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Unraveling the Sea Slug Fauna from an Extremely Variable Environment, The 'Passetto' Rocky Tide Pools (North Adriatic Sea)

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Abstract: The Mediterranean Sea is known to be a hot spot for marine biodiversity, especially if considering the highly specialized Heterobranchia mollusks. In recent years, there has been increasing effort to fill some knowledge gaps existing on Mediterranean heterobranch diversity, but, to date, several potentially interesting habitats remain unexplored. In fact, most studies have been focused on sea slugs inhabiting coastal areas and lakes, but those living in extremely variable areas like Mediterranean rocky tide pools remain almost completely ignored even if it is reported worldwide that they can host a high sea slug diversity. In this context, the rocky tide pool system near the 'Passetto' urban beach (Ancona, Italy) in the North Adriatic Sea (Mediterranean Sea) represents a biodiversity hot spot, highlighting the importance of the conservation of this peculiar habitat. A preliminary quantitative survey on the associated Heterobranchia unveiled a thriving community residing in this limited and fragile habitat, and it inspired a more detailed investigation as performed in the present study. In order to reveal the presence of species that have gone unnoticed, an in-depth study was carried out between 2018 and 2022, which aimed to amplify the knowledge on this vulnerable environment and the fauna associated with this as yet poorly known habitat. Tide pooling activities corroborated by photographic analyses allowed the recording of 45 taxa in total and the addition of 25 species to the previously known list. Within those, (i) two species were potentially new to science, (ii) one was recorded in the Mediterranean Sea for the first time, (iii) two species were added to the Italian fauna, and (iv) one species constituted a new record for Sector 9 of the Italian Seas.

Keywords: sea slug; Nudibranchia; checklist; Mediterranean Sea; biodiversity

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1. Introduction

The Mediterranean Sea only covers 0.82% of the global oceanic surface, but it shows a high biodiversity, since more than 4% of all known marine species live in this basin [1–3]. In this context, one of the most diversified animal groups is the Mollusca, represented by about 2113 Mediterranean species [4–12]. Among mollusks, the marine Heterobranchia (class Gastropoda) could be considered one of the most specialized groups consisting of species that have reduced or completely lost their shell in the adult stage, a keystone for the evolution of unique alternative defensive strategies [13]. Marine Heterobranchia, commonly known as 'sea slugs', inhabit almost all marine environments from the shallows

to the deep (e.g., [14–22]). The systematics of this group has exponentially improved in recent years thanks to the development of so-called 'integrative taxonomy', which allows us to investigate the evolutionary relationships between different taxa by integrating multiple sources of data (from morphology, genetics, ecology, ethology, chemistry, etc.) [23–27]. Even if several steps forward have been made and complicated cases of troubled taxonomy have been resolved [10,24,26–29], gaps in the knowledge of these intriguing mollusks are far from being filled.

To date, most Mediterranean heterobranch inventories have focused on coastal areas [27,30–33], coastal lakes [34], and only recently deep sea [8,22], but fauna from a lot of other less-known habitats remains mostly unexplored. Mediterranean rocky tide pools are a very promising study area due to their geomorphological heterogeneity that can host a high Heterobranchia diversity as already reported in similar extra-Mediterranean areas [35–40]. However, the state of knowledge on intertidal Mediterranean Heterobranchia comes from a few single and/or occasional records [33,41], except for one study by Riccardi and collaborators [42], which specifically aimed to list the sea slug species characterizing the above-mentioned marine environment. In the latter study, the authors explored the limestone rocky tide pool system near the 'Passetto' urban beach (Ancona, Italy) in the Adriatic Sea (Mediterranean Sea), documenting the presence of a total of 20 Heterobranchia [42]. However, considering that the Conero Mount coastline is a crucial stepping stone for larval dispersion in the western Adriatic Sea [43] and rocky tide pools can provide refuge, feeding, and nursery grounds for many other intertidal invertebrates [44,45], an in-depth study of this environment can lead to a better understanding of Heterobranchia diversity in the whole Adriatic area. Interestingly, this tide pool system represents one of the many intertidal rocky environments characterizing the Conero Mount Regional Natural Park [46,47], suggesting that this environment could be more dynamic than expected, with shifts in Heterobranchia fauna according to changes in the sessile dominant communities due to different local conditions like currents, seasons, and salinity. Furthermore, it is noteworthy that this limestone rocky coastline extends for 15 km and represents the largest natural hard coastal substrate in the North-Western Adriatic Sea, contrary to the rest of the coastline that is mainly characterized by sandy and pebble beaches [48], so it could also function as a refuge for a lot of benthic heterobranchs that need sessile species to feed on. Finally, considering that a total of 72 sea slug species were reported from all coastal areas of Conero Riviera [17,49], it could be hypothesized that the current list of 20 species from the 'Passetto' rocky tide pools is an underestimation and a hidden diversity still remains to be revealed from this poorly characterized habitat. For all these reasons, the main aims of the present study are to (i) update the current list of Heterobranchia species living in the rocky tide pools in Ancona; (ii) improve the knowledge of the geographical distribution ranges of the species; (iii) generate an iconographic list of all the species found in the study area; and (iv) compare the Heterobranchia here reported with those already known from the same area and from similar environments in extra-Mediterranean localities.

