatica*: (A) *F. stellaris stellaris* entire; (B) *F. stellaris stellaris*, particular of the anterior end ventral view; (C) *F. stellaris adriatica* entire; (D) *Brifacia aragonensis* entire; (E) *B. aragonensis* particular of the anterior end ventral view; (F) *Parafabricia mazzellae* entire; (G) *P. mazzellae* particular of the anterior end without crown; (H) *Novafabricia posidoniae* entire; (I) *N. posidoniae* particular of the anterior end ventral view; (L) *Novafabricia infratorquata* entire; (M) *Rubifabriciola tonerella* entire; (N) *R. tonerella* particular of the anterior end dorsal view.

two thoracic chaetigers (Figures 4D, 5A). Branchial crown with three pairs of radioles, each with 7–8 pairs of pinnules terminating at the same height. Branchial hearts present. Ventral filamentous appendages absent. Dorsal lips erect, well developed, triangular ridges with dorsal margins separated from proximal-most pinnules of dorsal radioles (Figures 4E, 5B, C). Anterior margin of anterior peristomial ring as a low ridge dorsally and laterally. Collar well developed ventrally as a triangular lobe (Figure 5B). One pair of black eyes along anterior margin of posterior peristomial ring. Chaetigers 5–6 longer than others. One pair of black pygidial eyes. Superior thoracic notochaetae elongate, narrowly hooded, 3 per fascicle. Inferior thoracic notochaetae of chaetigers 2 and 8 shorter and narrowly hooded, in number of 1–2 per fascicle. Only one broadly hooded pseudospatulate chaeta per fascicle (Figure 5E) present in chaetigers 3–7. Acicular uncini in chaetigers 2–8, in single row of 8–9 per fascicle, with main fang slender, single large tooth over the main fang followed by a series of small teeth; hood present (Figure 5D). Abdominal neuropodia with elongate narrowly hooded chaetae, 2–3 per fascicle. Abdominal uncini with dentate region with 7–8 rows of teeth in profile, 3–4 teeth

per row, manubrium as long as dentate region slightly expanded proximally (Figure 5F). From 18, 16 and 15 abdominal uncini in each torus, proceeding from chaetigers 9–11. Males with spermiogenesis occurring in chaetigers 4–8. Distribution of eggs was not observed.

Dark brown pigment covering peristomial rings and the first thoracic chaetigers.

ETYMOLOGY

The species is named after the type locality, the Castello Aragonese d'Ischia. The Castello, an ancient fortress, is named after the Spanish royal dynasty from Aragona that dominated this area (1443–1504) and that rearranged the castle settlement (an ancient Roman *castrum*) in its present state.

REMARKS

This genus is new for Mediterranean polychaete fauna. The genus *Brifacia* was described for the New South Wales (Australia) for the species *B. metastellaris* Fitzhugh, 1998, which was found inhabiting intertidal algal mats (Fitzhugh, 1998). The diagnosis of the genus was identical to that of

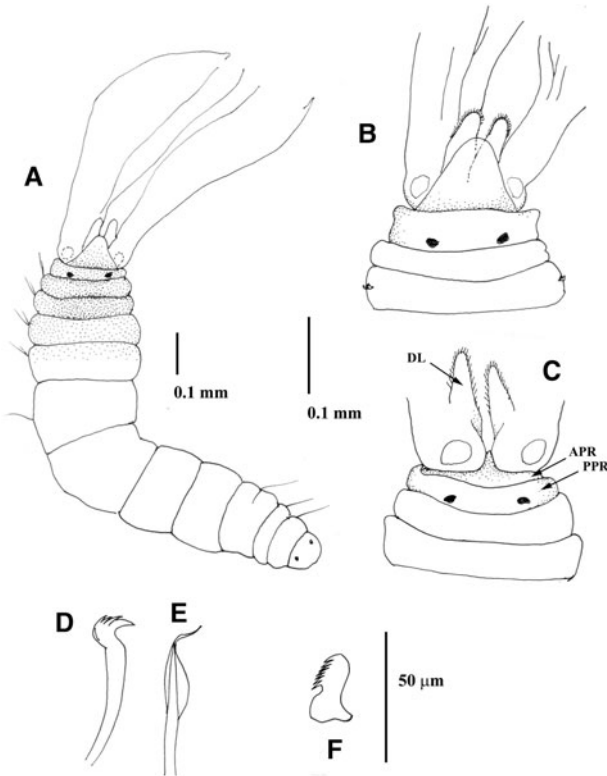


Fig. 5. *Brifacia aragonensis* sp. nov.: (A) entire worm ventral view; (B) anterior end ventral view showing the dorsal lips; (C) anterior end dorsal view showing the dorsal lips; (D) thoracic uncinus; (E) pseudospatulate chaeta; (F) abdominal uncinus. APR, anterior peristomial ring; PPR, posterior peristomial ring; DL, dorsal lip.

