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Project "Biodiversity MARE Tricase": a biodiversity inventory of the coastal area of Tricase (Ionian Sea, Italy) – Mollusca: Heterobranchia

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Abstract
The marine biodiversity of the Tricase coastal area (Ionian Sea, Italy) was investigated at the MARE Outpost (Avamposto MARE) between 2016 and 2017, with the help of citizen scientists and trained taxonomists. Among the most interesting groups encountered, heterobranch molluscs deserve a special mention. Altogether, 268 specimens were ascribed to this group and referred to 49 taxa. Notwithstanding the extensive literature on Mediterranean heterobranchs, two species proved to be new to the Italian coasts, the records of eight species represented their easternmost sightings in the Mediterranean Sea, and 13 taxa represented new records for the Ionian Sea. In addition, several feeding habits and phenological events were new to science. Although the European Union's Marine Strategy Framework Directive lists "Biodiversity is maintained" as the first descriptor in achieving "Good Environmental Status", our results highlighted the presence of conspicuous gaps in the knowledge of species distribution, taxonomy and ecology of heterobranch molluscs, indicating the necessity of even small-scale species checklists to understand biodiversity changes in worldwide biota.

Keywords: *Mediterranean Sea, biodiversity, Avamposto MARE, checklist, sea slugs*

Introduction

Biodiversity is the result of billions years of evolution and supplies all the goods and services humans need to live (Haines-Young & Potschin 2010). The European Union fully recognises the importance of biodiversity, but the unsustainable development of human societies threats its integrity (Sandifer et al. 2015; Thiede et al. 2016). Along this line, the European Union's Marine Strategy Framework Directive lists "Biodiversity is maintained" as the first descriptor in achieving "Good Environmental Status" (EU Marine Strategy Framework Directive 2008). In spite of the stated importance of biodiversity, and even within the widely studied Mediterranean fauna, important gaps still exist in basic knowledge, such as taxonomy, distribution, abundance and temporal trends of almost all taxonomic groups (Coll et al. 2010). In addition, there is now enough evidence that over the last few decades the distribution, phenology and physiology of many animal and plant species have been altered due to climate change, with feedbacks on local abundances and geographic ranges (Hughes 2000; Puce et al. 2009; Rivetti et al. 2014; Boero et al. 2016). Therefore, there is an urgent need for data, together with long-term data sets to assess the impact of global climate change on ecosystems (Visser & Both 2005; Boero et al. 2015). In 2015, the University of Salento, in collaboration with the CIHEAM IAM Bari (Mediterranean Agronomic

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Institute of Bari), the Regional Park "Costa Otranto – Santa Maria di Leuca e Bosco di Tricase", the Municipality of Tricase and the local association "Magna Grecia Mare" established the MARE Outpost (Avamposto Mare) to study the local marine biodiversity. The MARE Outpost is situated in the northern portion of the Ionian Sea (Tricase Porto, Salento Peninsula, Italy), according to the traditional borders of this sea, along the eastern coast of Italian Peninsula, in the middle of the Mediterranean Sea. It is not only a research facility for biodiversity research and promotion, but also a place of international cooperation for the sustainable development of Mediterranean coastal communities. The project "Biodiversity MARE Tricase" aims at monitoring Mediterranean biodiversity, promoting its wise management. In the framework of this project, species were collected whenever possible in collaboration with "citizen scientists" (European Citizen Science Association 2015) (e.g. fishermen, divers, local citizens, tourists, bathers, school students and any other any non-professional scientist who becomes engaged in a scientific enterprise, providing data and observations to professional scientists) and further analysed with the help of taxonomists. Results obtained so far have led to many interesting records, revealing a still-fragmented knowledge not only of the local fauna but of the Italian marine fauna as a whole. Among the explored taxa, the study of sea slugs (Mollusca: Gastropoda: Heterobranchia), formerly known as Opisthobranchia, led to novel results.

According to recent phylogenetic analyses based on morphological, embryological and molecular data, the taxon Opisthobranchia was found to be not monophyletic, and sea slugs are now placed within the clade Heterobranchia (Schrödl et al. 2011; Wägele et al. 2014). A large literature regarding these molluscs covers the Mediterranean Sea, especially concerning taxonomy, faunal studies and, more recently, non-indigenous species (see discussions in Gosliner et al. 2008). Regarding Italian waters, biodiversity assessments were recently carried out in different geographic areas (e.g. Trainito & Doneddu 2015; Furfaro & Mariottini 2016; Vitale et al. 2016; Zenetos et al. 2016), although the absence of specialists in the Salento Peninsula led to the fact that this taxonomic group has been poorly studied in the past, with the main articles published so far dating back to around 30 years ago (e.g. Perrone 1983, 1986). The scope of the present work, a starting point towards creating an overall biodiversity inventory of Tricase (Apulia, Italy), is to provide a preliminary checklist of sea slugs inhabiting the Tricase coastal area. In addition, remarks regarding taxonomy, reproduction, biogeography and feeding behaviour of selected species are provided so as to fill gaps in current knowledge of Mediterranean heterobranchs.

Materials and methods

Study site and samplings

Samplings were carried out along the coast between Otranto and Santa Maria di Leuca (Lecce, Italy), in the Otranto Channel (Ionian Sea), between 25 March 2016 and 16 September 2017. Most samplings were conducted in an area of ~33 km^2 here defined as the Tricase area (T), having as its northern boundary the latitude 39°59′30.7"N (WGS84), its southern boundary the latitude 39°54′13.5"N, its western boundary the coastline, and its eastern boundary a bathymetric depth of 70 m. The area has been defined as such because for the species found by citizen scientists (i.e. local fishermen) the exact sampling location was not available. The investigated site is characterised by steeply slanting rocky substrates from the surface to about 18 m depth. Below, the slope decreases and coralligenous platforms alternate with sand and mud down to about 70 m depth. Many marine karst caves and karst freshwater springs are also scattered along the whole coast. Moreover, the study site includes the harbour of Tricase, a small marina composed by an exposed natural inlet with a cement quay (the old harbour) and a semiclosed artificial harbour (the new harbour), characterised by the presence of several freshwater springs (Parenzan 1983; Onorato et al. 1999). In addition to the Tricase area, four sites located within the Otranto Channel were sampled, namely: (1) Acquaviva's cave (A) – 39°59′32.4"N, 18°24′55.5"E; (2) Bortones' cave (B) – 40° 03′43.9"N, 18°28′46.6"E; (3) Ciolo's cave (C) – 39°50′39.4"N, 18°23′10.4"E; (4) Castro's mussel farm (M) – 39°59′40.3"N, 18°25′37.0"E (Figure 1).