2. Materials and Methods

2.1. Study Site

The studied area consisted of a natural rocky tide pool system located near the 'Passetto' urban beach (Ancona, Italy; 43.618947 N, 13.531880 E). The surrounding area has been extensively modified by anthropogenic activities, with several artificial fishermen caves and concrete docks, and it is characterized by intense tourism during the summer season [50]. The whole area, as a consequence of its peculiar microclimate and its morphology, is characterized by high seasonal variability in both salinity and temperature [51]. These abiotic factors are even more variable inside tide pool systems due to the freshwater contamination on rainy days and high evaporation rates on warm sunny days [52]. The selected tide pools have covered a slightly wider area than the object of the previous study [42], with the inclusion of the small pool system near the northern crevice and of the shallow channel (Figure 1), both characterized by environmental conditions perfectly

matching those reported in the previous study (i.e., a semi-closed system that forms pools completely isolated from the sea during low tide). All rock pools have an elongated shape extending sub-parallelly and often intercommunicating, corresponding to the complex originated comb-shaped morphotype in the classification system used for Italian rock pools [53]. The channel connects the open sea to a large, always submerged, semi-closed pool. Despite being deeper than the pool system, this channel completely emerges during daily minimum low tide, allowing the formation of smaller pools between the rocks. The study site is characterized by a very shallow depth, with a variable maximum between 0.2 and 0.4 m [42].

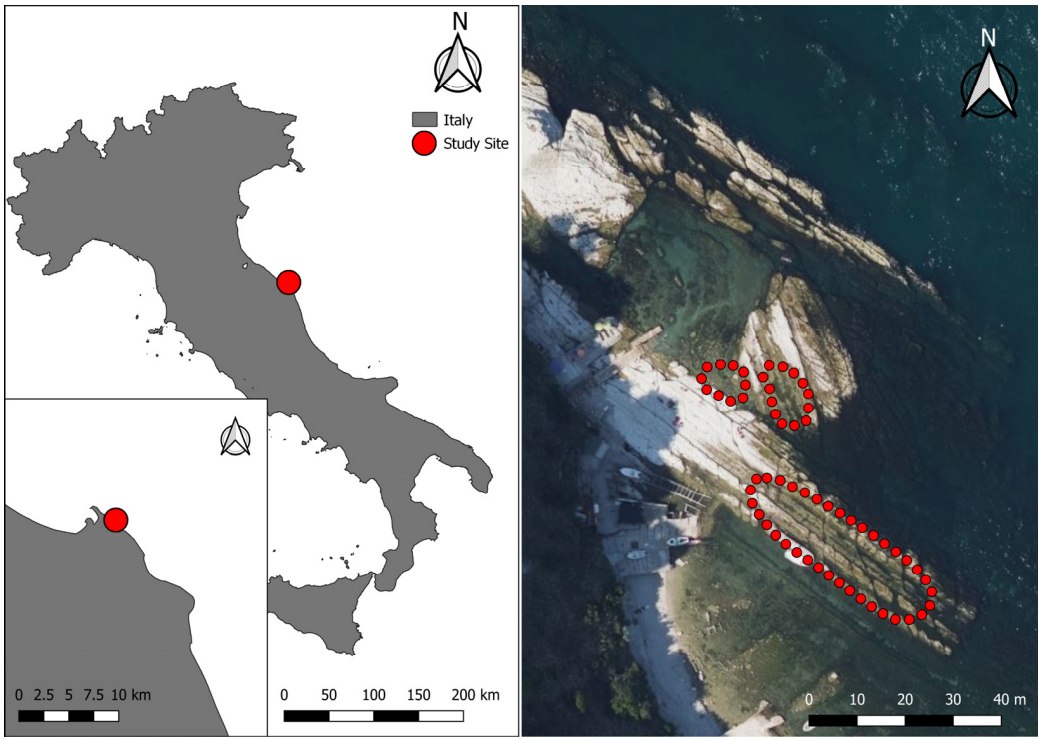

Figure 1. Map of the study area. Geographical location (**left**) and satellite orthophotography at a higher magnification level (**right**). The red dots define the area covered by this work, i.e., the system of semi-closed rocky tide pools that forms isolated pools during low tide. Satellite picture is copyright of Microsoft Bing, Microsoft Corporation.

