

## Article

# Alien Travel Companies: The Case of Two Sea Slugs and One Bryozoan in the Mediterranean Sea

Erika Mioni <sup>1</sup> and Giulia Furfaro <sup>2,\*</sup> <sup>1</sup> Istituto Comprensivo Statale “ISA2”, 19122 La Spezia, Italy<sup>2</sup> Department of Biological and Environmental Sciences and Technologies-DiSTeBA, University of Salento, Via Prov. le Lecce-Monteroni, 73100 Lecce, Italy

\* Correspondence: giulia.furfaro@unisalento.it; Tel.: +39-0832-29-8660

**Abstract:** Mediterranean marine fauna is constantly changing due to the entry of non-indigenous (NI) species and the loss of endemic biodiversity. In this framework, it is very important to monitor this constant change and investigate possible new pathways of dispersion. Marinas and ports are considered key stations to detect and study some important ecological aspects, such as NI and invasive species, the effects of climate change, and pollution. Here, we reported the case of a group of NI species that presumably reached the Mediterranean Sea together, each of them being ecologically associated with one another. The bryozoan *Amathia verticillate* and the sea slugs *Favorinus ghanensis* and *Polycerella emertoni* were found in the shallow waters of Fezzano’s marina in the gulf of La Spezia (Ligurian Sea, Mediterranean Sea). Molecular analyses were carried out to exclude cryptic diversity and to investigate the phylogenetic relationships occurring between closely related taxa. The spreading of these two NI sea slugs into the Mediterranean Sea was confirmed and the first record of *P. emertoni* from the Ligurian Sea reported. These findings shed some light on the poorly known ecology of these species that could be useful for future monitoring and conservation strategies.

**Keywords:** Heterobranchia; Nudibranchia; NIS; trophic chain; citizen science; Ligurian Sea



**Citation:** Mioni, E.; Furfaro, G. Alien Travel Companies: The Case of Two Sea Slugs and One Bryozoan in the Mediterranean Sea. *Diversity* **2022**, *14*, 687. <https://doi.org/10.3390/d14080687>

Academic Editor: Bert W. Hoeksema

Received: 11 July 2022

Accepted: 18 August 2022

Published: 21 August 2022

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

The Mediterranean Sea is a semi-closed basin that is considered an important hotspot for marine biodiversity and endemism [1–3]. In fact, it is currently accepted that, with only 0.82% of the global oceanic surface, the Mediterranean Sea has more than 4% of all known marine species, with a rate of endemism estimated at 45% [1,4–6]. While the Mediterranean Sea is considered a hotspot for biodiversity, it is also highly affected by invasions of allochthonous organisms. This tendency has increased in the last decades, especially in the European Atlantic and Mediterranean waters, as suggested by the growing literature that has been published on this issue lately [7–11]. This problematic threat is favored by the abundant presence of lagoons, estuaries, and marinas [12]; favorable geographic and climatic conditions [13]; and intense human activity [10]. In fact, the anthropic impact and the constantly growing marine human activities (freight transport, ferries, cruise ships, and yachting) constitute a powerful drive that has increased the spread of non-indigenous (NI) species with negative effects on Mediterranean marine biodiversity and ecosystem functioning [14]. In this context, an important role is played by harbors and marinas, considered as being particularly vulnerable habitats to biological invasion since they are subject to the high pressures of boating and cruising, which can act as vectors for alien species distribution [15].

Mollusks are one of the most representative groups in terms of diversity, with about 100,000 identified species and ca. 2500 inhabiting the Mediterranean basin, of which 71% live in Italian waters [16], making the Italian malacofauna a very remarkable part of the Mediterranean fauna. Mediterranean mollusks also include the greatest number of invading taxa, with 156 alien, NI species recently catalogued [9,10,17,18], of which 44 were

reported from Italian seas [16]. Among Gastropoda, the amount of alien species to date reported is 101, with new Heterobranchia constantly being added [18–25]. Nudibranch sea slugs are known to show specialized feeding strategies that directly influence species' distribution patterns [26]. This ecological characteristic could represent one of the main limits to the spreading of NI species that, in fact, need to find sources of available food to settle and establish in a new, invaded habitat. Cases that confirm this behavior are new alien food webs with prey–predator relationships involving only NI species [20,21,25,27,28]. These new trophic chains, usually composed by a NI sessile prey eaten by an allochthonous sea slug species, have mainly been reported from small lakes, marinas, or harbor areas near mussel farms and characterized by high human impact [20,21,27,28]. Considering that shipping activities are the most plausible vector of introduction and that, frequently, the same vector can introduce whole clusters of associated organisms, as widely demonstrated also for algal species [29], it could be extremely important to monitor these anthropogenic habitats to detect new possible cases of biological invasions. A sustainable monitoring program that promotes the long-lasting engagement of volunteers in citizen science research activities can represent the opportunity of an early warning system [30]. Regarding this topic, the coastal monitoring program of the project “Percorsi nel Blu” (“Blue Paths”), promoted by the ISA2 Institute of La Spezia, Italy [24,31], can be considered a valid support for scientific investigation and to check the spreading of alien species [23,24,32,33]. Since 2011, the project has been setting up a network among schools, institutions, and research centers for citizen science research activities throughout the coastal areas located in the Ligurian and Tyrrhenian Seas and surrounding the “Pelagos” Mammals’ Sanctuary [24,31,34]. Furthermore, since November 2019, the project has been promoting “Fantastic Creatures and where to find them”, an operative program focused on the coastal monitoring of the fouling community along artificial floating pontoons in marinas located in the Gulf of La Spezia [23,24], which achieved interesting results on the presence of indigenous and non-indigenous marine Heterobranchia, including the first Italian report of *Favorinus ghanensis* Edmunds, 1968 [23,24].

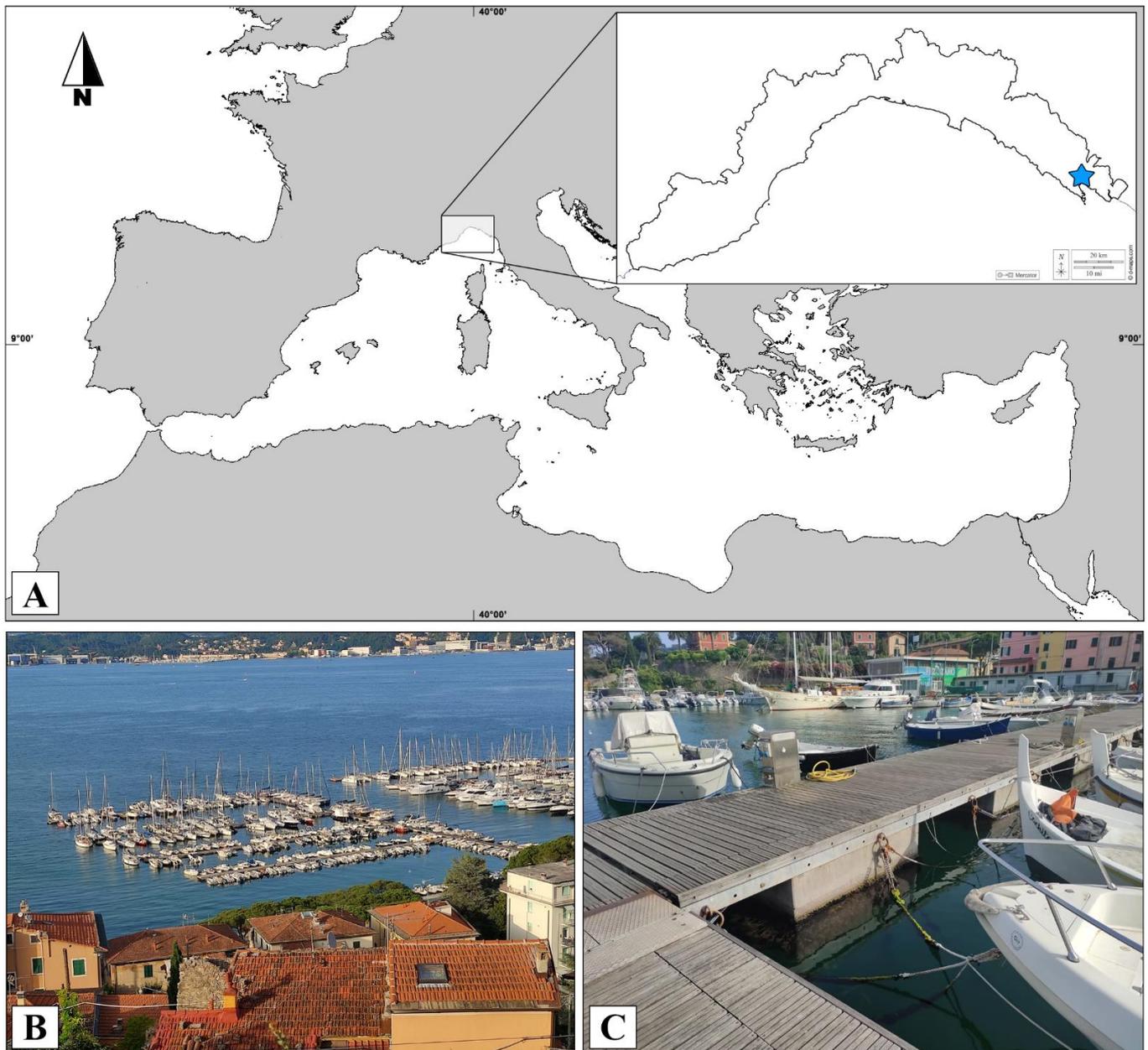
Taking all these considerations into account, the main aims of this study are to: (1) investigate the possible presence of new NI species, (2) unravel possible cases of new NI trophic webs not reported before from the studied area, (3) investigate the phylogenetic relationships between NI species and their closely related taxa, and (4) speculate on the possible way of introduction to promote alternative and more efficient future conservation strategies.