Specimens were collected by skin diving, scuba diving, nylon gillnets and trammels. In addition, citizen scientists (e.g. fishermen, divers, local citizens, tourists, bathers, etc.) were involved in the collection of animals. Overall, there were 72 sampling sessions. Samplings were not uniform during the year and most of them were performed during spring (52 during the month of April, May, and June). When possible, organisms were photographed in the field before collection with Canon Power-Shot D30, Sony Rx100 and Nikon AW130 cameras.

Laboratory work

Once sampled, organisms were brought to the laboratory of Avamposto MARE, maintained in

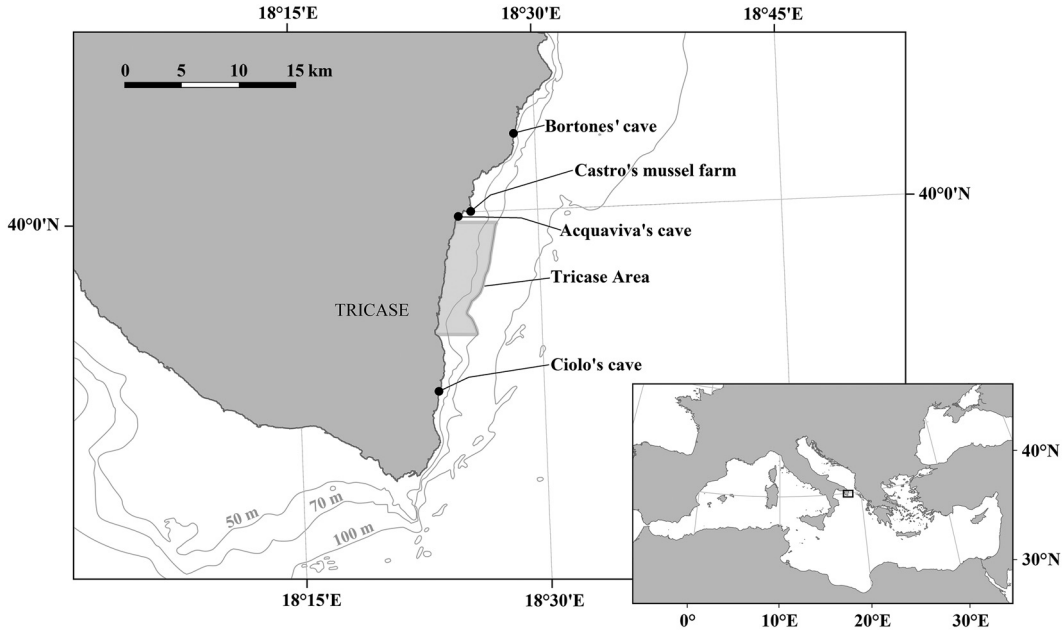

Figure 1. Map of the south-eastern tip of the Italian Peninsula. The Tricase Area is indicated in light gray; black dots indicate the additional study sites. Inset: Location of the study area in the Mediterranean Sea.

aerated tanks, and identified alive. Soon after, they were either released into the sea or fixed in EtOH 80% for further analysis. Species identification was made using books (e.g. Cattaneo-Vietti et al. 1990; Trainito & Doneddu 2014) and/or original descriptions and taxonomic literature (see below under single taxa). Species were referred to higher taxa following recent reviews and molecular phylogenies (e.g. Bouchet et al. 2017 and references therein), whereas lower taxonomy and nomenclature follow the World Register of Marine Species (WoRMS Editorial Board 2017).

Results

Altogether, 268 heterobranch specimens were found. They were referred to 49 taxa (Table I; Figures 2–4). Citizen scientists collected 11 species (22%). Most specimens were identified at the species level, but four were only tentatively assigned to a species due to their complex taxonomy. In addition, four specimens were identified to family or genus level only: an unidentified Onchidorididae specimen, a *Trinchesia* and two *Haminoea* species (Figure 4).

The 49 taxa censused here belong to two main clades (cohorts Ringipleura and Tectipleura), seven orders (Pleurobranchida, Nudibranchia, Umbraculida, Cephalaspidea, Runcinida, Aplysiida and Sacoglossa) (Table I; Figure 2), 27 families, and at least 34 genera. The orders showing the highest biodiversity were Nudibranchia, with 17 families and 30 species, followed by Cephalaspidea and Sacoglossa, with three families and five species each, and by Runcinida, with one family and four species (Table I; Figure 2). Regarding families, the richest in species number was Chromodorididae with five taxa, followed by Runcinidae and Dotidae, with four species each (Table I).

From an ecological point of view, *Doto pygmaea* was very common, with 145 individuals found in 15 sampling events, followed by *Cratena peregrina* with 15 individuals, and *Felimare picta* with 13 individuals. All the other species were rarely encountered. In addition, 10 feeding habits and five phenological events were recorded, some of which are new to science.

The records of two species are new for the Italian coasts: *Runcina* cf. *brenkoae* and *Runcina* cf. *ornata*. In fact, despite their tentative identification, no *Runcina* specimens referable to our species have ever before been reported from Italy. However, their identification may be assessed with certainty only after a molecular review of the Mediterranean Runcinidae is carried out. The records of eight species represented their easternmost sightings in the Mediterranean Sea (*Doto acuta*, *D.* cf. *koenneckeri*, *D. paulinae*, *D. pygmaea*, *Runcina adriatica*, *R.* cf. *brenkoae*, *R.* cf. *ornata* and *Ercolania viridis*), and all of them are also new records for the Salento Peninsula and the Ionian Sea, together with

Table I. List of the heterobranch taxa found during the "Biodiversity MARE Tricase" project, with notes on depth or depth range (in metres), substrate, phenology and finding area. New records marked in bold (see Taxonomic remarks). Phenology: Mat. – Mating event. Area: A – Acquaviva's cave; B – Bortones' cave; C – Ciolo's cave; M – Castro's mussel farm; T – Tricase area. In phenology, roman numerals indicate the month of observation.