2.2. Data Collection and Analysis

This study lasted five years and was conducted from 2018 to 2022, with some lack of data from March to June 2020 due to local restrictions during the global SARS-CoV-2 pandemic. The rocky tide pools were monitored from January to June by spotting and photographing all the heterobranchs. The months from July to December were not considered due to harmful algae blooms (HABs) of the benthic dinoflagellate *Ostreopsis ovata* Fukuyo, 1981, which have negative effects on human health and cause a die-off in most marine invertebrates [54], and due to frequent autumnal sea storms that make the site unworkable. Surveys took place during daily minimum low tide, with a wave motion of a maximum coastal height of 0.3 m to avoid rippling of the water in the pools and to allow specimens to be found. Tide and wave forecasts were checked with windy.app "https://windy.app/it" (accessed on 10 July 2022) and RMN—Rete Mareografica Nazionale, ISPRA "https://www.mareografico.it" (accessed on 10 July 2022). The mean survey duration was 1 h and 30 min, and it always ended with the first signals of rising tide, which usually corresponded to an oily and opaque surface layer accumulating in the inner pools. Specimens were photographed using three different underwater cameras: Sony α68, Sony RX100 mk, and Ricoh GX-200, Sony Europe B.V., Milano, Italy. The lighting system was

Inon z-240 type IV for Sony users, and Sea&Sea DS1, SEA&SEA Co., Ltd., Tokyo, Japan, for other users. Identification photographs were taken of all the observed species. Specimens were identified using morphological descriptions from the scientific literature [4,6,55] and Mediterranean Sea slug guides [17,31,49,56] and following the nomenclature proposed by WORMS "www.marinespecies.org" (accessed on 6 June 2024). When necessary, specimens were collected, preserved in EtOH 96%, and stored in the collection of the Department of Biological and Environmental Sciences and Technologies, DiSTeBA, of the University of Salento (Lecce, Italy) for possible future anatomical and molecular studies.

3. Results

The study area did not show any significant variation in the main structure of local communities during the five years of survey: the semi-closed submerged environment immediately adjacent to the study site was characterized by macroalgal assemblage dominated by *Gongolaria barbata* (Stackhouse) Orellana & Sansón, 2019, while the investigated pool system was the same as previously described by Riccardi et al. [42] and characterized by a cnidarian-dominated benthic fauna, with the anthozoans *Exaiptasia diaphana* (Rapp, 1829), *Anemonia viridis* (Forsskål, 1775), and *Actinia mediterranea* Schmidt, 1971, and hydrozoans of the genus *Obelia* Peron & Lesueur 1810. Other sessile common species included bryozoans such as *Bugula neritina* (Linnaeus, 1758), bivalves like *Mytilus galloprovincialis* Lamarck, 1819, balanid crustaceans like *Perforatus perforatus* (Bruguière, 1789), and various species of sea sponges (Porifera). The vagile benthic community included crustaceans like decapods *Macropodia* sp. Leach, 1814 [in Leach, 1813–1815], *Pachygrapsus marmoratus* (J.C. Fabricius, 1787), and *Palaemon elegans* Rathke, 1836; the amphipods *Caprella* spp. Lamarck, 1801, and *Jassa marmorata* Holmes, 1905; many different Gammaridae Latreille, 1802; and shelled gastropods, mainly Rissoidea Gray, 1847. The fish population mainly included Blenniidae Rafinesque, 1820, and Gobiidae Cuvier, 1816: *Aidablennius sphynx* (Valenciennes, 1836), *Lipophrys trigloides* (Valenciennes, 1836), *Parablennius incognitus* (Bath, 1968), *Salaria pavo* (Risso, 1810), and *Gobius cobitis* Pallas, 1814.

The results allowed us to confirm the presence of all 20 previously reported species [42] and to add another 25, increasing the total number of Heterobranchia currently known in the studied area to 45 (belonging to five different orders) (Table 1).