## 2. Materials and Methods

Marina of Fezzano is in an inlet on the western side of the Gulf of La Spezia facing northeast (44° 4' 52.2" N, 9° 49' 37.8" E). It houses the mooring of the residents' boats and is subject to intense maritime traffic due to the nearby cruise terminal of the port of La Spezia. Fezzano's marina is a complex of floating piers made with one principal pier from which four secondary ones branch off (Figure 1).

The pier's boardwalk floats on parallelepiped-shaped, concrete pontoons with dimensions of 180 × 225 × 100 cm (width × depth × height) (Figure 1C). This kind of pier provides protection from the wind and shelter for the boats, creating a harbor characterized by shallow water and a quiet sea surface that represents the ideal condition for the spreading of the bryozoan *Amathia verticillata* (delle Chiaje, 1822). The monitoring surveys were realized at the inner and central areas of the floating complex on 4 September and 10 October 2021. During the surveys, five floating units with a draft of 50 cm depth each were analyzed within a linear transect of 20 m long. Sampling was carried out on the bryozoan *A. verticillata* by leaning out from the edge of the pier and hand-picking at the lateral side of the artificial floating units in a depth range of 0–30 cm. The specimens collected were observed under a ‘Solomark Microscope’ stereomicroscope, photographed using the camera of a Smartphone Xiaomi Note 10T Lite, preserved in 96% ethanol (EtOH),

and deposited in the Heterobranchia collection of the Department of Science, University of Roma Tre, Rome, Italy (Vouchers RM3\_2283-2293).



**Figure 1.** (A) Map showing the geographical position of the marina of Fezzano ( $44^{\circ}4'52.2''$  N,  $9^{\circ}49'37.8''$  E) highlighted by the blue star, located in the Gulf of La Spezia in the eastern portion of the Ligurian Sea (north of Mediterranean Sea); (B) photograph of the marina of Fezzano; (C) floating piers of the marina of Fezzano bordering the monitoring site, characterized by shallow water and a quiet sea surface.

### 2.1. DNA Barcoding

DNA was extracted from a small piece of tissue by using the 'salting out' procedure [35], following the same steps described in Furfaro et al. [36]. Amplifications of the barcode fragment of the cytochrome oxidase subunit I (COI) were performed by PCR using universal primers: LCO1490 and HCO2198 [37]. The universal primers pairs 16Sar-L and 16Sbr-H [38] and H3AD-F and H3BD-R [39] were used for the mitochondrial 16S and the nuclear H3 markers, respectively. The PCR reaction mix had a final volume of 20  $\mu$ L and

consisted of 14.6  $\mu\text{L}$   $\text{dH}_2\text{O}$ , 4.0  $\mu\text{L}$  5 $\times$  FIREPol Mastermix (5 $\times$  reaction buffer (0.4 M tris-HCl, 0.1 M  $(\text{NH}_4)_2\text{SO}_4$ , and 0.1% *w/v* Tween-20), 12.5 mM  $\text{MgCl}_2$ , and 1 mM dNTP), 0.2  $\mu\text{L}$  each of the forward and reverse primers (20  $\mu\text{M}$ ), and 1.0  $\mu\text{L}$  DNA [36]. PCR conditions were the same for the three markers and included an initial denaturation step at 94  $^\circ\text{C}$ , which lasted 5 min. This step was followed by 35 cycles consisting of 30 s at 94  $^\circ\text{C}$  for the denaturation step, 60 s at an annealing temperature of 46–50  $^\circ\text{C}$  (48 $^\circ$ , 46 $^\circ$ , and 50 $^\circ$  for the COI, 16S, and H3, respectively), and 60 s of elongation at 72  $^\circ\text{C}$ . After this last cycle, the temperature was held for an additional 7 min at 72  $^\circ\text{C}$ . Once all these steps were completed, the amplified products were cooled down at 10  $^\circ\text{C}$  [40]. All amplicons were sequenced at the European Division of Macrogen Inc. (Amsterdam, The Netherlands). The resulting sequences were assembled and edited with Staden Package 2.0.0b9 [41]. A BLASTN [42] search was conducted in the GenBank database to confirm the identity of the sequenced fragments and to exclude contamination. COI consensus sequences were aligned with GenBank (<https://www.ncbi.nlm.nih.gov/nucleotide/>) (accessed on 1 August 2022) sequences using the Muscle algorithm implemented in MEGA 6.0 [43]. Since the COI mitochondrial marker is the most-used for species barcoding in Heterobranchia [44–47], a comparison between the new sequences here reported and the ones already deposited in GenBank was carried out. An ASAP (available at <http://www.wabi.snv.jussieu.fr/public/abgd/>) (accessed on 10 August 2022) species delimitation analysis was conducted to detect the barcode gap in the distribution of pairwise distances calculated on the COI sequence alignment [48,49]. The ASAP analysis was performed on the ingroup dataset using Jukes–Cantor genetic distance and the default setting parameters.

## 2.2. Phylogenetic Analyses

Two different COI datasets were generated. One included all *Favorinus* Gray, 1850 species already available in GenBank; members belonging to the *Flabellina* McMurtrie, 1831 genus (*sensu* Furfaro et al. [46]); and *Coryphella verrucosa* (M. Sars, 1829). *Duvaucelia striata* (Haeufelfinger, 1963) was used as the outgroup for this analysis [46]. The second dataset was focused on *P. emertoni* specimens in addition to representatives of the most-related genera: *Palio* Gray, 1857; *Polycera* Cuvier, 1816; and *Thecacera* J. Fleming, 1828. *Thecacera pennigera* (Montagu, 1813) was used as the outgroup for this second dataset. The best-fitting evolutionary model for each dataset was determined using JModelTest version 2.1.10 under the BIC model [50]. Two distinct phylogenetic analyses were carried out per each dataset: a Bayesian inference (BI) analysis and a maximum likelihood (ML) analysis. The former was carried out using the program of MrBayes (v. 3.2.6) [51]. Four independent MCMC (Markov-Chain-Monte-Carlo) runs were conducted with four five million generations each, a sample frequency of one tree per 1000 generations, and a burn-in of 25%. The maximum likelihood analysis was performed using raxmlGUI 1.5b2 [52], a graphical front-end for RAXML 8.2.1 [53], with 100 independent ML searches and 1000 bootstrap replicates. FigTree (v. 1.4.3), a tree figure drawing tool (available at <https://github.com/rambaut/figtree/releases/tag/v1.4.3>) (accessed on 1 August 2022), was used to visualize the resulting topologies.

## 3. Results

During the sampling activities that were carried out between September and October 2021, a great number of individuals belonging to *Favorinus ghanensis* and *Polycerella emertoni* Verrill, 1880 (Gastropoda and Nudibranchia), were observed under the pier of Fezzano's marina in the Gulf of La Spezia (Ligurian Sea, Mediterranean Sea). The specimens collected were all together on a single unit of the artificial floating pontoon (44 $^\circ$ 04'44" N, 9 $^\circ$ 49'43" E) (Figure 1C). Four specimens of *F. ghanensis* 5–9 mm long (vouchers RM3\_2290–2293) and seven *P. emertoni* individuals 2–3 mm long (vouchers RM3\_2283–2289) were collected, photographed, and stored in the collection of the department of Science of the Roma Tre University (Table 1).

**Table 1.** Species names and voucher codes, as well as COI, 16S, and H3 GenBank accession numbers, of the specimens collected from the marina of Fezzano (Ligurian Sea) are listed. In bold are the specimens that were molecularly analyzed.

Species Name	Voucher	COI	16S	H3
<i>Favorinus ghanensis</i>	<b>RM3_2290</b>	OP223449	OP221319	OP251348
	<b>RM3_2291</b>	OP223450	OP221320	OP251349
	<b>RM3_2292</b>	OP223451	OP221321	OP251350
	RM3_2293	—	—	—
<i>Polycerella emertoni</i>	RM3_2283	—	—	—
	RM3_2284	—	—	—
	<b>RM3_2285</b>	OP218058	OP218055	OP251346
	<b>RM3_2286</b>	OP218059	OP218056	OP251347
	RM3_2287	—	—	—
	RM3_2288	—	—	—
	RM3_2289	—	—	—