Taxa	Depth	Substrate	Phenology	Area
Cohort RINGIPLEURA				
Subcohort NUDIPLEURA				
Order PLEUROBRANCHIDA				
Family PLEUROBRANCHAEIDAE				
Pleurobranchaea meckeli (Blainville, 1825)	34	On sand	-	M
Order NUDIBRANCHIA				
Family CALYCIDORIDIDAE				
***Diaphorodoris papillata* Portmann & Sandmeier, 1960**	30	On coralligenous formations	-	T
Family ONCHIDORIDIDAE				
Onchidoridae ind.	70	Amidst dead coralligenous formations	-	T
Family DORIDIDAE				
***Doris ocelligera* (Bergh, 1881)**	40	Amidst dead coralligenous formations	-	T
Family DISCODORIDIDAE				
Peltodoris atromaculata Bergh, 1880	18–30	On *Petrosia ficiformis* (Porifera)	Eggs (VI)	T
Platydoris argo (Linnaeus, 1767)	22	On coralligenous formations	-	T
Family DENDRODORIDIDAE				
Dendrodoris grandiflora (Rapp, 1827)	4	On rocks	-	B
Family PHYLLIDIIDAE				
Phyllidia flava Aradas, 1847	23	Feeding on *Acanthella acuta* (Porifera)	-	T
Family POLYCERIDAE				
Kaloplocamus ramosus (Cantraine, 1835)	70	On *Cellaria salicornioides* (Bryozoa)	-	T
Polycera quadrilineata (O. F. Müller, 1776)	40	Amidst dead coralligenous formations	-	T
Family CHROMODORIDIDAE				
Felimare picta (Schultz in Philippi, 1836)	8–18	On *Peyssonnelia* sp. (Rhodophyta)	-	T
Felimare tricolor (Cantraine, 1835)	14	On algae	-	T
Felimare villafranca (Risso, 1818)	10	On *Peyssonnelia* sp. (Rhodophyta)	-	T
Felimida krohni (Vérany, 1846)	40	Amidst dead coralligenous formations	-	T
Felimida luteorosea (Rapp, 1827)	40	On coralligenous formations	-	T
Family DOTIDAE				
***Doto acuta* Schmekel & Kress, 1977**	1	Feeding on *Corydendrium parasiticum* (Hydrozoa)	-	T
***Doto* cf. *koenneckeri* Lemche, 1976**	2	Feeding on *Aglaophenia octodonta* (Hydrozoa)	-	T
***Doto paulinae* Trinchese, 1881**	2	Feeding on *Aglaophenia octodonta* (Hydrozoa)	-	T
***Doto pygmaea* Oken, 1815**	0	Feeding on *Clytia hemisphaerica* (Hydrozoa)	Eggs (VII)	T
Family TRITONIIDAE				
Tritonia manicata Deshayes, 1853	1	On algae	-	T
Family FLABELLINIDAE				
Calmella cavolini (Vérany, 1846)	1	On *Eudendrium* sp. (Hydrozoa)	-	T
Flabellina affinis (Gmelin, 1791)	10	On *Eudendrium* sp. (Hydrozoa)	-	T, B
Family SAMLIDAE				
Luisella babai (Schmekel, 1972)	22	On coralligenous formations	-	T
Family AEOLIDIIDAE				
Berghia coerulescens (Laurillard, 1832)	1	On algae	-	T
Spurilla neapolitana (Delle Chiaje, 1841)	4	On rocks, in a marine cave	-	A, T
Family FACELINIDAE				
Cratena peregrina (Gmelin, 1791)	21–25	Feeding on *Eudendrium racemosum* (Hydrozoa)	-	T
***Facelina annulicornis* (Chamisso & Eysenhardt, 1821)**	4	On rocks	-	B
Family EUBRANCHIDAE				
***Eubranchus exiguus* (Alder & Hancock, 1848)**	0	Feeding on *Clytia hemisphaerica* (Hydrozoa)	-	T
Family FIONIDAE				
***Fiona pinnata* (Eschscholtz, 1831) complex**	0	Feeding on *Lepas pectinata* (Crustacea)	Eggs (V)	T
Family TRINCHESIIDAE				
Trinchesia caerulea (Montagu, 1804)	2	Feeding on *Sertularella ellisii* (Hydrozoa)	-	T
Trinchesia sp.	34	On the bottom of a mussel farm	-	M
Cohort TECTIPLEURA				
Subcohort EUOPISTHOBRANCHIA				
Order UMBRACULIDA				
Family UMBRACULIDAE				

(*Continued*)

Table I. (Continued).

Taxa	Depth	Substrate	Phenology	Area
Umbraculum umbraculum (Lightfoot, 1786)	1–3	On *Spirastrella cunctatrix* (Porifera)	-	T
Order CEPHALASPIDEA				
Family AGLAJIDAE				
Aglaja tricolorata Renier, 1807	34	On sand	-	M
Family BULLIDAE				
Bulla striata Bruguière, 1792	10	-	-	T
Family HAMINOEIDAE				
Haminoea sp. 1	40	Amidst dead coralligenous formations	-	T
Haminoea sp. 2	1	On *Corydendrium parasiticum* (Hydrozoa)	-	T
Weinkauffia turgidula (Forbes, 1844)	40	On coralligenous formations	-	T
Order RUNCINIDA				
Family RUNCINIDAE				
***Runcina adriatica* T**. Thompson 1980	20–40	On coralligenous formations	-	T
***Runcina* cf. *brenkoae* T**. Thompson 1980	2	On algae	-	T
Runcina ferruginea Kress, 1977	40	Amidst dead coralligenous formations	-	T
***Runcina* cf. *ornata* (Quatrefages, 1844**)	20	On coralligenous formations	-	T
Order APLYSIIDA [ANASPIDEA]				
Family APLYSIIDAE				
Aplysia depilans Gmelin, 1791	2	On algae	Mat. (IX)	T
Aplysia fasciata Poiret, 1789	3	On algae	Mat. (VII)	T
Aplysia cf. *parvula* Mörch, 1863	10	Feeding on Laurencieae (Florideophyceae)	Mat. (II)	T, A
Subcohort PANPULMONATA				
Superorder SACOGLOSSA				
Family LIMAPONTIIDAE				
***Ercolania viridis* (A. Costa, 1866)**	0	Feeding on *Chaetomorpha linum* (Chlorophyta)	-	T
Family BOSELLIIDAE				
Bosellia mimetica Trinchese, 1891	21	On *Flabellia petiolata* (Chlorophyta)	Eggs (VI)	T
Family PLAKOBRANCHIDAE				
Elysia timida (Risso, 1818)	7	On algae	-	T
Elysia viridis (Montagu, 1804)	10	Feeding on *Flabellia petiolata* (Chlorophyta)	-	T
Thuridilla hopei (Vérany, 1853)	7	On algae	-	T