Fabricia, which is currently monotypical, except for the distribution of spermiogenesis, which in *Fabricia* occurs in chaetigers 3–8, and for the length of the manubrium of abdominal uncini, which in *Fabricia* is two times longer than the dentate regions. Dorsal lips of this new taxon are also more developed than those of *F. stellaris stellaris*, with a higher ratio between the length of dorsal lips and the length of the first thoracic chaetiger. Specifically, this value was 4 in *B. aragonensis* and about 2 in *B. metastellaris*. The difference in this value between the two species is because the first two chaetigers appear more compressed (more wide than long) in *B. metastellaris*. Indeed *B. aragonensis* is about half the size of *B. metastellaris*, although its appearance is more enlarged. *Brifacia aragonensis* also has a more developed ventral collar lobe, a shorter tip of broadly hooded pseudospatulate chaetae, and a smaller number of thoracic notochaetae (up to only 5 in the Mediterranean taxon, compared to 15 in the Australian taxon). Moreover, the thoracic hooks are arranged in single row, respect to the arrangement in irregular double row shown by the Australian taxon. Lastly, there are fewer abdominal uncini compared to the Australian taxon, as well as fewer teeth of the abdominal uncini (7–8 compared to 9–10).

Genus *Parafabricia* Fitzhugh, 1992
Parafabricia mazzellae sp. nov.

MATERIAL EXAMINED:

110 specimens; ambient pH stations: N1d: 4 individuals; S1a: 1; S1c: 4; low pH stations: S2a: 6; S2b: 1; extreme low pH stations: N3d: 3; S3c: 4; S3d: 93.

Holotype from sample S3d (extreme low pH), MNCN 16.01/14698; paratypes from sample S3d (extreme low pH) deposited at MNCN 15333. Remaining specimens deposited in PCZL collection at University of Salento (Lecce, Italy), and MCG collection at Stazione Zoologica Anton Dohrn (Ischia, Napoli).

DESCRIPTION

Holotype complete with eight thoracic and three abdominal segments. Branchial crown length 0.5 mm, body length 2.1 mm, maximum width 0.2 mm. Body slender (Figures 4F, 6A). Branchial crown with three pairs of radioles, each radiole with 6–7 pairs of pinnules all terminating at the tip of radioles. Branchial hearts present. Ventral filamentous appendages absent. Dorsal lips erect and well developed, distally rounded with dorsal margins separated from proximal-most pinnules of dorsal radioles (Figure 6C). Anterior margin of anterior peristomial ring with well developed ridges dorsally and laterally. Anterior peristomial ring developed ventrally as broad, thin lobe widest proximally and broadly rounded distally, at least two times longer than posterior peristomial ring (Figure 6A–D). One pair of black eyes along anterior margin of the posterior peristomial ring. First chaetiger short, all the remaining chaetigers about four times longer than chaetiger 1, giving an elongate appearance.