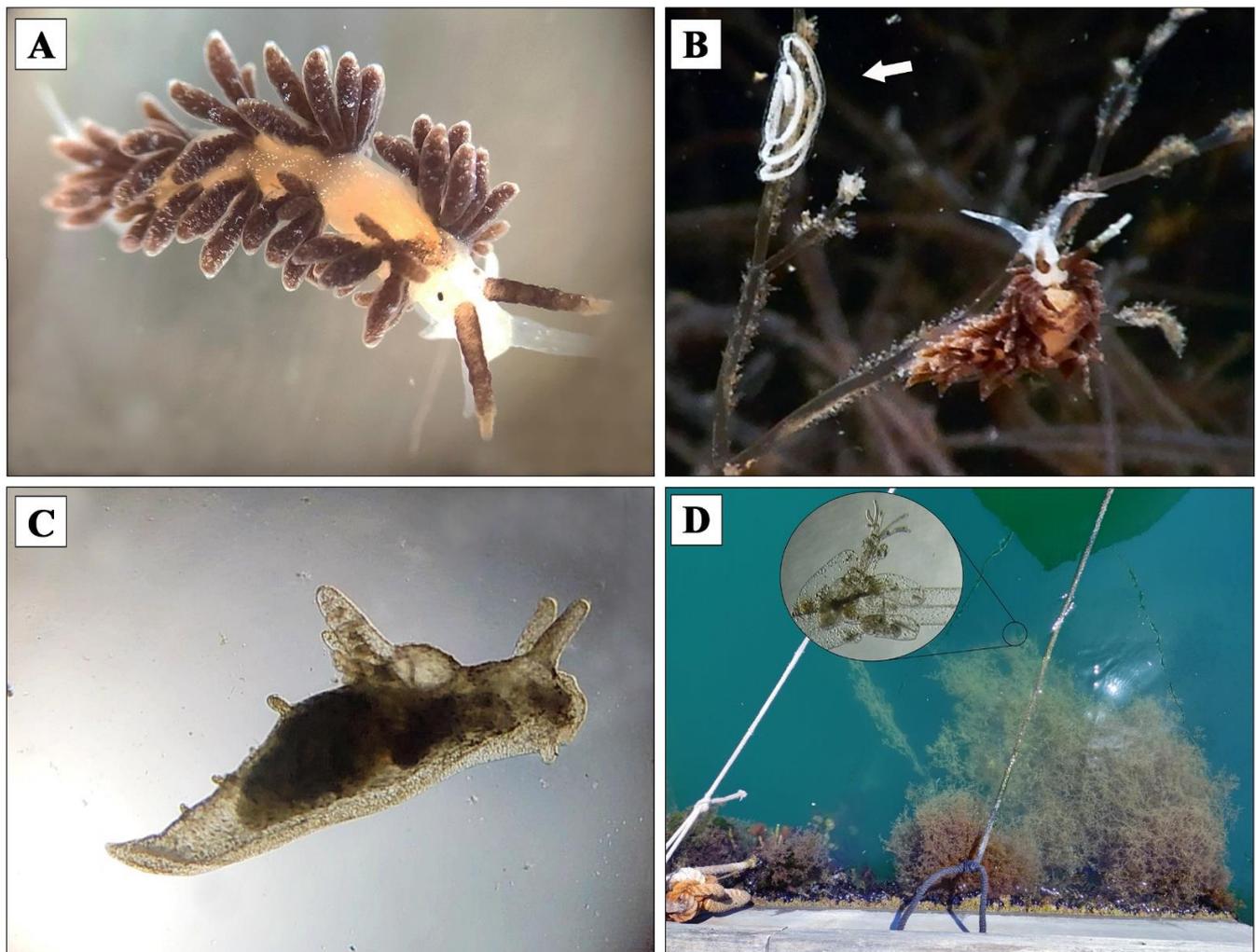
### 3.1. Morphological Identification

*Favorinus ghanensis* was originally described by Edmunds [54] based on 13 specimens from Thema Harbor, Ghana [54]: “The animals are translucent greyish white with a few white dots on the head, oral tentacles, back and tail. The rhinophores have three small swellings on them. The tip is clear greyish with white dots, the rest of the stalk is purple-brown or maroon (the colour being in closely set dots). The liver ducts are only normally visible in the body of live animals if they are old and unhealthy when they appear as dark lines joining the bases of the cerata. The liver in the cerata is cream or brown with purple-brown blotches, so that its general appearance is purple-brown. The cnidosac is minute, 0.08–0.09 mm long, and not visible at all in the living animal. The cerata have a few white dots on the surface, and a few white glands at the tip. The cerata are arranged in arches towards the front of the animal, in rows further back”. This morphological description perfectly matches with the color pattern and body shape characterizing the Ligurian specimens (Figure 2A,B). After its description, *F. ghanensis* was recorded only twice: Ben Souissi et al. [55] reported a high number of specimens from Rade’s Harbor (Tunisia) and Tamsouri et al. (2014) from the marina of Agadir (Morocco).

*Polycerella emertoni* was originally described by Verrill [56] (1880) from Connecticut. This species has a widespread distribution [28], being reported as common from Massachusetts to Brazil [57] and having been reported from Agadir, Morocco [28], at Thema Harbor, Ghana [58], along the Atlantic coast of the Iberian Peninsula [59–63], and in the Mediterranean Sea from Alfacs bay, Ebro Delta, (Spain) [64], Fusaro lake, and the Tuscany coasts of Italy [65,66], Malta [67,68], Greece [7,69], and Tunisia [70]. The external morphology of the collected *Polycerella* individuals is coherent with those reported in the original description and in the following published papers focused on this species (Figure 2C), leaving no doubt on its morphological identification. Its finding in the marina of Fezzano constitutes the first record of this species in the Ligurian Sea.

### 3.2. Molecular Analyses

Three sequences per each COI, 16S, and H3 (517 bp, 425 bp, and 261 bp, respectively) from *F. ghanensis* individuals and two COI, 16S, and H3 (637 bp, 411 bp, and 324 bp, respectively) from *P. emertoni* specimens were obtained (Tables 1 and 2).



**Figure 2.** (A,B) Pictures of *F. ghanensis* obtained in laboratory (A) and in situ (B). Highlighted by the white arrow is an egg ribbon, possibly laid by *F. ghanensis*. (C) *Polycerella emertoni* photographed in laboratory under a stereomicroscope. (D) Colonies of *A. verticillata* living in the marina of Fezzano.

**Table 2.** Species names, collection localities, vouchers, and COI accession numbers of the specimens included in the molecular analyses. The ‘\*’ symbol indicates the outgroups. In bold are the Ligurian specimens that were molecularly analyzed.

Species Name	Locality	Voucher	COI
<i>Favorinus auranoralis</i>	Pacific Ocean: Marshall Islands, Kwajalein, Atoll, Loi Island, South Loi sandspit	CASIZ 181353	JX220463
<i>Favorinus auritulus</i>	Philippines: Luzon, Batangas Province, Maricaban Island, Bethlehem	CASIZ 182795	JX220462
	Atlantic Ocean: North Atlantic Ocean, Bermuda, Hamilton Parish, Shelly Bay	CASIZ 181172	JX220476
	Atlantic Ocean: North Atlantic Ocean, Bermuda, Hamilton Parish, Walsingham Bay	CASIZ 181196	JX220475
	Atlantic Ocean: North Atlantic Ocean, Bermuda, Hamilton Parish, Walsingham Bay	CASIZ 181202	JX220474
	Atlantic Ocean: North Atlantic Ocean, Bermuda, Hamilton Parish, Walsingham Bay	CASIZ 181203	JX220473
<i>Favorinus blianus</i>	United Kingdom: Scotland, Outer Hebrides, St. Kilda, Hirta, Village Bay	SRR1950950	KX889733
		CASIZ 118892	JX220472
<i>Favorinus branchialis</i>	Italy: Latium, Sabaudia, Lago di Paola	BAU2676	LT596562
	Spain: Cadiz	MNCN15.05/53695	HQ616761

Table 2. Cont.

Species Name	Locality	Voucher	COI
<i>Favorinus bullitis</i>	Pacific Ocean: Marshall Islands, Kwajalein, Atoll, Gugeegue Island, Gugeegue sandspit	CASIZ 181361	JX220461
	Pacific Ocean: Marshall Islands, Kwajalein, Atoll, Little Shell sandspit	CASIZ 185769A	JX220460
	Pacific Ocean: Marshall Islands, Kwajalein, Atoll, Little Shell sandspit	CASIZ 185769B	JX220459
	Pacific Ocean: Marshall Islands, Kwajalein, Atoll, Little Shell sandspit	CASIZ 185769C	JX220458
	Philippines: Luzon, Batangas Province, Calumpan, Peninsula, Balayan Bay	CASIZ 186045	JX220457
<i>Favorinus elenalexiarum</i>	Costa Rica: Guanacaste, Isla Plata	CASIZ178875	HM162755
	Mexico: Jalisco, Bahia de Banderas, Puerto Vallarta, Marietas and Los Arcos	CASIZ 174060A	JX220471
<i>Favorinus fuscumaris</i>	Philippines: Luzon, Batangas Province, Balayan, Bay, Anilao, Anilao Harbor	CASIZ 186046	JX220455
<i>Favorinus ghanensis</i>	Italy: Liguria, La Spezia, Fezzano's marina	RM3_2290	OP223449
	Italy: Liguria, La Spezia, Fezzano's marina	RM3_2291	OP223450
	Italy: Liguria, La Spezia, Fezzano's marina	RM3_2292	OP223451
<i>Favorinus inflatus</i>	Philippines: Luzon, Batangas Province, Balayan, Bay, Anilao, Anilao Harbor	CASIZ 186438	JX220456
<i>Favorinus japonicus</i>	Philippines: Luzon, Batangas Province, Maricaban Island, Devil's Point	CASIZ 177286	JX220470
	USA: Hawaii, Maui, Airport Beach	CASIZ 180309	JX220469
	Philippines: Sibuyan Sea, Romblon Province, Tablas Island, north tip of Tablas Island	CASIZ 182291	JX220468
<i>Favorinus mirabilis</i>	Philippines: Luzon, Batangas Province, Calumpan Peninsula, Maricaban Strait, Twin Rocks	CASIZ 177474	JX220467
	Philippines: Luzon, Batangas Province, Maricaban Island, Devil's Point	CASIZ 177672A	JX220466
	Pacific Ocean: Marshall Islands, Kwajalein Atoll, Kwajalein Harbor	CASIZ 181347A	JX220465
	Pacific Ocean: Marshall Islands, Kwajalein Atoll, North Loi Island	CASIZ 182194A	JX220464
	Indonesia	Fasp_17Bu-1	MK514329
<i>Favorinus sp.</i>	Indonesia	Fasp_15Bu-1	MK514328
<i>Favorinus sp.</i>	French Polynesia: Moorea	PW-2014	KJ522462
<i>Favorinus tsuruganus</i>	Philippines: Luzon, Batangas Province, Maricaban Island, Bethlehem	CASIZ 177373	JX220454
	Philippines: Luzon, Batangas Province, Maricaban Island, Bethlehem	CASIZ 177374	JX220453
	Philippines: Luzon, Batangas Province, Balayan Bay, east of Dive'n Trek	CASIZ 177652	JX220452
	Philippines: Sibuyan Sea, Romblon Province, Romblon Island, Bon Bon Beach	CASIZ 182284	JX220451
	Philippines: Luzon, Batangas Province, Maricaban Island, Caban Island, Kirby's Rock	CASIZ 186044	JX220450
<i>Coryphella verrucosa</i>		WS14499	MZ832555
<i>Flabellina affinis</i>		ZMBN106122	MZ832557
	Italy: Latium, Ponza Is.	BAU2806	LT718555
	Italy: Latium, Ponza Is.	BAU2807	LT718556
	Greece: Lefkada	BAU2808	LT718557
	Spain: Cadiz	BAU2809	LT718558
<i>Flabellina cavolini</i>	Italy: Sardinia, Tavolara	RM3_1390	LR813668
	Italy: Liguria, La Spezia, Scoglio Ferale	RM3_1560	LR813669
	Italy: Liguria, Genova, Portofino, Il faro	RM3_1751	LR813670
<i>Flabellina gaditana</i>	Italy: Apulia, Tremiti Islands	RM3_461	LR813671
	Italy: Sardinia, Tavolara Island	RM3_783	LR813672
	France: Arcachon	MCNCN/ADN51998	JX087557
<i>Duvaucelia striata</i> *	Italy: Tuscany, Giannutri Is.	BAU2695	LT596540
	Italy: Grosseto, Le Formiche Is.	BAU2696	LT596541
<i>Palio dubia</i>	USA: Maine, Portland	CASIZ 182030	MZ382786
	Russia: White Sea	ZMMU:Op-751	MZ425304