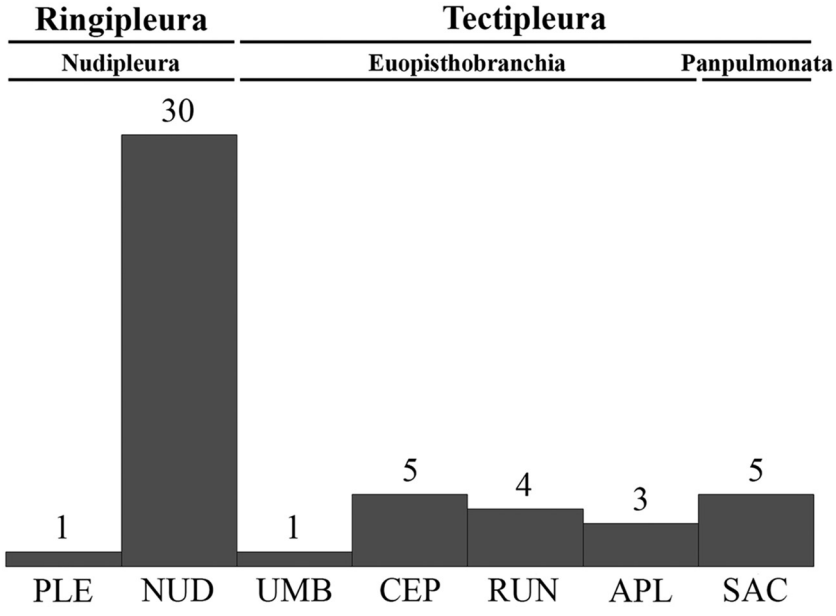

Figure 2. Main clades belonging to the heterobranch assemblage found during the "Biodiversity MARE Tricase" project and the overall number of species (divided per orders). Abbreviations used: PLE – Pleurobranchida; NUD – Nudibranchia; UMB – Umbraculida; CEP – Cephalaspidea; RUN – Runcinida; APL – Aplysiida; SAC – Sacoglossa.

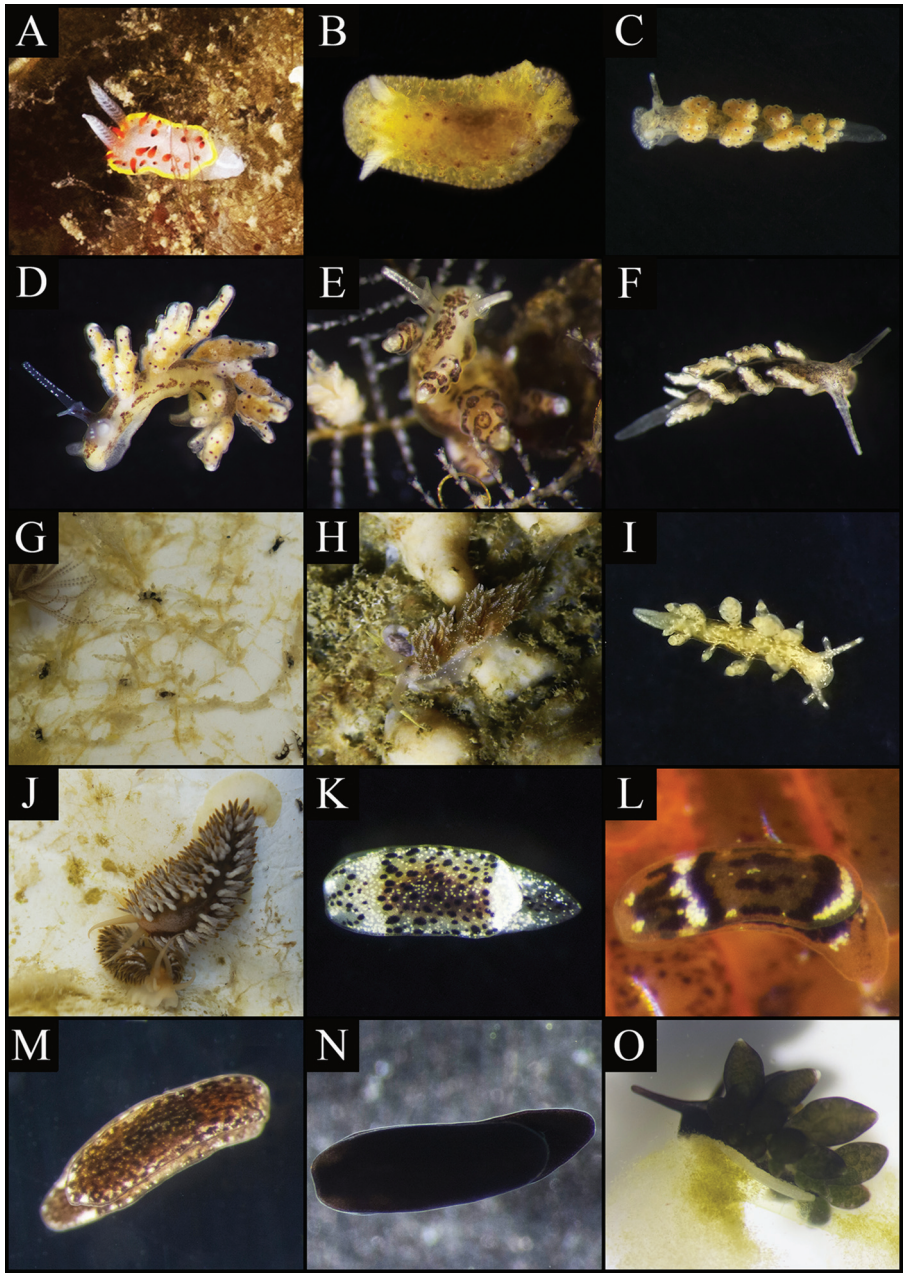

Figure 3. Heterobranch taxa whose geographic distribution is better understood due to the "Biodiversity MARE Tricase" project. Specimens not to scale. (A) *Diaphorodoris papillata*; (B) *Doris ocelligera*; (C) *Doto acuta*; (D) *Doto* cf. *koenneckeri*; (E) *Doto paulinae*; (F) *Doto pygmaea*; (G) *Doto pygmaea* assemblage on plastic debris. (H) *Facelina annulicornis*; (I) *Eubranchus exiguus*; (J) *Fiona pinnata;* (K) *Runcina adriatica*; (L) *Runcina capreensis* sensu *auctores* (= *Runcina adriatica*); (M) *Runcina* cf. *brenkoae*; (N) *Runcina* cf. *ornata*; (O) *Ercolania viridis*. A = 9 mm; B = 8.7 mm; C = 5.4 mm; D = 2.8 mm; E = 2.7 mm; F = 3.8 mm; H = 32 mm; I, N = 3 mm; J = 15 and 31 mm; K = 2.4 mm; L = 1 mm; M = 2 mm; O = 4 mm.