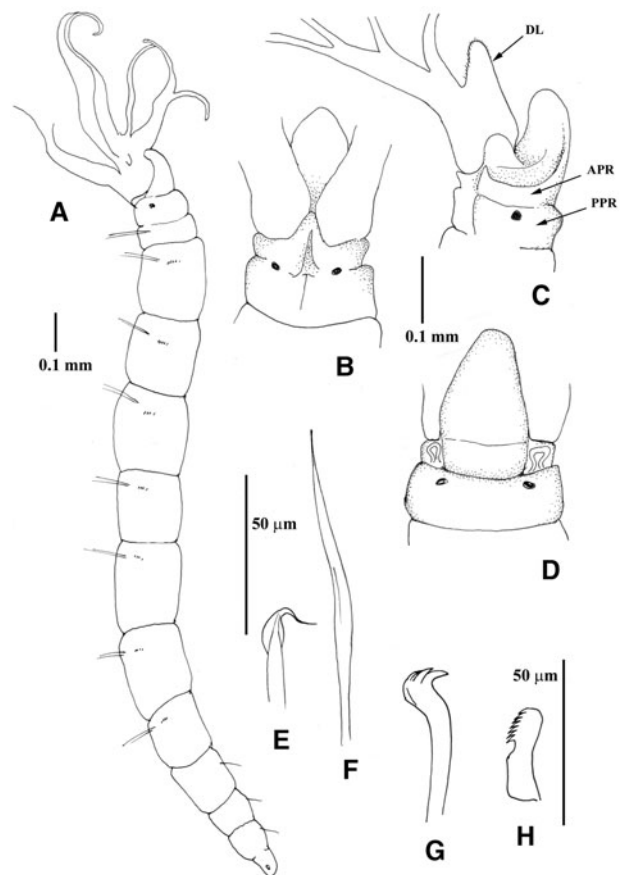


Fig. 6. *Parafabricia mazzellae* sp. nov.: (A) entire worm ventral view; (B) particular of the anterior end dorsal view; (C) particular of the anterior end lateral view without half crown, showing the dorsal lip; (D) particular of the anterior end ventral view; (E) pseudospatulate chaeta; (F) abdominal chaeta; (G) thoracic uncinus; (H) abdominal uncinus. APR, anterior peristomial ring; PPR, posterior peristomial ring; DL, dorsal lip.

One pair of black pygidial eyes. Superior thoracic notochaetae elongate, narrowly hooded (Figure 6F), 3 per fascicle. Inferior thoracic notochaetae of chaetigers 2 and 8 shorter and narrowly hooded, 1–2 per fascicle. One broadly hooded pseudospatulate chaeta in chaetigers 3–7 (Figure 6E). Acicular uncini in chaetigers 2–8, 10–12 per fascicle, main fang slender, single large tooth over the main fang followed by a series of small teeth; hood present (Figure 6G). Abdominal neuropodia with elongate narrowly hooded chaetae (Figure 6F), 2–3 per fascicle. Abdominal uncini with dentate region with 7–8 rows of teeth in profile, 2–3 teeth per row, manubrium 1.5 longer than dentate region (Figure 6H), in number of 18 to 25 per torus. Body wall without dark pigmentation in the anterior end of some individuals, others with just light brown pigmentation. Eggs in chaetigers 3–4. Vascular loops visible in the peristomial ring above the eyes (Figure 6D).

ETYMOLOGY

The species is named in memory of Dr Lucia Mazzella (head of the benthic ecology Laboratory of the Stazione Zoologica Anton Dohrn of Naples from 1986 to 1999), who initiated the first ecological studies at the Castello Aragonese area in the mid-1970s.

REMARKS

The genus was previously unreported in the Mediterranean. This genus was described from the Great Barrier Reef of Australia with the species *P. ventricingulata* inhabiting the intertidal algal mat (Fitzhugh, 1992). The diagnosis of the genus was identical to that of *Fabricia*, except for the shape of the ventral lobe collar, which is broad and thin, and for the length of the manubrium of abdominal uncini, which is shorter than in *Fabricia*. The specimens from our study area were attributed to the genus *Parafabricia* according to the flattened appearance of the ventral collar lobe coupled with the length of the manubrium of the abdominal uncini. *Parafabricia mazzellae* differs from *P. ventricingulata* in its longer size with a more elongated appearance, in the more developed ventral lobe collar, in the longer manubrium of the abdominal uncini (however, always shorter than in the genus *Fabricia*), but especially in the absence of the glandular ridge on chaetiger 5. At a first superficial view its slender appearance led to identify our specimens as another monotypic genus endemic to the Mediterranean Sea, *Pseudofabricia aberans* Cantone, 1972, especially for the development of ventral collar lobe (Figure 4G). However, despite this apparent similarity this taxon clearly distinguishes from *Pseudofabricia* genus for the presence of pseudospatulate chaetae in the thorax.

Genus *Novafabricia* Fitzhugh, 1990

Novafabricia posidoniae Giangrande & Licciano, 2006

MATERIAL EXAMINED

17 specimens; ambient pH stations: S1b: 5 individuals; low pH stations: N2c: 10; extreme low pH stations: S3c: 2.

A description of this taxon is given in Licciano & Giangrande (2006).