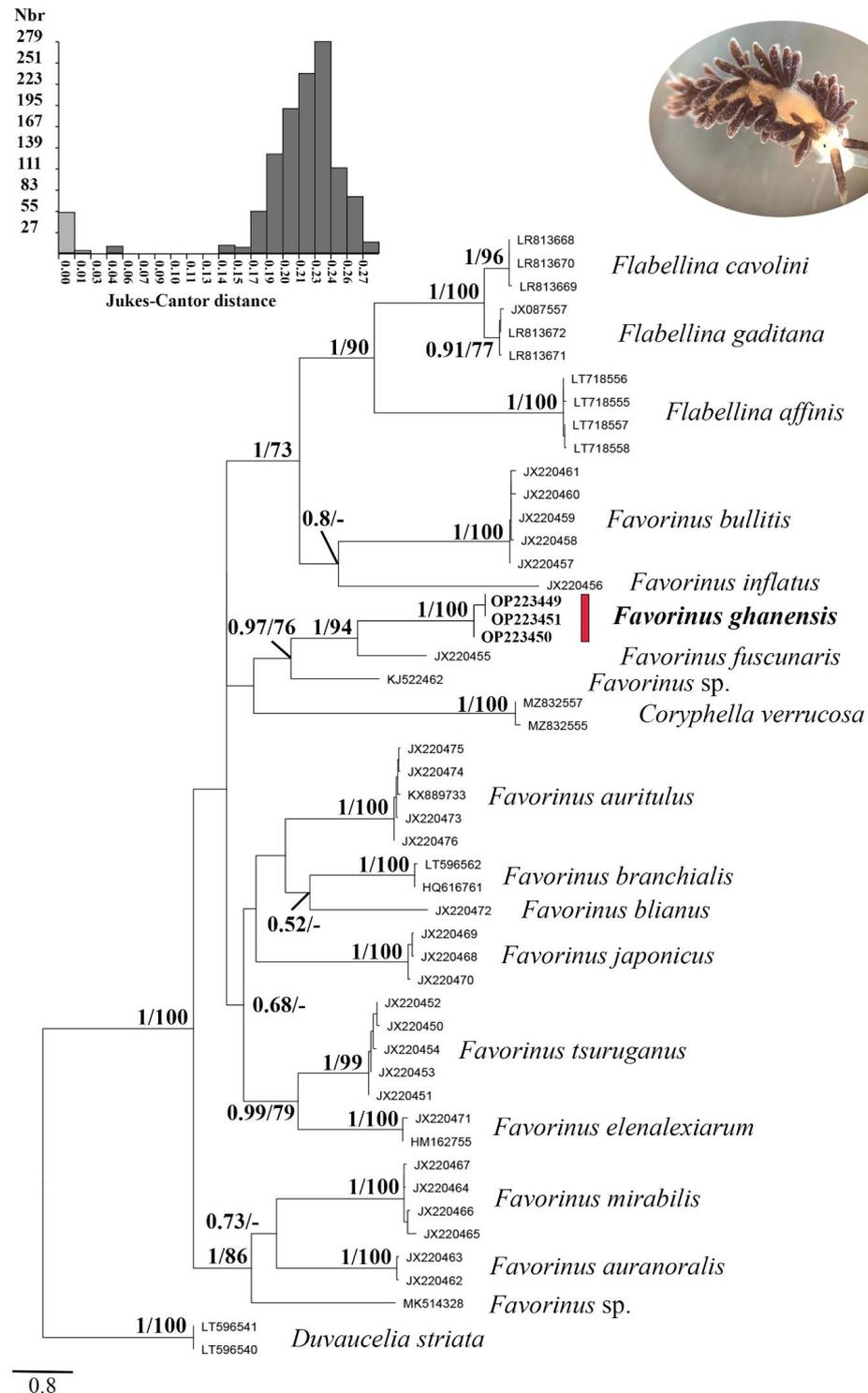
Table 2. Cont.

Species Name	Locality	Voucher	COI
	Canada: Quebec, Baie Ste-Marguerite, Trait 7	CCDB-15498-E04	KF644300
	Canada: Quebec, Baie Ste-Marguerite, Trait 7	CCDB-15498-E07	KF643719
	Canada: Quebec, Baie Ste-Marguerite, Trait 7	CCDB-15498-E06	KF643686
	Sweden: Kristineberg, Bohuslän	MNCN:15.05/55467	JX274100
	Sweden: Kristineberg, Bohuslän	—————	AJ223272
<i>Polycera atra</i>	USA: California, San Francisco Bay, USA, San Francisco Marina	CASIZ 170506b	JX274085
	USA: California, San Francisco Bay, USA, San Francisco Marina	CASIZ 170506a	JX274084
<i>Polycera quadrilineata</i>	Sweden: Gothenborg, Tjarno	MNCN:15.05/55466	JX274073
	Sweden: Gothenborg, Tjarno	MNCN:15.05/55463	JX274074
<i>Polycerella emertoni</i>	<b>Italy: Liguria, La Spezia, Fezzano's marina</b>	<b>RM3_2285</b>	<b>OP218058</b>
	<b>Italy: Liguria, La Spezia, Fezzano's marina</b>	<b>RM3_2286</b>	<b>OP218059</b>
	Spain: Cadiz, Santi Petri, Pantalan	MNCN:15.05/55482	JX274099
	Spain: Cadiz, Santi Petri, Pantalan	MNCN:15.05/55482	JX274098
	Spain: Cadiz, Santi Petri, Pantalan	MNCN:15.05/55479.2	JX274097
	Spain: Cadiz, Santi Petri, Pantalan	MNCN:15.05/55479.1	JX274096
	Spain: Cadiz, Santi Petri	MNCN:15.05/55480	JX274095
	Spain: Cadiz, Andalusia	—————	AJ223273
<i>Thecacera pennigera</i> *	Spain: Cadiz, Andalusia	—————	AJ223277
	South Africa: Cape Province, Atlantic Coast, Oudekraal	CASIZ 176285	JX274094