Diaphorodoris papillata, *Doris ocelligera*, *Facelina annulicornis*, *Eubranchus exiguus* and specimens belonging to the *Fiona pinnata* complex. Finally, *Kaloplocamus ramosus* deserves a mention: already reported from the Ionian Sea on the Amendolara Seamount at about 70 m depth (Perrone 1985), our record constitutes a confirmation of its presence in the area, 32 years since the first sighting. It was based on a single specimen found 26 May 2016 at 70 m depth on a colony of the bryozoan *Cellaria salicornioides* Lamouroux, 1816, a food item recently reported by Vanhaelen et al. (2014).

Data reported above are fully explained in the Faunal remarks section.

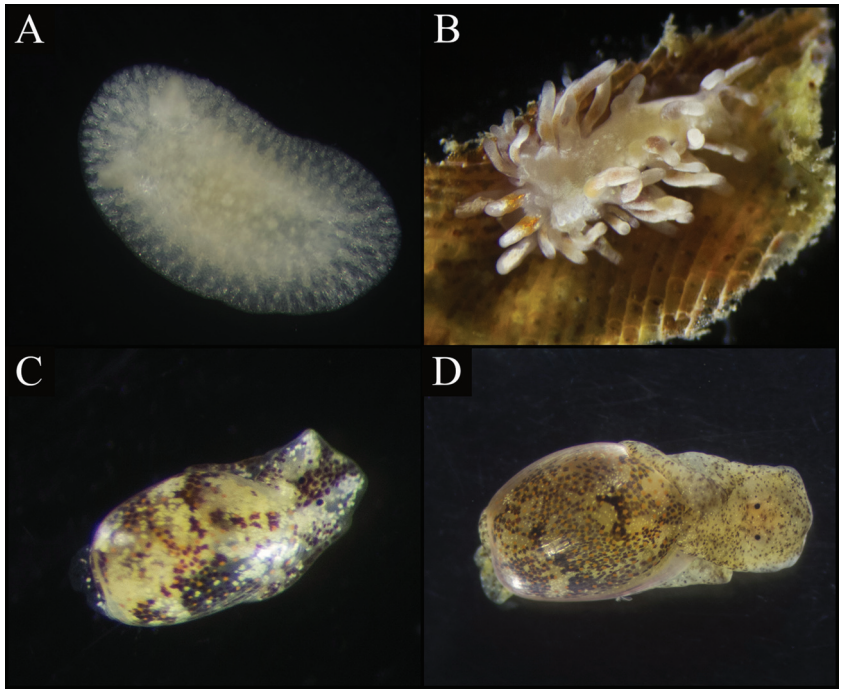

Figure 4. Unidentified heterobranch taxa found during the "Biodiversity MARE Tricase" project. Specimens not to scale. (A) Onchidoridae ind.; (B) *Trinchesia* sp.; (C) *Haminoea* sp. 1; (D) *Haminoea* sp. 2. A = 0.7 mm; B = 7.2 mm; C = 2 mm; D = 9 mm.

Faunal remarks

Family **Calycidoridae** Roginskaya, 1972
Genus ***Diaphorodoris*** Iredale & O'Donoghue, 1923
Diaphorodoris papillata Portmann & Sandmeier, 1960
(Figure 3A)

Material examined. One specimen, 9 mm length, Rio (39°55′09.6"N, 18°23′55.0"E), 16 september 2017, 30 m depth, on algal turfs on coralligenous formations.

Mediterranean distribution. Diaphorodoris papillata has a wide Mediterranean distribution, ranging from the western to the eastern parts of the basin, including the Adriatic Sea (e.g. Sammut & Perrone 1998; Cervera et al. 2004; Mienis 2015; Ciriaco & Poloniato 2016; Zenetos et al. 2016). In Italy, it was recorded in the Ligurian Sea, the Tyrrhenian Sea and the Adriatic Sea (e.g. Schmekel & Portmann 1982; Cattaneo-Vietti et al. 1990; Trainito & Doneddu 2015; Ciriaco & Poloniato 2016; Furfaro & Mariottini 2016 – specimen from Sistiana (Trieste): Ciriaco & Poloniato pers. comm.; Betti et al. 2017), whilst Cattaneo-Vietti and Giovine (2008) listed its presence in "sector 5" (south-eastern tip of Sicily, Pelagie Islands, and the Maltese archipelago), but this record refers to Malta (see Sammut & Perrone 1998).

Family **Doridae** Rafinesque, 1815
Genus ***Doris*** Linnaeus, 1758
Doris ocelligera (Bergh, 1881)
(Figure 3B)

Material examined. One specimen, 8.7 mm length, Funnuvojere's shoal (39°53′11.0"N, 18°24′51.9"E), 6 August 2017, 40 m depth, amidst dead coralligenous formations mostly composed by the arborescent bryozoans *Schizoretepora serratimargo* (Hincks, 1886) and *Myriapora truncata* (Pallas, 1766).

Mediterranean distribution. Doris ocelligera has a wide Mediterranean distribution, ranging from the western to the eastern parts of the basin, including the Adriatic Sea (e.g. Koutsoubas & Koukouras 1993; Sammut & Perrone 1998; Cervera et al. 2004; Zenetos et al. 2016). In Italy, it was recorded in the Ligurian Sea, the Tyrrhenian Sea and the Adriatic Sea (e.g. Sordi & Majidi 1956; Macali et al. 2013; Trainito & Doneddu 2015; Zenetos et al. 2016), whilst its presence in "sector 5"

(south-eastern tip of Sicily, Pelagie Islands, and the Maltese archipelago) listed by Cattaneo-Vietti and Giovine (2008) refers to Malta (see Sammut & Perrone 1998).

Family **Dotidae** Gray, 1853
Genus ***Doto*** Oken, 1815
Doto acuta Schmekel & Kress, 1977
(Figure 3C)

Material examined. Ten specimens, 2.5–5.4 mm length, new harbour of Tricase (39°55′53.4"N, 18° 23′44.6"E), 13 September 2017, 1 m depth, feeding on the hydrozoan *Corydendrium parasiticum* (Linnaeus, 1767).

Mediterranean distribution. Doto acuta has a restricted Mediterranean distribution, being only known from the western part of the basin (e.g. Schmekel & Portmann 1982; Cervera et al. 2004; Trainito & Doneddu 2015). In Italy, it was only recorded in the Tyrrhenian Sea (Schmekel & Portmann 1982; Trainito & Doneddu 2015).