The species was described from the Tyrrhenian Sea (Ponza Island, Italy).

No differences were recorded in the material collected in the present study from previous findings. The species is

easily recognizable within the genus due to the pointed ventral collar lobe (Figure 4H, I).

Novafabricia infratorquata (Fitzhugh, 1983)

MATERIAL EXAMINED

8 specimens; low pH stations: S2c: 6; S2d: 2.

A redescription of this taxon is already present in Bick (2005) and in Licciano & Giangrande (2006).

REMARKS

The present record represents the second one for the Western Mediterranean basin. The species was described in the Caribbean Sea by Fitzhugh (1983) and then reported in the Western Mediterranean by Bick (2005) and in the Eastern basin by Giangrande & Licciano (2006).

The limited number of specimens in the present study had an absence of pigmentation, similar to the observed specimens from the Eastern basin (Figure 4L) (Licciano & Giangrande, 2006). This is in contrast to what was reported by Bick (2005) for the Western basin, and highlights some degree of variability within this taxon.

Genus *Rubrifabriciola* Huang, Fitzhugh & Rouse, 2011

Rubrifabriciola tonerella (Banse, 1959). Type locality: Tyrrhenian Sea (Italy)

MATERIAL EXAMINED

15 specimens; low pH stations: N2c: 4 individuals; S2a: 1; extreme low pH stations: N3b:1; N3c: 6; N3d: 3.

A description of this taxon is present in Bick (2005).

REMARKS

The genus was recently erected to accommodate several species previously ascribed to *Fabriciola* (Huang *et al.*, 2011). The synapomorphy for this group is the presence of red eyes, which persist after fixation (in formalin) and storage in alcohol (Figure 4M, N), in addition to some unique reproductive characters (Huang *et al.*, 2011). Most of the species included in this genus also have an abdominal pin-head neurochaetae. In the Mediterranean Sea, there are two known species of this genus: *R. tonerella* is the most common and is an autochthonous species (Banse, 1956), while *R. ghardaqa* is an introduced species from the Suez Canal (lessepsian migrant), which to date has only been reported in the Southern Adriatic Sea (Zenetos *et al.*, 2011).

Here we provide an identification key for the Mediterranean genera and species of Fabriciidae, which including the two new described taxa account for a total of 8 genera and 11 species (Giangrande *et al.*, 2013).

KEY TO FABRICIIDAE GENERA AND SPECIES UP TO NOW RECORDED FOR THE MEDITERRANEAN SEA

1. a. Thoracic uncini with distinct main fang surmounted by a series of small teeth. Up to four abdominal uncini and presence of short unbranched filament (ventral filamentous appendage).....*Echinofabricia rousei*
- b. Thoracic uncini with tooth decreasing in size over the main fang. Only three abdominal chaetigers 2
2. a. Pseudospatulate chaetae present, thoracic uncini with only one asymmetrical large tooth over the main fang,

- anterior peristomial ring collar developed as a ventral lobe, ventral filamentous appendages absent 3
- b. Pseudospatulate chaetae absent, some genera can have flagellate chaetae but never pseudospatulate, variable shape of thoracic uncini and collar..... 8
3. a. Pseudospatulate chaetae in chaetigers 3–6. Dorsal lips low and fused to proximal-most pinnule. Abdominal uncini with few large teeth in profile in the dentate region *Novafabricia* 4
- b. Pseudospatulate chaetae in chaetigers 3–7. Dorsal lips well developed and separate from the proximal pinnule. Abdominal uncini with a large number of small teeth in profile in the dentate region..... 5
4. a. Ventral collar lobe very pointed. Manubrium of abdominal uncini as long as dentate region *N. posidoniae*
- b. Ventral collar lobe more rounded. Manubrium of abdominal uncini two times longer than dentate region *N. infratorquata*
5. a. Anterior peristomial ring collar broad and flattened *Parafabricia mazzellae*
- b. Anterior peristomial ring collar low, triangular..... 6
6. a. Manubrium of abdominal uncini at least two times longer than dentate region *Fabricia* 7
- b. Manubrium of abdominal uncini about the same length of dentate region *Brifacia aragonensis*
7. a. Crown long with 6–7 pinnules in each radiole *F. stellaris stellaris*
- b. Crown short with only 3 pinnules in each radiole *F. stellaris adriatica*
8. a. Anterior peristomial ring collar as a ventral elongate and flattened lobe. Thoracic uncini with an asymmetric large tooth over the main fang. Ventral filamentous appendages very short *Pseudofabricia aberans*
- b. Anterior peristomial ring collar developed as a membranous collar, thoracic uncini with two large symmetrical teeth over the main fang 9
9. a. Membranous collar with a dorsal gap, ventral filamentous appendages long as radioles, peristomial red eyes present also after fixation *Rubifabriciola* 10
- b. Membranous collar entire all around, ventral filamentous appendages absent..... *Pseudofabriciola* 11
10. a. Collar higher ventrally, thoracic flagellate chaetae present *R. tonerella*
- b. Collar at the same height dorsally and ventrally, dorsal gap very wide *R. gardaqa*
11. a. Pygidium enlarged *P. analis*
- b. Pygidium elongated *P. longipyga*