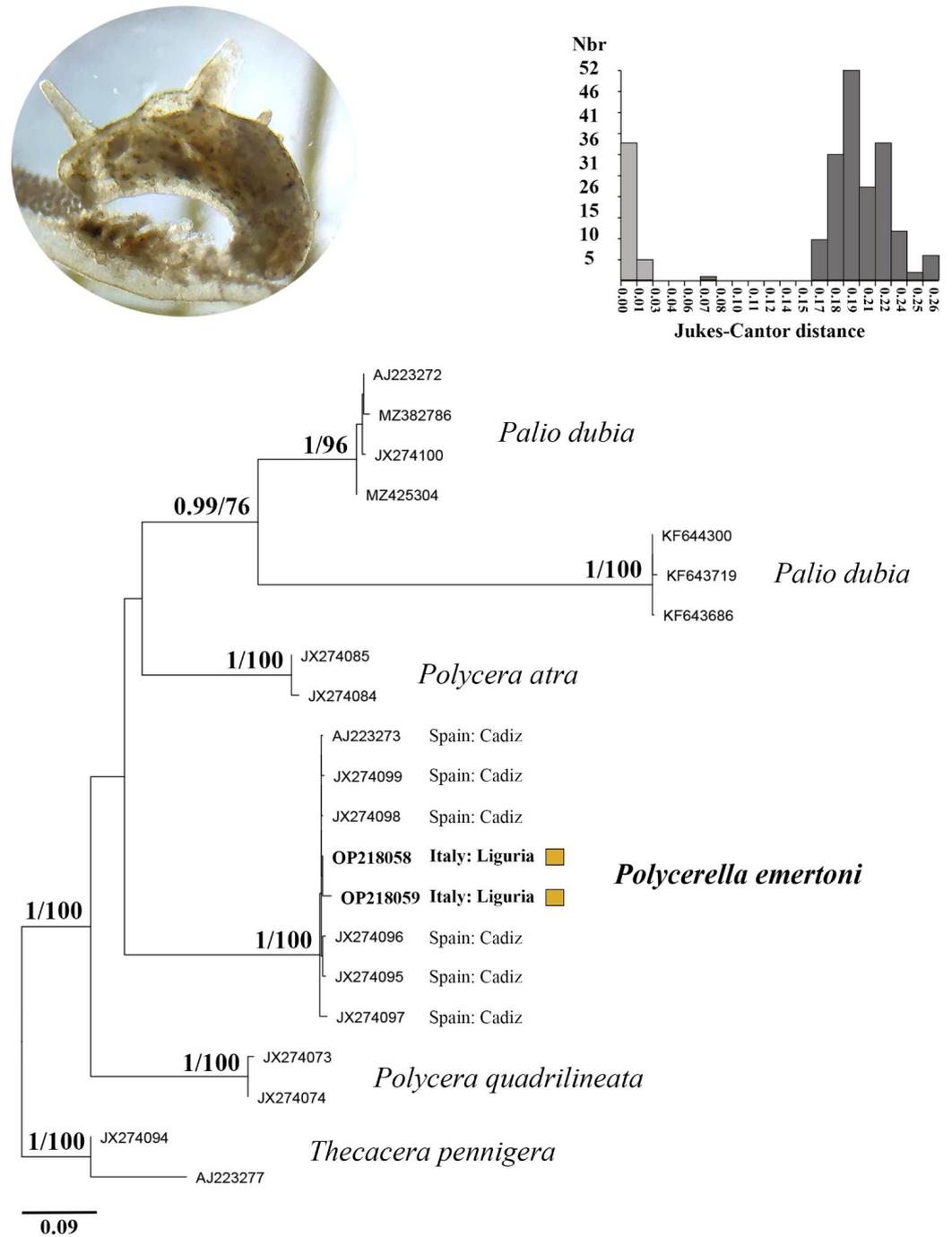
The molecular analysis on the *F. ghanensis* dataset involved 51 COI sequences (657 bp), 37 of which are currently ascribed to 15 *Favorinus* species (Table 2). TPM3uf + I + G resulted to be the best evolutionary model for this dataset. The Bayesian inference (BI) and maximum likelihood (ML) analyses were congruent with each other (Figure 3), with most of the relationships weakly supported. The ingroup taxa consisted of a strongly supported monophyletic clade (BI = 1, ML = 100), which in turn included two distinct clades: a basal one (BI = 1, ML = 86) composed of *F. mirabilis* Baba, 1955; *F. auranoralis* West and Gosliner, 2012; and a *Favorinus* sp.; as well as a second big clade with no statistical support, that included all the remaining *Coryphella*, *Favorinus*, and *Flabellina* species. All the terminal nodes were strongly supported, being informative at a species taxonomic level, while the internal nodes were weakly resolved. The clades that were statistically supported were the *Flabellina* one (BI = 1, ML = 90), which included the sisters *F. cavolini* (Vérany, 1846) and *F. gaditana* (Cervera, García-Gómez, and F. J. García, 1987) (BI = 1, ML = 100), in turn sister (BI = 1, ML = 90) to the species *F. affinis* (Gmelin, 1791) (BI = 1, ML = 100), and the monophyletic clade composed by *Favorinus ghanensis* (BI = 1, ML = 100), sister to *F. fuscunaris* West and Gosliner, 2012, forming a monophyletic clade (BI = 1, ML = 92) with a *Favorinus* sp. as the sister taxon (BI = 0.97, ML = 76). The third statistically supported internal node (BI = 1, ML = 73) grouped *Flabellina* spp. in a monophyletic clade with *Favorinus bullitis* West and Gosliner, 2012, and *F. inflatus* West and Gosliner, 2012, as the sister group. These results suggested a polyphyletic condition of the *Favorinus* genus that should deserve future in-depth analyses. Future comparisons with *F. ghanensis* specimens from the type locality are also desirable to exclude possible cases of cryptic diversity.

The *Polycerella emertoni* dataset included a total of 21 sequences (618 bp) belonging to four ingroup taxa. TrN + I + G resulted to be the best evolutionary model for this dataset. Results from the *P. emertoni* BI and ML analyses were congruent and confirmed the morphological identification of this species (Figure 4). In fact, Ligurian *P. emertoni* specimens were grouped in a well-supported monophyletic clade (BI = 1, ML = 100), which included other conspecifics collected from Atlantic Spain. The *P. emertoni* COI distance values were within the currently accepted range of intraspecific variability [45,46]. Phylogenetic relationships with other genera were not resolved and in-depth analyses, including additional molecular markers and species, are needed to clarify the evolutionary relationships occurring between *P. emertoni* and its closely related genera. All the species included in the analyses were monophyletic except *Palio dubia* (M. Sars, 1829), which was separated into two distinct, well-supported phylogenetic lineages (BI = 1, ML = 96 and BI = 1, ML = 100

respectively). Additional morphological and molecular analyses (that are beyond the scope of the present study) are needed to exclude possible cases of misidentification and to resolve the systematic statuses of these two potential separated species.



**Figure 3.** Maximum likelihood topology based on the COI dataset with results from species delimitation analyses (left, top). Numbers at nodes indicate Bayesian posterior probability (BPP; left) of the BI and bootstrap support (BP; right) from the ML. BPP < 0.50 and BP < 50% were not reported. The histogram in the upper left part shows the distribution of the pairwise genetic distances (JC69) in intraspecific (left, light grey) and interspecific (right, dark grey) comparisons. The red-colored rectangle highlights *F. ghanensis* specimens. The '-' symbol indicates unsupported values.



**Figure 4.** Maximum likelihood topology based on the COI dataset with results from species delimitation analyses (right, top). Numbers at nodes indicate Bayesian posterior probability (BPP; left) of the BI and bootstrap support (BP; right) from the ML. BPP < 0.50 and BP < 50% were not reported. The histogram in the upper right part shows the distribution of the pairwise genetic distances (JC69) in intraspecific (left, light grey) and interspecific (right, dark grey) comparisons. The orange-colored quadrants highlight *P. emertoni* Ligurian specimens.

#### 4. Discussion

Investigations on non-indigenous (NI) organisms carried out during citizen science programs in the Gulf of La Spezia have allowed the unravelling of an NI trophic web new to the Mediterranean Sea that was here reported and investigated. Two NI nudibranchs entered the Mediterranean Sea travelling on a NI bryozoan. These NI travellers, the cryp-

togenic bryozoan *Amathia verticillata*, the rare *Favorinus ghanensis* (to date reported only from this Italian locality [23,24]), and *Polycerella emertoni* (commonly found in association with this widespread bryozoan and reported here for the first time in the Ligurian Sea) are linked to each other by deep prey–predator relationships. Interestingly, the same food web was already reported by Tamsouri et al. [28] from Agadir (Morocco). In their paper, the authors state that *A. verticillata* was preyed upon by *P. emertoni*, while they hypothesized that *F. ghanensis* could eat the eggs laid by *P. emertoni*. A recent paper [58] demonstrated that, contrary to what was expected, *P. emertoni* is a micro-herbivore feeding on the periphyton covering *A. verticillata*, with several diatom taxa also found in its stomach contents. This is quite surprising and opens new perspectives on the need to investigate the epiphytic algal community associated with NI and invasive taxa. Regarding the trophic behavior of *F. ghanensis*, our observations support the hypothesis made by Tamsouri et al. [28], considering that the egg masses of *P. emertoni* are the main prey of *F. ghanensis*. It is interesting to note that three out of the total four records of *F. ghanensis* have reported it in sympatry with *P. emertoni*. Even in the Tunisian record of this species [55], which is the only one that does not explicitly report this sympatric association, the first record of *P. emertoni* was made six years later than that of *F. ghanensis* from the same locality. Considering that the authors themselves stated that ‘The species may have been present in the Bay of Tunis for a long time and remained unnoticed because it is extremely inconspicuous’ [70], it could be hypothesized that *P. emertoni* was already present when *F. ghanensis* was firstly recorded, making 100% of its records in sympatry with *P. emertoni*. This observation could indicate a strong and not fully understood ecological association between these two elusive and inconspicuous sea slugs that remains unnoticed so far. In fact, it could be speculated that *F. ghanensis* shows a monophagous diet by selectively preying on the eggs laid by *P. emertoni*, making the presence of the latter species essential for the former sea slug. Another possibility is that *F. ghanensis* shares the same ecological niche characterizing *P. emertoni* but with low plasticity. This last hypothesis is also supported by the fact that *Favorinus* spp. are known to secondarily feed on cnidarians to store nematocysts into their small cnidosacs for defensive purposes [71]. However, the difficulty of studying in a natural condition and the high number of ecological parameters involved make it difficult to exclude one possibility or another. Furthermore, considering the increasing number of Mediterranean nudibranch cryptic species recently revealed by molecular methods [36,45,72,73], we investigated the Ligurian specimens using a molecular approach to exclude possible cases of cryptic diversity and to investigate on the phylogenetic relationships occurring with closely related taxa. Results from Bayesian and maximum likelihood molecular analyses, as well as species delimitation methods, confirmed the identity of *P. emertoni* and demonstrated *P. emertoni*’s Ligurian population to be genetically linked to the eastern Atlantic one, although further studies are needed to confirm the ampho-atlantic status of this widespread species. The lack of GenBank reference sequences ascribed to *F. ghanensis* prevented excluding cryptic diversity within this species, but the presence of characteristic diagnostic external features and the perfect match between the Ligurian phenotype and the one originally reported for the species left no doubt on the morphological identification of this favorinid sea slug. Sequences obtained from the Ligurian specimens were deposited in GenBank for the first time, which could be useful for future molecular comparison between populations from different geographical areas. Interestingly, molecular analyses carried out on a broad dataset, which included all the *Favorinus* species to date available, revealed a possible polyphyletic condition of the genus *Favorinus* that needs to be clarified. Indeed, an unexpected closely relationship was revealed here between some *Favorinus* species and members of the family Flabellinidae that deserves future in-depth phylogenetic investigations.