Remarks. Doto acuta feeds on hydroids (McDonald & Nybakken 1997). In particular, Schmekel and Portmann (1982) reported *Obelia geniculata* (Linnaeus, 1758) as its food item. To the best of our knowledge, our sightings not only widen its known food items but overall constitute the first report of a nudibranch feeding on *C. parasiticum*.

Doto cf. ***koenneckeri*** Lemche, 1976
(Figure 3D)

Material examined. One specimen, 2.8 mm length, Monte Purtusu (39°55′34.9"N, 18°23′45.6"E), 20 April 2016, 2 m depth, feeding on the hydrozoan *Aglaophenia octodonta* Heller, 1868.

Mediterranean distribution. Doto koenneckeri has a restricted Mediterranean distribution, being only known from the western part of the basin (e.g. Cervera et al. 2004; Betti et al. 2015; Trainito & Doneddu 2015). In Italy, it was only recorded in the Ligurian Sea and the Tyrrhenian Sea (Betti et al. 2015; Trainito & Doneddu 2015).

Remarks. Doto koenneckeri feeds exclusively on hydroids of the genus *Aglaophenia* Lamouroux, 1812. In the Mediterranean Sea, this species is known to feed on *A. octodonta*, and other *Aglaophenia* taxa (McDonald & Nybakken 1997; Pujals et al. 2014; Betti et al. 2015).

Doto paulinae Trinchese, 1881
(Figure 3E)

Material examined. One specimen, 2.7 mm length, Avamposto MARE (39°55′36.1"N, 18°23′45.2"E), 23 April 2016, 2 m depth, feeding on the hydrozoan *Aglaophenia octodonta* Heller, 1868.

Mediterranean distribution. Doto paulinae has a restricted Mediterranean distribution, being only recorded in the western Mediterranean Sea (e.g. Trinchese 1881; Haefelfinger 1960; Schmekel & Portmann 1982; Cervera et al. 2004; Ballesteros et al. 2016). In Italy, it was only recorded in the Ligurian Sea and the Tyrrhenian Sea (e.g. Trinchese 1881; Schmekel & Portmann 1982; Trainito & Doneddu 2015).

Remarks. Doto paulinae is an almost unmistakable species, only showing slight similarities with *Doto floridicola* Simroth, 1888. However, *D. paulinae* specimens figured in the current literature show some differences (e.g. see Trinchese 1881; Marín & Ros 1991; Trainito & Doneddu 2015; Ballesteros et al. 2016; present paper), which led to the suspicion that undiscovered cryptic diversity may be present within this taxon. *Doto paulinae* is known to feeds on hydroids (McDonald & Nybakken 1997); reported food items are *Aglaophenia pluma* (Linnaeus, 1758), *Obelia geniculata* (Linnaeus, 1758), and *Eudendrium* Ehrenberg, 1834 taxa (Schmekel & Portmann 1982; Marín & Ros 1991). *Aglaophenia octodonta* proved to be an additional food item.

Doto pygmaea Oken, 1815
(Figure 3F,G)

Material examined. One hundred and forty-five specimens, 1.1–9.9 mm length, Tricase area, between 3 May 2016 and 20 June 2017, 0 m depth, on floating debris, feeding on the hydrozoan *Clytia hemisphaerica* (Linnaeus, 1767).

Mediterranean distribution. Doto pygmaea has a restricted Mediterranean distribution, being only recorded in the western Mediterranean Sea (e.g. Schmekel & Portmann 1982; Ballesteros et al. 2016). In Italy, it was only recorded in the Tyrrhenian Sea (Schmekel & Portmann 1982).

Remarks. Doto pygmaea can be easily identified by the characteristic shape of the cerata, showing an asymmetric form with the internal side very arched, without tubers and pseudobranchs (Bergh 1871; Ortea et al. 1997). It was found commonly and in high densities during our samplings, mostly living on floating objects, such as plastic debris, and always feeding on *C. hemisphaerica* stolons (Figure 3G). Previously, *D. pygmaea* was only reported to feed

on *Aglaophenia pluma* (Linnaeus, 1758) and *Obelia geniculata* (Linnaeus, 1758) (Schmekel & Portmann 1982; McDonald & Nybakken 1997); therefore, our sightings widen its food range. Egg masses were found in July.

Family **Facelinidae** Bergh, 1889
Genus ***Facelina*** Alder & Hancock, 1855
Facelina annulicornis (Chamisso & Eysenhardt, 1821)
(Figure 3H)

Material examined. One specimen, 32 mm length, Bortones' cave (40°03′43.9"N, 18°28′46.6"E), 22 March 2017, 4 m depth, on a limestone boulder.

Mediterranean distribution. Facelina annulicornis has a wide Mediterranean distribution, ranging from the western to the eastern parts of the basin, including the Adriatic Sea (e.g. Cervera et al. 2004; Öztürk et al. 2014; Zenetos et al. 2016). In Italy, it was recorded in the Tyrrhenian Sea, the Strait of Messina and the Adriatic Sea (e.g. Cattaneo-Vietti et al. 1990; Vazzana 2010; Zenetos et al. 2016), whilst its presence in "sector 5" (south-eastern tip of Sicily, Pelagie Islands and the Maltese archipelago) listed by Cattaneo-Vietti and Giovine (2008) refers to Malta (see Sammut & Perrone 1998).

Family **Eubranchidae** Odhner, 1934
Genus ***Eubranchus*** Forbes, 1838
Eubranchus exiguus (Alder & Hancock, 1848)
(Figure 3I)

Material examined. Two specimens, 3–4 mm length, Tricase area, 2 June 2017, on a floating plastic bag, feeding on the hydrozoan *Clytia hemisphaerica* (Linnaeus, 1767).

Mediterranean distribution. Eubranchus exiguus has a wide Mediterranean distribution, ranging from the western to the eastern parts of the basin, including the Adriatic Sea (e.g. Cervera et al. 2004; Crocetta et al. 2015; Zenetos et al. 2016). In Italy, it was recorded in the Tyrrhenian Sea and the Adriatic Sea (e.g. Schmekel & Portmann 1982; Cattaneo-Vietti et al. 1990; Trainito & Doneddu 2015; Zenetos et al. 2016), whilst its presence in "sector 5" (south-eastern tip of Sicily, Pelagie Islands and the Maltese archipelago) listed by Cattaneo-Vietti and Giovine (2008) refers to Malta (see Sammut & Perrone 1998).