Nudibranchia was by far the most represented order (35 species represented in Figures 2–4), followed by Sacoglossa (6 species shown in Figure 4). Pleurobranchida consisted of two species (Figure 4), while Aplysiida and Cephalaspidea had one species each (Figure 1). The nudibranch family mostly represented was Trinchesiidae (six species), followed by Facelinidae (five species), and the equally represented Chromodorididae and Eubranchidae (four species). Regarding the Sacoglossa order, the family mostly represented was Limapontiidae with four species.

Within the newly recorded species, six proved particularly interesting.

Okenia mediterranea (Inhering, 1886) is recorded here for the first time in Sector 9 of the Italian Seas (according to [57]). This species has already been reported in the Adriatic Sea and in the waters beneath Conero Mount [49], which is about 10 km south of the study site and currently included in Sector 8 of the Italian Seas (Central Adriatic).

Palio nothus (G. Johnston, 1838) is an Atlantic species that is reported here for the first time in Italy. The only two known Mediterranean records of this species are from Vir Island in Croatia [58] and Cap de Creus in Spain [59].

Table 1. List of the Heterobranchia species recorded in this study from the semi-closed rocky pool system at Passetto (Ancona, Italy). Species that were previously known in the studied area [42] are highlighted in the last column with an 'x'. Taxa signed with an asterisk (*) are discussed in detail in the Results and Discussion sections.

Order	Suborder	Family	Species	[42]
APLYSIIDA		Aplysiidae	*Aplysia punctata*	
CEPHALASPIDEA		Haminoeidae	*Haminoea* sp. *	
NUDIBRANCHIA	DORIDINA	Chromodorididae	*Felimare villafranca*	x
			Felimida krohni	
			Felimida luteorosea	
			Felimida purpurea	
		Dorididae	*Doris ocelligera*	
		Discodorididae	*Jorunna tomentosa*	x
			Paradoris indecora	
		Goniodorididae	*Okenia mediterranea* *	
			Pelagella castanea	
		Polyceridae	*Palio nothus* *	
			Polycera quadrilineata	x
	CLADOBRANCHIA	Aeolidiidae	*Spurilla neapolitana*	
		Calmidae	*Calma glaucoides*	
		Dotidae	*Doto cervicenigra*	x
			Doto coronata	x
		Eubranchidae	*Eubranchus exiguus*	
			Eubranchus farrani	
			Eubranchus linensis *	x
			Eubranchus viriola *	x
		Flabellinidae	*Edmundsella pedata*	x
			Flabellina affinis	
			Flabellina cavolini *	
		Facelinidae	*Cratena peregrina*	x
			Facelina dubia	x
			Facelina rubrovittata	x
			Facelina vicina	x
			Favorinus branchialis	x
		Janolidae	*Antiopella cristata*	x
		Tethydidae	*Tethys fimbria*	
		Trinchesiidae	*Trinchesia diljuvia* *	x
			Trinchesia cuanensis *	
			Trinchesia genovae *	
			Trinchesia morrowae	x
			Trinchesia sp. 1 *	
			Trinchesia sp. 2 *	
PLEUROBRANCHIDA		Pleurobranchidae	*Berthella perforata* *	x
			Berthella stellata	
SACOGLOSSA		Limapontiidae	*Calliopaea bellula* *	
			Ercolania viridis	x
			Limapontia capitata *	
			Placida dendritica	x
		Plakobranchidae	*Elysia viridis*	x
			Thuridilla hopei	