From a conservation strategy point of view, it is interesting to note that the role of *A. verticillata* as a powerful vector able to introduce multiple NI species through transport on vessel hulls, vessel fouling, and mussel imports, has been previously demonstrated [21,74,75] and was here confirmed. This should be considered to promote targeted monitoring programs and more effective environmental protection actions. In fact, even if it is usually

observed that this characteristic pattern of multiple colonization takes advantage of the availability of ‘empty’ ecological niches, with apparently no negative effects for endemic fauna, in most cases a newly realized NI niche is generated as a response to the new hosting habitat features [21,76]. Moreover, *A. verticillata*, supposed to be native to the Caribbean ecoregion [77] but cautiously considered as cryptogenic (i.e., a species with uncertain geographical origin [78]), is distributed worldwide and, consequently, able to support epiphytic fauna from all over the world. However, the shift observed in Ligurian Sea from an endemic Mediterranean food web to a new and NI one perfectly describes the high increase in change within the long story of Mediterranean faunal transformation, whose consequence is an impoverishment of indigenous biodiversity and of endemic habitats and whose sequel is unknown and yet unpredictable [25]. Finally, the results here presented confirmed the importance of harbors and marinas, such as the marina of Fezzano, as strategic key stations for the early detection of NI species. Furthermore, considering the numerous boats hosted in this Ligurian marina, we could also speculate that recreational vessels, and not only commercial boats, can effectively facilitate alien species dispersal [15]. Indeed, it is worth mentioning that the NI species reported here appeared to be introduced from the Atlantic Ocean instead of the Suez Canal, and this is quite rare considering the large amount of alien species that reach the Mediterranean Sea using this latter entry point. In this framework, it is important to highlight how a sustainable coastal monitoring project scheduling operative surveillance plans within public research activities of citizen science, as “Percorsi nel Blu” project does, can play a fundamental key role in monitoring and conservation strategies. Citizen science monitoring programs focused on these kinds of ‘sentinel stations’ are helpful to increase and update data collection, to deepen the knowledge of ecological aspects linked to such interesting anthropogenic marine habitats, to detect new NI taxa, and to promote effective and suitable conservation strategies.

## 5. Conclusions

In conclusion, with this study, an NI trophic chain new to the Mediterranean Sea was reported, which involved three different actors: *Amathia verticillata*, *Polycerella emertoni* (which feeds on the diatom community associated to this bryozoan), and *Favorinus ghanensis* (which feeds mainly on the eggs laid by the polycerid sea slug). The two travelling NI sea slugs were investigated from morphological and molecular points of view, and phylogenetic relationships with closely related taxa were reported. The rare *F. ghanensis* was morphologically identified, and molecular data from Ligurian individuals were reported and deposited for the first time. *Polycerella emertoni* was recorded for the first time from the Ligurian Sea, expanding its current known range of distribution, and molecular intraspecific divergence within the Mediterranean and Atlantic populations unraveled. Finally, the key role of marinas and ports in the early warning of alien invasions was confirmed, and the need to implement research activities and monitoring strategies also involving citizen science was highlighted.

**Author Contributions:** Conceptualization, E.M. and G.F.; methodology, G.F.; formal analysis, G.F.; investigation, E.M.; resources, E.M. and G.F.; data curation, G.F.; writing—original draft preparation, G.F.; writing—review and editing, E.M. and G.F.; supervision, G.F.; funding acquisition, G.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Italian Ministry of Education, University, and Research (MIUR, PON 2014–2020, grant number AIM 1848751-2, Linea 2); the APC was funded by MIUR (PON 2014–2020).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** G.F. and E.M. are grateful to the four anonymous reviewers whose help greatly improved the present paper and to Arianna Reato, who corrected the English language of the manuscript. The authors gratefully thank the staff of voluntary operators involved in the surveys carried out at the marina of Fezzano: Isabella Vacchetti, Vittoria Guani, Rachele Guani, Matilde Olmi, Giacomo Frione, Tiziano Scimone, Pietro Orlandi, Christian Sbernadori, Houssein Kadraoui, Pietro Barbati, Francesca Battini, Silvio Guani, Giorgio Ghinelli, Alteo Ruci, Ami Ruci and Roberto Traverso. G.F. wishes to thank the Scubalandia Team for technical underwater support. G.F. is supported by funds from the Italian Ministry of Education, University, and Research (MIUR, PON 2014–2020, grant AIM 1848751-2, Linea 2).

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

- Coll, M.; Piroddi, C.; Steenbeek, J.; Kaschner, K.; Ben Rais Lasram, F.; Aguzzi, J.; Ballesteros, E.; Bianchi, C.N.; Corbera, J.; Dailianis, T.; et al. The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. *PLoS ONE* **2010**, *5*, e11842. [[CrossRef](#)] [[PubMed](#)]
- Danovaro, R.; Company, J.B.; Corinaldesi, C.; D’Onghia, G.; Galil, B.; Gambi, C.; Gooday, A.J.; Lampadariou, N.; Luna, G.M.; Morigi, C.; et al. Deep-Sea Biodiversity in the Mediterranean Sea: The Known, the Unknown, and the Unknowable. *PLoS ONE* **2010**, *5*, e11832. [[CrossRef](#)] [[PubMed](#)]
- Lejeusne, C.; Chevaldonné, P.; Pergent-Martini, C.; Boudouresque, C.F.; Pérez, T. Climate change effects on a miniature ocean: The highly diverse, highly impacted Mediterranean Sea. *Trends Ecol. Evol.* **2010**, *25*, 250–260. [[CrossRef](#)] [[PubMed](#)]
- Bianchi, C.N.; Morri, C. Marine Biodiversity of the Mediterranean Sea: Situation, Problems and Prospects for Future Research. *Mar. Pollut. Bull.* **2000**, *40*, 367–376. [[CrossRef](#)]
- Mouillot, D.; Albouy, C.; Guilhaumon, F.; Lasram, F.B.R.; Coll, M.; Devictor, V.; Meynard, C.N.; Pauly, D.; Tomasini, J.A.; Troussellier, M.; et al. Protected and Threatened Components of Fish Biodiversity in the Mediterranean Sea. *Curr. Biol.* **2011**, *21*, 1044–1050. [[CrossRef](#)]
- Costello, M.J.; Tsai, P.; Wong, P.S.; Cheung, A.K.L.; Basher, Z.; Chaudhary, C. Marine biogeographic realms and species endemism. *Nat. Commun.* **2017**, *8*, 1057. [[CrossRef](#)]
- Zenetos, A.; Cinar, M.E.; Pancucci-Papadopoulou, M.A.; Harmelin, J.G.; Furnari, G.; Andaloro, F.; Bellou, N.; Streftaris, N.; Zibrowius, H. Annotated list of marine alien species in the Mediterranean with records of the worst invasive species. *Mediterr. Mar. Sci.* **2005**, *6*, 63–118. [[CrossRef](#)]
- Zenetos, A.; Meriç, E.; Verlaque, M.; Galli, P.; Boudouresque, C.F.; Giangrande, A.; Çinar, M.E.; Bilecenoglu, M. Additions to the annotated list of marine alien biota in the Mediterranean with special emphasis on Foraminifera and Parasites. *Mediterr. Mar. Sci.* **2008**, *9*, 119–166. [[CrossRef](#)]
- Zenetos, A.; Gofas, S.; Verlaque, M.; Cinar, M.E.; Raso, J.E.G.; Bianchi, C.N.; Morri, C.; Azzurro, E.; Bilecenoglu, M.; Frogli, C.; et al. Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union’s Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. *Mediterr. Mar. Sci.* **2010**, *11*, 381. [[CrossRef](#)]
- Zenetos, A.; Gofas, S.; Morri, C.; Rosso, A.; Violanti, D.; Raso, J.G.; Çinar, M.E.; Almogi-Labin, A.; Ates, A.S.; Azzurro, E.; et al. Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union’s Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. *Mediterr. Mar. Sci.* **2012**, *13*, 328–352. [[CrossRef](#)]
- Galil, B.S. Taking stock: Inventory of alien species in the Mediterranean sea. *Biol. Invasions* **2009**, *11*, 359–372. [[CrossRef](#)]
- Galil, B.S. A sea under siege—alien species in the Mediterranean. *Biol. Invasions* **2000**, *2*, 177–186. [[CrossRef](#)]
- Bianchi, C.N.; Morri, C.; Chiantore, M.; Montefalcone, M.; Parravicini, V.; Rovere, A. Mediterranean Sea biodiversity between the legacy from the past and a future of change. In *Life in the Mediterranean Sea: A Look at Habitat Changes*; Nova Science Publishers: Hauppauge, NY, USA, 2012; Volume 1, p. 55.
- Ameer, A.; Olof, L. Maritime Traffic Effects on Biodiversity in the Mediterranean Sea: Review of Impacts, Priority Areas and Mitigation Measures, Malaga, Spain. Ph.D. Thesis, IUCN Centre for Mediterranean Cooperation, Gland, Switzerland, 2008; p. 184.
- Ferrario, J.; Caronni, S.; Occhipinti-Ambrogi, A.; Marchini, A. Role of commercial harbours and recreational marinas in the spread of non-indigenous fouling species. *Biofouling* **2017**, *33*, 651–660. [[CrossRef](#)]
- Renda, W.; Amati, B.; Bogi, C.; Bonomolo, G.; Capua, D.; Dell’angelo, B.; Furfaro, G.; Giannuzzi-Savelli, R.; La Perna, R.; Nofroni, I.; et al. The new Checklist of the Italian Fauna: Marine Mollusca. *Biogeogr. J. Integr. Biogeogr.* **2022**, *37*, ucl005. [[CrossRef](#)]
- Sabelli, B.; Taviani, M. The making of the Mediterranean molluscan biodiversity. In *The Mediterranean Sea*; Springer: Dordrecht, The Netherlands, 2014; pp. 285–306.
- Zenetos, A.; Galanidi, M. Mediterranean non indigenous species at the start of the 2020s: Recent changes. *Mar. Biodivers. Rec.* **2020**, *13*, 10. [[CrossRef](#)]
- Crocetta, F.; Macali, A.; Furfaro, G.; Cooke, S.; Villani, G.; Valdes, A. Alien molluscan species established along the Italian shores: An update, with discussions on some Mediterranean “alien species” categories. *ZooKeys* **2013**, *277*, 91. [[CrossRef](#)]