Remarks. Eubranchus exiguus has a wide diet breadth, composed by hydrozoans of the genera *Abietinaria* Kirchenpauer, 1884, *Bougainvillia* Lesson, 1830, *Clytia* Lamouroux, 1812, *Cordylophora* Allman, 1844, *Coryne* Gaertner, 1774, *Halecium* Oken, 1815, *Hydrallmania* Hincks, 1868, *Kirchenpaueria* Jickeli, 1883, *Laomedea* Lamouroux, 1812, *Obelia* Péron & Lesueur, 1810, *Plumularia* Lamarck, 1816, *Sertularia* Linnaeus, 1758, and *Tubularia* Linnaeus, 1758 (Todd 1981; McDonald & Nybakken 1997). It was previously found on *C. hemisphaerica*, but never feeding on it (Swennen 1961; Swennen & Dekker 1987).

Family **Fionidae** Gray, 1857
Genus ***Fiona*** Alder & Hancock [in Forbes & Hanley], 1853
Fiona pinnata (Eschscholtz, 1831) complex
(Figure 3J)

Material examined. Four specimens, 15–31 mm length, Tricase area, 23 May 2017 and 29 May 2017, on a floating plastic object and on a floating piece of wood, feeding on the cirriped crustacean *Lepas pectinata* Spengler, 1793.

Mediterranean distribution. Fiona pinnata had been widely recorded from the Mediterranean Sea, from the western to the eastern parts of the basin, but never from the Adriatic Sea (e.g. Barash & Danin 1972; Cervera et al. 2004; Crocetta et al. 2015; Zenetos et al. 2016). In Italy, it was recorded in the Ligurian Sea and the Tyrrhenian Sea (e.g. Trainito & Doneddu 2015; Betti et al. 2017), whilst its presence in "sector 5" (south-eastern tip of Sicily, Pelagie Islands and the Maltese archipelago) listed by Cattaneo-Vietti and Giovine (2008) refers to Malta (see Sammut & Perrone 1998).

Remarks. Fiona pinnata occurs exclusively on macroalgal rafts and other floating substrates. Although commonly considered a cosmopolitan species, recent studies suggest it could be a species complex (Trickey et al. 2016). It is known to feed on both neustonic colonial hydrozoans such as *Porpita porpita* (Linnaeus, 1758) and *Velella* Lamarck, 1801 taxa, and on cirriped crustaceans, such as those belonging to the genera *Alepas* Rang, 1829, *Dosima* Gray, 1825, and *Lepas* Linnaeus, 1758 (McDonald & Nybakken 1997). *Lepas pectinata* proved here to be an additional food item. Egg masses were found in May.

Family **Runcinidae** H. Adams & A. Adams, 1854
Genus ***Runcina*** Forbes [in Forbes & Hanley], 1851
Runcina adriatica T. Thompson, 1980
(Figure 3K,L)

Material examined. Three specimens, 1–2.4 mm length, Funnuvojere's shoal (39°53′11.0"N, 18° 24′51.9"E), 6 August 2017, 40 m depth, on dead

coralligenous formations; one specimen, 1 mm length, Isola locality (39°56′49.9"N, 18°24′13.0"E), 19 June 2016, 20 m depth, on red algae from coralligenous formations entangled in fishermen's nets.

Mediterranean distribution. *Runcina adriatica* has a wide Mediterranean distribution, ranging from the western to the central parts of the basin, including the Adriatic Sea (e.g. Thompson 1980; Cachia et al. 2001; Ballesteros et al. 2016; Zenetos et al. 2016). In Italy, it was recorded in the Tyrrhenian Sea (e.g. Schmekel & Cappellato 2002; Klussmann-Kolb 2004), whilst its presence in "sector 5" (south-eastern tip of Sicily, Pelagie Islands and the Maltese archipelago), and "sectors 8 and 9" (central and northern Adriatic Sea, from the Gulf of Manfredonia to Istria) listed by Cattaneo-Vietti and Giovine (2008) refer to Malta (see Sammut & Perrone 1998), Croatia (Thompson 1980) and Slovenia (Lipej et al. 2008), respectively. These two latter records were mistakenly included by Zenetos et al. (2016) as held in the Italian Adriatic Sea, based on a misreading of Cattaneo-Vietti and Giovine (2008).

Remarks. The three specimens found showed a variegated colour pattern. Whilst one specimen clearly belongs to *R. adriatica*, another is very similar to specimens widely recorded in the older literature from Spain and Malta as *Runcina capreensis* (Mazzarelli, 1894) (references in Cervera et al. 2004; Sammut & Perrone 1998; colour photos of specimens from Spain in Correa et al. 2012; from Malta in www.naturamalta.com). However, such a colour pattern does not match that originally described by Mazzarelli (1894), although clearly showing the big black patches near the eyes highlighted in its original description. Finally, the third specimen shows an almost intermediate colour pattern between *R. adriatica* and specimens previously ascribed to *R. capreensis* sensu *auctores* (Figure 3L). This is in agreement with Cervera et al. (2004), who first suggested that Spanish records of *R. capreensis* were doubtful, and with Ballesteros et al. (2016), who considered *R. capreensis* sensu *auctores* a misidentification of *R. adriatica*.

Runcina cf. ***brenkoae*** T. Thompson, 1980 (Figure 3M)

Material examined. One specimen, 2 mm length, Monte Purtusu (39°55′34.9"N, 18°23′45.6"E), 9 June 2016, 2 m depth, on algae.

Mediterranean distribution. *Runcina brenkoae* is a poorly known species described from Croatia and subsequently recorded only on the Mediterranean coastline of Spain and France (Thompson 1980; Thompson & Brodie 1988; Schmekel & Cappellato 2002; Ballesteros et al. 2016), as its presence in "sectors 8 and 9 (central and northern Adriatic Sea, from the Gulf of Manfredonia to Istria)" listed by Cattaneo-Vietti and Giovine (2008) refers to Croatia. These records were mistakenly included by Zenetos et al. (2016) as held in the Italian Adriatic Sea, based on a misreading of Cattaneo-Vietti and Giovine (2008).

Runcina cf. ***ornata*** (Quatrefages, 1844) (Figure 3N)

Material examined. One specimen, 3 mm length, Isola locality (39°56′49.9"N, 18°24′13.0"E), 19 June 2016, 20 m depth, on red algae from coralligenous formations entangled in fishermen's nets.

Additional material: five specimens, 1–4 mm length, San Foca, southern Adriatic Sea (40° 18′19.5"N, 18°24′40.0"E), 4 m depth, in *Posidonia oceanica* (Linnaeus) Delile meadows.