DISCUSSION

Fabriciidae were particularly abundant and diverse in the study area. Almost all species collected were distributed across the entire acidification gradient, including the most

acidified zones (extreme low pH), with the exception *Novafabricia infratorquata*. The species belonging to genus *Novafabricia* Fitzhugh, 1990 appear to be more sensitive to low pH, since *N. posidoniae* were also less abundant in the most acidified zones, although this trend cannot be statistically tested.

By contrast the two new taxa described here (*Brifacia aragonensis* sp. nov. and *Parafabricia mazzellae* sp. nov.) were more abundant in the extreme low pH zone than in ambient pH conditions, with *P. mazzellae* being the most abundant among the Fabriciidae species in these zones. This trend suggests that these species are not only capable of coping with such harsh environmental conditions, but that they may actually benefit from reduced pH environments. This could be due to specific eco-physiological adaptation or acclimation to low pH conditions, as observed in the sabeliid *A. mediterranea* (Calosi *et al.*, 2013) which was also found in high abundance in the extreme low pH zones (Kroeker *et al.*, 2011; Ricevuto *et al.*, 2012). Because these fabriciid polychaetes are relatively uncommon under normal conditions (although they are often under-reported), their increased abundance in extreme low pH could also be due to increased competitive ability in the novel conditions and/or less competition with other taxa that are less tolerant of the harsh pH conditions (community compensation, Kroeker *et al.*, 2011). Another possible reason for the relatively high abundance of Fabriciidae in low and extreme low pH is the differences in macroalgal composition and cover among the pH zones. The diversity of macroalgae is reduced in the acidified conditions (Porzio *et al.*, 2011), and the resulting communities are quite simplified and homogeneous, dominated by filamentous algae and fleshy erect algae (Kroeker *et al.*, 2013), which mainly provide habitat for relatively small organisms and interstitial taxa such as Fabriciidae. Indeed, there was a high abundance of other small-sized species (e.g. *A. mediterranea*, various small amphipods species) and interstitial taxa (e.g. polychaete Exogoninae) in the same plots where fabriciids were abundant, which could signal that the invertebrates were responding indirectly to seawater chemistry via changes in habitat structure (Kroeker *et al.*, 2011; Ricevuto *et al.*, 2012, Gambi *et al.*, unpublished data).

Because so little is known about the ecology of this often neglected or under-reported group of small polychaetes, it is hard to compare the results from this study with other areas of the Mediterranean. However, a high abundance of some fabriciids belonging to *Fabricia* and *Augeneriella* has been reported in other potentially physiologically stressful conditions, such as sheltered environments with a large silt input (Lewis, 1968) or in brackish waters (Gitay, 1970), while species of the genus *Manayunkia* are common in freshwater (e.g. *M. aestuarina* Bourne, 1883) or in salt lakes (e.g. *M. athalassia* Hutchings, Denuer & Geddes, 1981). All of these examples suggest that adaptation to stressful conditions might be a common trait within this family.