20. Vitale, D.; Giacobbe, S.; Spinelli, A.; De Matteo, S.; Cervera, J.L. “Opisthobranch” (mollusks) inventory of the Faro Lake: A Sicilian biodiversity hot spot. *Ital. J. Zool.* **2016**, *83*, 524–530. [[CrossRef](#)]
21. Furfaro, G.; De Matteo, S.; Mariottini, P.; Giacobbe, S. Ecological notes of the alien species *Godiva quadricolor* (Gastropoda, Nudibranchia) occurring in Faro Lake (Italy). *J. Nat. Hist.* **2018**, *52*, 645–657. [[CrossRef](#)]
22. Azzola, A.; Furfaro, G.; Trainito, E.; Doneddu, M.; Montefalcone, M. Sea water warming favours the northward range extension of Lessepsian species in the Mediterranean Sea: A further example with the cephalaspidean *Lamprohaminoea ovalis*. *JMBA* **2022**, *102*, 1–7.
23. Mioni, E.; Furfaro, G.; Merlino, S.; Mannino, A.M. Project “Percorsi nel Blu”: Ocean Literacy & Citizen Science as a valid tool to monitor Marine Heterobranchia. The case study of the first records of the alien species *Favorinus ghanensis* and the Mediterranean *Okenia* cf. *longiductis*, as updating records for the Italian coast. In Proceedings of the EMSEA Virtual Conference 2021, Gdynia, Poland, 7–8 October 2021.
24. Mioni, E. “Percorsi nel Blu” (“Blue Paths”): A long-lasting project to integrate Ocean Literacy and Marine Citizen Science into school curricula. *Mediterr. Mar. Sci.* **2022**, *23*, 405–416. [[CrossRef](#)]
25. Trainito, E.; Doneddu, M.; Furfaro, G. Aliens in changing seascapes: A newly reported non-native sacoglossan (Mollusca, Heterobranchia) in the western Mediterranean Sea. *Check List* **2022**, *18*, 545–551. [[CrossRef](#)]
26. Canessa, M.; Bavestrello, G.; Cattaneo-Vietti, R.; Furfaro, G.; Doneddu, M.; Navone, A.; Trainito, E. Rocky substrate affects benthic heterobranch assemblages and prey/predator relationships. *Estuar. Coast. Shelf Sci.* **2021**, *261*, 107568. [[CrossRef](#)]
27. Cervera, J.L.; Tamsouri, N.; Moukrim, A.; Villani, G. New records of two alien opisthobranch molluscs from the north-eastern Atlantic: *Polycera hedgpethi* and *Godiva quadricolor*. *Mar. Biodivers. Rec.* **2010**, *3*, e51. [[CrossRef](#)]
28. Tamsouri, N.; Carmona, L.; Moukrim, A.; Cervera, J.L. *Polycerella emertoni* and *Favorinus ghanensis*: Two new alien sea slug molluscs from the Moroccan Atlantic coasts. *Mar. Biodivers. Rec.* **2014**, *7*, e13. [[CrossRef](#)]
29. Verlaque, M. Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. *Oceanol. Acta* **2001**, *24*, 29–49. [[CrossRef](#)]
30. Garcia-Soto, C.; van der Meeren, G.I.; Busch, J.A.; Delany, J.; Domegan, C.; Dubsky, K.; Gorsky, G.; von Juterzenka, K.; Malfatti, F.; Mannaerts, G.; et al. *Advancing Citizen Science for Coastal and Ocean Research*; Position Paper 23; French, V., Kellett, P., Delany, J., McDonough, N., Eds.; European Marine Board, European Science Foundation: Ostend, Belgium, 2017; p. 112.
31. Mioni, E.; Merlino, S.; Giovacchini, A. Engaging way to help students develop skills, interest and methodological research approaches in marine and environmental science. In *Advances in Higher Education*; de la Poza, E., Domènech, J., Lloret, J., Vincent-Vela, M.C., Zuriaga, E., Eds.; Editorial Universitat Politècnica de València: València, Spain, 2016; pp. 400–408.
32. Mioni, E.; Mannino, A.M.; Merlino, S. First record of *Aplysia dactylomela* (Rang, 1828) (Heterobranchia, Aplysiidae) from the island of Pianosa (Alto Tirreno). In Proceedings of the Conference of the Italian Society of Marine Biology SIBM, Cesenatico, Italy, 4–8 June 2018; p. 224.
33. Mioni, E.; Merlino, S.; Balistreri, P.; Mannino, A.M. First record of the invasive crab *Percnon gibbesi* (H. Milne Edwards, 1853) at Pianosa Island: The second goal reached by the innovative Marine Citizen Science Literacy Project “PERCORSI NEL BLU” (“BLUE PATHS”). In Proceedings of the 11th International Conference on Biological Invasions, Vodice, Croatia, 15–18 September 2020; p. 150.
34. Mioni, E.; Stroobant, M.; Locritani, M.; Merlino, S.; Traverso, R. Blue Paths: 5 years motivating the participation of citizens in research activities for coastal monitoring of the Benthos. In Proceedings of the First Citizen Science Conference, Rome, Italy, 23–25 November 2017; p. 64.
35. Aljanabi, S.M.; Martinez, I. Universal and Rapid Salt-Extraction of High Quality Genomic DNA for PCR-Based Techniques. *Nucleic Acids Res.* **1997**, *25*, 4692–4693. [[CrossRef](#)]
36. Furfaro, G.; Schreier, C.; Trainito, E.; Pontes, M.; Madrenas, E.; Girard, P.; Mariottini, P. The Sea Slug *Doriopsilla areolata* Bergh, 1880 (Mollusca, Gastropoda) in the Mediterranean Sea: Another Case of Cryptic Diversity. *Diversity* **2022**, *14*, 297. [[CrossRef](#)]
37. Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA Primers for Amplification of Mitochondrial Cytochrome c Oxidase Subunit I from Diverse Metazoan Invertebrates. *Mol. Mar. Biol. Biotechnol.* **1994**, *3*, 294–299.
38. Palumbi, S.R.; Martin, A.; Romano, S.; Mc Millan, W.O.; Stice, L.; Grabowski, G. *The Simple Fool’s Guide to PCR*; Department of Zoology and Kewalo Marine Laboratory, University of Hawaii: Honolulu, HI, USA, 2002.
39. Colgan, D.J.; McLauchlan, A.; Wilson, G.D.F.; Livingston, S.P.; Edgecombe, G.D.; Macaranas, J.; Cassis, G.; Gray, M.R. Histone H3 and U2 SnRNA DNA Sequences and Arthropod Molecular Evolution. *Aust. J. Zool.* **1999**, *46*, 419–437. [[CrossRef](#)]
40. Furfaro, G.; Oliverio, M.; Mariottini, P. The southernmost record of *Felimida elegantula* (Philippi, 1844) (Gastropoda: Nudibranchia). *Mar. Biodivers.* **2017**, *47*, 579–584. [[CrossRef](#)]
41. Staden, R.; Beal, K.F.; Bonfield, J.K. The Staden package, 1998. *Methods Mol. Biol.* **2000**, *132*, 115–130. [[CrossRef](#)] [[PubMed](#)]
42. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [[CrossRef](#)]
43. Tamura, K.; Stecher, G.; Peterson, D.; Filipiński, A.; Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [[CrossRef](#)]
44. Furfaro, G.; Mariottini, P. A New *Dondice* Marcus Er. 1958 (Gastropoda: Nudibranchia) from the Mediterranean Sea Reveals Interesting Insights into the Phylogenetic History of a Group of Facelinidae Taxa. *Zootaxa* **2020**, *4731*, 1–22. [[CrossRef](#)]