Mediterranean distribution, and remarks. *Runcina ornata* is a poorly known species described from the French Atlantic coasts (Quatrefages 1844) and only recorded in the Mediterranean Sea from the Strait of Gibraltar (Cervera et al. 2004). However, specimens almost identical to ours have been recorded from Malta as *Runcina* [cf.] *zavodniki* Thompson, 1980 (Sammut & Perrone 1998; Cachia et al. 2001; photos of specimens from Malta in www.naturamalta.com), although this identification was subsequently questioned and virtually moved to *R. ornata* by Rudman (1999). Cattaneo-Vietti and Giovine (2008) noticed this, and listed its presence in "sector 5" (south-eastern tip of Sicily, Pelagie Islands and the Maltese archipelago) as doubtful, referring to Malta. Further specimens that may be conspecific to ours are those from Croatia described by Thompson (1980) as *R. zavodniki*, although this taxon was subsequently considered by Gosliner (1990) and by Schmekel and Cappellato (2002) to be a synonym of *Runcina ferruginea* (Kress, 1977), originally described from Plymouth (Great Britain). We provisionally decided to report this taxon as "*Runcina* cf. *ornata* (Quatrefages, 1844)" to highlight the occurrence of a runcinid taxon with such a colour pattern in the studied area, pending further studies on the taxonomic relationships between topotypical *R. ferruginea*, *R. ornata* and *R. zavodniki* specimens, as well as on the specimens from the central Mediterranean Sea shown here and already recorded from Malta.

Family **Limapontiidae** Gray, 1847
Genus ***Ercolania*** Trinchese, 1872
Ercolania viridis (A. Costa, 1866)
(Figure 3O)

Material examined. One specimen, 4 mm length, Avamposto MARE (39°55′44.1”N, 18°23′41.2”E), 25 May 2016, tide level in a rock pool, grazing on the green algae *Chaetomorpha linum* (O.F. Müller) Kützing.

Mediterranean distribution. Ercolania viridis published distribution was restricted to the western part of the basin (e.g. Trinchese 1872; Schmekel & Portmann 1982; Cervera et al. 2004; Ballesteros et al. 2016) until Vitale et al. (2016) reported its presence from the central Mediterranean, based on samples from Faro Lake (Strait of Messina). Despite the fact that our sighting constitutes the easternmost Mediterranean record of this taxon, it is noteworthy to highlight that *E. viridis* has also been recorded from the Black Sea, where it is considered a non-indigenous species introduced through shipping from the Caribbean (Son 2010), and that its unpublished presence was noticed herein from Tunisia and Croatia (Rudman 2003; Ballesteros et al. 2012–2018).

Remarks. Ercolania viridis is known to graze on several Chlorophyta, such as *C. linum, Chaetomorpha aerea* (Dillwyn) Kützing and *Chaetomorpha capillaris* (Kützing) Børgesen, as well as on *Cladophora* Kützing, 1843 and *Cladophoropsis* Børgesen, 1905 taxa (Händeler & Wägele 2007).

Discussion and conclusions

Sea slugs are important components of marine biodiversity. However, although the Mediterranean malacofauna is the most studied worldwide, and heterobranch taxa, for their charm, attract interest even among SCUBA divers, the knowledge of Mediterranean sea slugs is still far from complete (Gosliner et al. 2008; Sabelli & Taviani 2014). Our results contribute to filling some gaps in the knowledge of distribution, taxonomy and ecology of these taxa. In fact, the finding of two species that are new for the Italian coasts during an 18-month project, as well as the new records for the eastern Mediterranean and the Ionian Sea, show that local heterobranch biodiversity, and more generally that of the eastern Mediterranean, has been poorly investigated so far. Indeed, even though an extensive literature is available from the Mediterranean Sea, most of the studies come from its westernmost areas, and in particular from the Gulf of Naples, the Iberian Peninsula and the French coast, places where sea slug specialists have widely worked in the past (Gosliner et al. 2008). This seems to be also confirmed by the fact that the majority of the species newly reported from the area are straightforward to identify and have already been censused along the Italian coastline, and mostly from the Tyrrhenian Sea and the Adriatic Sea. Therefore, they presumably have a distribution wider than reported but have simply been overlooked due to the absence of focused field studies.

A different situation regards species belonging to two families dealt with here. In particular, in the Mediterranean Sea, Runcinidae have been widely studied only in the Iberian Peninsula (e.g. Cervera et al. 1991; Ballesteros et al. 2016), the French coast (e.g. Schmekel & Cappellato 2002), and the northern Adriatic Sea (e.g. Thompson 1980, 1988). The almost complete lack of data from other biogeographic areas, including the whole eastern Mediterranean, explains our two new records for Italy. This is due to the shortage of experts able to identify these tiny molluscs, and to their cryptic behaviour and small sizes, which leave them overlooked during general biodiversity studies. This again suggests that these species are presumably much commoner than what the current literature reports. Dotidae is another heterobranch clade usually neglected because of a lack of taxonomists and because of the considerable complexity of their identification. All the species found during this project represented new records for the Ionian Sea and overall the easternmost records in the Mediterranean Sea. *Doto pygmaea*, usually living in association with pelagic *Sargassum* mats, deserves a mention. Once very rare in the Mediterranean Sea, it was recorded on almost every piece of floating plastic found in the area during this study. This suggests that at least this taxon may be a real newcomer in the area, and has presumably spread farther during the last decades in the Mediterranean basin, enhanced by the increase in plastic debris (Aliani & Molcard 2003).

This study confirms that despite the long history of faunistic studies in the Mediterranean Sea and the importance given to biodiversity during the last few decades, there is still much work to be done. In addition, the present study further corroborates the usefulness of science projects involving citizen scientists, not only in early warning and monitoring of marine invasive species (Boero 2013) but also as a potential tool for ecological data collection and environmental awareness enhancement. Increasing knowledge on species distribution and phenology is fundamental for understanding the effects of climate change and human actions on ecosystems and to assess “Good Environmental Status” as the Marine Strategy Framework Directive requires. Therefore, there is an urgent need for long-term biodiversity

monitoring, observational articles and new taxonomists able to identify species and perform research on marine organisms (Giangrande 2003; Boero 2013). Inspired by the seminal work held by Salvatore Lo Bianco in the Gulf of Naples (Lo Bianco 1909), this biodiversity project represents an important addition to the knowledge of local heterobranch biodiversity, a starting point for several new studies, and, at the same time, a way to promote nature and biodiversity conservation in the Italian peninsula and the Mediterranean Sea.

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