The present paper documents a higher number of fabriciids in the Mediterranean than previously recorded, bringing the current estimates to eight genera and 11 species. Both the new genera presently reported for the Mediterranean were until now considered monotypical, belonging to an assemblage of other genera, all monotypical, in which individuals have the following combination of features: (1) triangular dorsal lips; (2) inner, ventral margins of branchial lobes adjacent to the dorsal lips, as opposed to developed as ventral

filamentous appendages; and (3) anterior peristomial ring collar in the form of a ventral lobe. This group includes *Fabricia* Blainville, 1828, *Parafabricia* Fitzhugh, 1992, *Brifacia* Fitzhugh, 1998 and *Raficiba* Fitzhugh, 2001. Pseudospatulate chaetae are present in chaetigers 3–7 in members of *Fabricia*, *Parafabricia* and *Brifacia* and in chaetigers 2–8 in members of *Raficiba* (Huang *et al.*, 2011). The cladistics analysis of Huang *et al.* (2011) revealed that the difference among these taxa is comparable to the differences existing among species in other genera (e.g. *Novafabricia*). Therefore, it is likely that the monotypical status of some genera should be reconsidered. As suggested by Fitzhugh (2010), however, the presence of several monotypical genera characterizing this group could be an artefact of the Linnean nomenclature for the scientific recognition of taxa.

The occurrence of these two new taxa in the Mediterranean is intriguing, considering that the co-generic species of both taxa are distributed in the austral hemisphere. Such a disjunct distribution is often the result of a transoceanic dislocation, such as the one that occurred for the sabellid *Laonome triangularis* Murray & Hutchings, 1984 (Çinar, 2009), or the most recent example of *Laonome calida* Capa, 2007 (Capa *et al.*, 2014), from Australia to the Mediterranean. The isopod genus *Mesanthura* Barnard, 1914 is another example of an originally-Australian taxon that was recently reported in the Mediterranean and showed features closely related to the Australian species *Mesanthura romulea* Poore & Lew Ton, 1986, although it was not possible to exclude the presence of a cryptic species (Lorenti *et al.*, 2009). In the case of the two species identified in this study, it is difficult to imagine an introduction of these two Fabriciidae to the Mediterranean, although it is not unusual that an undescribed species attracts attention only after introduction. This is the case for the sabellid *Bispira polyomma*, which was recently recorded by Faasse & Giangrande (2012). However, it is more likely that both these new taxa were already present in Mediterranean but previously confused with other Mediterranean species. In particular, *Brifacia aragonensis* sp. nov. could easily be misidentified as *Fabricia stellaris*, and *Parafabricia mazzellae* sp. nov. as *Pseudofabricia aberans* Cantone, 1972.

As a whole, the limited knowledge on the actual number of species existing across the world prevents any useful biogeographical inferences. Our understanding of Fabriciidae distribution is still far from exhaustive due to the difficulty in sampling and identifying these small and cryptic taxa. For example, the genus *Echinofabricia* Huang, Fitzhugh & Rouse, 2011 was recently reported for Mediterranean (Giangrande *et al.*, 2013) based on material collected 25 years ago, when the only other species in this genus had been reported on the Hawaiian coast. Other than the Mediterranean taxon, there are only three known species that are distributed throughout the world, including Australia. Moreover, our understanding of their distribution is complicated due to the fact that fabriciids are direct developers (Rouse & Fitzhugh, 1994), which increases the potential for their geographical isolation and speciation.

Finally, the occurrence and high abundance of new species in the extremely acidic conditions may also be related to the fact that unique habitats, such as the natural volcanic vents, have not been studied in great detail. As such, our understanding of the species capable of surviving in such harsh pH conditions is still scarce. Along these lines, a new Acoela interstitial worm (*Philactinoposthia ischia* Nilsson,

Wallberg & Jondelius, 2011) was recently described in the coarse sandy sediments in the north acidified area of the Castello Aragonese (Nilsson *et al.*, 2011). In addition, some morphological and genetic differentiation has been observed for the brown alga *Sargassum vulgare*, which represents one of the few macroalgae that thrives in the most acidified areas associated with the CO₂ vents (Porzio *et al.*, 2011; Chiarore *et al.*, 2012). Finally, transplant experiments of selected polychaetes from the CO₂ vent habitat along the pH gradient suggest that some of the species studied are either acclimated or locally adapted to low pH, showing exclusive genotypes in the acidified area (Calosi *et al.*, 2013). All these results suggest that the CO₂ vent system of Ischia represents a unique habitat that can be used as a natural laboratory to study also the evolutionary effects of ocean acidification. The evolutionary implications of ocean acidification for diversity and adaptation of the marine biota, recently highlighted by Sunday *et al.* (2011, 2013), remain, in fact, one of the main issues for future investigations on the far-reaching biological effects of one of the main aspects of oceanic global climate change.

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