45. Furfaro, G.; Salvi, D.; Trainito, E.; Vitale, F.; Mariottini, P. When Morphology Does Not Match Phylogeny: The Puzzling Case of Two Sibling Nudibranchs (Gastropoda). *Zool. Scr.* **2021**, *50*, 439–454. [[CrossRef](#)]
46. Furfaro, G.; Salvi, D.; Mancini, E.; Mariottini, P. A multilocus view on Mediterranean aeolid nudibranchs (Mollusca): Systematics and cryptic diversity of Flabellinidae and Piseinotecidae. *Mol. Phylogenetic Evol.* **2018**, *118*, 13–22. [[CrossRef](#)]
47. Galià-Camps, C.A.R.L.E.S.; Cervera, J.L.; Valdés, Á.; Ballesteros, M. Attack on crypsis: Molecular and morphological study of *Dendrodoris Ehrenberg*, 1831 (Mollusca: Gastropoda: Nudibranchia) from the Mediterranean Sea and Northern Atlantic Ocean reinstates *Dendrodoris temarana* Pruvot-Fol, 1953. *Zootaxa* **2022**, *5133*, 383–406. [[CrossRef](#)]
48. Puillandre, N.; Lambert, A.; Brouillet, S.; Achaz, G. ABGD, Automatic Barcode Gap Discovery for Primary Species Delimitation. *Mol. Ecol.* **2012**, *21*, 1864–1877. [[CrossRef](#)]
49. Puillandre, N.; Modica, M.V.; Zhang, Y.; Sirovich, L.; Boisselier, M.-C.; Cruaud, C.; Holford, M.; Samadi, S. Large-Scale Species Delimitation Method for Hyperdiverse Groups. *Mol. Ecol.* **2012**, *21*, 2671–2691. [[CrossRef](#)]
50. Posada, D. JModelTest: Phylogenetic Model Averaging. *Mol. Biol. Evol.* **2008**, *25*, 1253–1256. [[CrossRef](#)] [[PubMed](#)]
51. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Syst. Biol.* **2012**, *61*, 539–542. [[CrossRef](#)] [[PubMed](#)]
52. Silvestro, D.; Michalak, I. RaxmlGUI: A Graphical Front-End for RAxML. *Org. Divers. Evol.* **2012**, *12*, 335–337. [[CrossRef](#)]
53. Stamatakis, A. RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [[CrossRef](#)]
54. Edmunds, M. Opisthobranchiate Mollusca from Ghana. *J. Molluscan Stud.* **1968**, *38*, 83–100. [[CrossRef](#)]
55. Ben Soussi, J.; Zaouali, J.; Rezig, M.; Bradai, M.N.; Quignard, J.P.; Rudman, B. Contribution à l'étude de quelques récentes migrations d'espèces exotiques dans les eaux Tunisiennes. *Rapp. Comm. Int. Pour L'exploration Sci. Mer Méditerran.* **2004**, *37*, 312.
56. Verrill, A.E. Notice of recent addition to the marine Invertebrata of the northeastern coast of America, with descriptions of new genera and species and critical remarks on others. Part II—Mollusca, with notes on Annelida, Echinodermata, etc., collected by the United States Fish Commission. *Proc. U. S. Natl. Mus.* **1880**, *3*, 356–409.
57. Valdés, A.; Hamann, J.; Behrens, D.W.; Dupont, A. *Caribbean Sea Slugs: A Field Guide to the Opisthobranch Mollusks from the Tropical Northwestern Atlantic*; Sea Challengers Natural History Books: Gig Harbor, WA, USA, 2006.
58. Edmunds, M. Larval development, oceanic currents, and origins of the opisthobranch fauna of Ghana. *J. Molluscan Stud.* **1977**, *43*, 301–308.
59. García, J.C.; Bobo, A. Un nuevo Doridaceo para el litoral Ibérico: *Polycerella emertoni* Verrill (1880) 1881 (Gastropoda: Nudibranchia). *Boll. Malacol.* **1986**, *22*, 49–56.
60. Cervera, J.L.; García-Gómez, J.C.; Toscano, F.; García, F.J. *Polycera hedgpethi* Marcus, 1964 (Gastropoda: Nudibranchia) an Indo-Pacific species discovered in the Mediterranean Sea. *Iberus* **1988**, *8*, 225–231.
61. Cervera, J.L.; García-Gómez, J.C.; Sánchez-Tocino, L. Familia Polyceridae. In *Moluscos Marinos de Andalucía*; Gofas, S., Moreno, D., Salas, C., Eds.; Servicio de Publicaciones e Intercambio Científico, Universidad de Málaga: Málaga, Spain, 2011; pp. 467–471.
62. García-Gómez, J.C.; Cervera, J.L.; García, F.J.; Ortea, J.A.; Garcia-Martin, S.F.; Medina, A.; Burnay, L.P. Resultados de la Campana Internacional de biología marina 'Algarve-88': Moluscos Opistobranquios. *Boll. Malacol.* **1991**, *27*, 125–138.
63. Megina, C.; Cervera, J.L. Diet, prey selection and cannibalism in the hunter opistobranch *Roboastra europaea*. *J. Mar. Biol. Assoc. UK* **2003**, *83*, 489–495. [[CrossRef](#)]
64. Camps-Castellà, J.; Ballesteros, M.; Trobajo, R.; Pontes, M.; Prado, P. Not all nudibranchs are carnivorous: Trophic ecology of *Polycerella emertoni* in the Ebro Delta. *Mar. Ecol. Prog. Ser.* **2020**, *645*, 67–82. [[CrossRef](#)]
65. Schmekel, L. Die gattung *Polycerella* Verrill im Mittelmeer (Gastrp. Opisthobranchia). *Pubbl. Della Stn. Zool. Napoli* **1965**, *34*, 226–234.
66. Cattaneo-Vietti, R.; Chemello, R.; Giannuzzi-Savelli, R. *Atlas of Mediterranean Nudibranchs*; La Conchiglia: Rome, Italy, 1990.
67. Sammut, C.R.; Perroneù, A.S. A preliminary check-list of Opisthobranchia (Mollusca, Gastropoda) from the Maltese Islands. *Basteria* **1998**, *62*, 221–240.
68. Sciberras, M.; Schembri, P.J. A critical review of records of alien marine species from the Maltese Islands and surrounding waters (Central Mediterranean). *Mediterr. Mar. Sci.* **2007**, *8*, 41–66. [[CrossRef](#)]
69. Koutsoubas, D.; Arvanitidis, C.; Dounas, C.; Drummond, L. Community structure and dynamics of the molluscan fauna in a Mediterranean lagoon (Gialova Lagoon, SW Greece). *Belg. J. Zool.* **2000**, *130*, 135–142.
70. Antit, M.; Gofas, S.; Salas, C.; Azzouna, A. One hundred years after *Pinctada*: An update on alien Mollusca in Tunisia. *Mediterr. Mar. Sci.* **2011**, *12*, 53–74. [[CrossRef](#)]
71. West, N.P. Systematics and phylogeny of *Favorinus*, a clade of specialized predatory nudibranchs. In *Collection: 2012 Biology: Concentration in Integrative Biology Masters Theses 2012*; San Francisco State University: San Francisco, CA, USA, 2012; Available online: <http://hdl.handle.net/10211.3/125190> (accessed on 1 August 2022).
72. Korshunova, T.; Picton, B.; Furfaro, G.; Mariottini, P.; Pontes, M.; Prkić, J.; Fletcher, K.; Malmberg, K.; Lundin, K.; Martynov, A. Multilevel fine-scale diversity challenges the 'cryptic species' concept. *Sci. Rep.* **2019**, *9*, 6732. [[CrossRef](#)]
73. Pola, M.; Paz-Sedano, S.; Macali, A.; Minchin, D.; Marchini, A.; Vitale, F.; Licchelli, C.; Crocetta, F. What is really out there? Review of the genus *Okenia* Menke, 1830 (Nudibranchia: Goniodorididae) in the Mediterranean Sea with description of two new species. *PLoS ONE* **2019**, *14*, e0215037. [[CrossRef](#)]

74. Marchini, A.; Ferrario, J.; Minchin, J.D. Marinas may act as hubs for the spread of the pseudo-indigenous bryozoan *Amathia verticillata* (Delle Chiaje, 1822) and its associates. *Sci. Mar.* **2015**, *79*, 355–365. [[CrossRef](#)]
75. Dailianis, T.; Akyol, O.K.A.N.; Babali, N.; Bariche, M.; Crocetta, F.; Gerovasileiou, V.; Ghanem, R.; Gökoğlu, M.; Hasiotis, T.; Izquierdo-Munoz, A.; et al. New Mediterranean biodiversity records (July 2016). *Mediterr. Mar. Sci.* **2016**, *17*, 608–626. [[CrossRef](#)]
76. Olenin, S.; Leppäkoski, E. Non-native animals in the Baltic Sea: Alteration of benthic habitats in coastal inlets and lagoons. *Hydrobiologia* **1999**, *393*, 233–243. [[CrossRef](#)]
77. Galil, B.S.; Gevili, R. *Zoobotryon verticillatum* (Bryozoa: Ctenostomatida: Vesiculariidae), a new occurrence on the Mediterranean coast of Israel. *Mar. Biodivers. Rec.* **2014**, *7*, e17. [[CrossRef](#)]
78. Carlton, J.T. Biological invasions and cryptogenic species. *Ecology* **1996**, *77*, 1653–1655. [[CrossRef](#)]