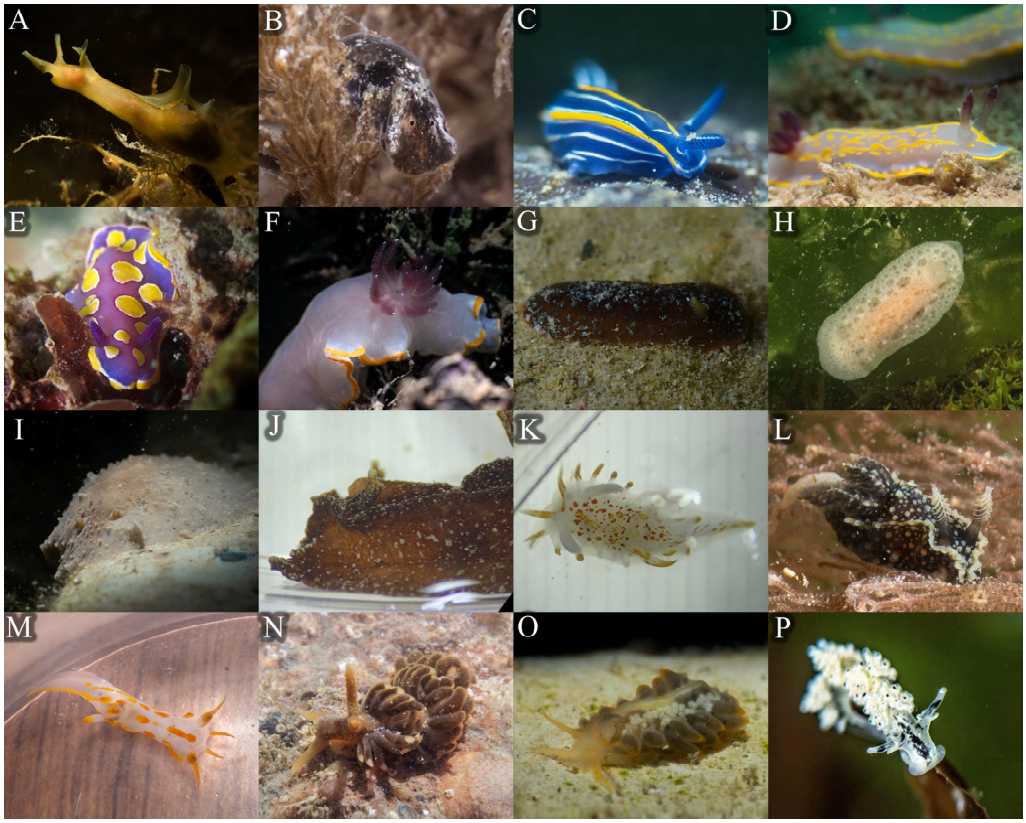

Figure 2. Heterobranchia found in the semi-closed rocky pool system at 'Passetto' (Ancona, Italy). (**A**) *Aplysia punctata*, (**B**) *Haminoea* sp., (**C**) *Felimare villafranca*, (**D**) *Felimida krohni*, (**E**) *Felimida luteorosea*, (**F**) *Felimida purpurea*, (**G**) *Doris ocelligera*, (**H**) *Jorunna tomentosa*, (**I**) *Paradoris indecora*, (**J**) *Pelagella castanea*, (**K**) *Okenia mediterranea*, (**L**) *Palio nothus*, (**M**) *Polycera quadrilineata*, (**N**) *Spurilla neapolitana*, (**O**) *Calma glaucoides*, (**P**) *Doto cervicenigra*.

Trinchesia cuanensis Korshunova, Picton, Furfaro, Mariottini, Pontes, Prkić, Fletcher, Malmberg, Lundin & Martynov, 2019, is a recently described species [55] very similar in its external morphology to other Mediterranean congeneric species such as *T. caerulea* (Montagu, 1804) and *T. morrowae* Korshunova, Picton, Furfaro, Mariottini, Pontes, Prkić, Fletcher, Malmberg, Lundin & Martynov, 2019. To date, it has never been reported in Italian waters, so the present record is the first one for Italian fauna. It is noteworthy to mention that the effective distribution of this species could be much wider than what is indicated in the literature since it could have been misidentified as Mediterranean congenerics; for this reason, a revision of the actual distribution of this species is required.

Trinchesia diljuvia Korshunova, Picton, Furfaro, Mariottini, Pontes, Prkić, Fletcher, Malmberg, Lundin & Martynov, 2019, was described for the first time with two specimens from the Yalta region in the Black Sea. This species was reported in Sicily [60], with a picture showing a whitish nudibranch identified by the authors as *Trinchesia* cf. *diljuvia*. However, the Sicilian specimen does not belong to *T. diljuvia* as the external morphology shows differences in the diagnostic characters. Rhinophores should be 1.5 times longer than the thin oral tentacles, while the Sicilian specimen has oral tentacles of the same length as the rhinophores and a thicker shape. Moreover, the formula of the cerata differs from the typical one, as does its body color pattern. In fact, the Sicilian specimen showed scattered white dorsal dots, instead of a thick white dorsal line. Considering that the author themself was not sure about the identification [60], it is reasonable to consider this record not to belong to *T. diljuvia* but to another *Trinchesia* not identified yet. On the other hand, specimens found in 'Passetto' tide pools perfectly match the morphology originally described for *T. diljuvia*, undoubtedly confirming it and the one previously reported from the same area as *Trinchesia* sp. [42] as the first report of this species in the whole Mediterranean Sea.

Figure 3. Heterobranchia found in the semi-closed rocky pool system at 'Passetto' (Ancona, Italy). (**A**) *Doto coronata*, (**B**) *Eubranchus farrani*, (**C**) *Eubranchus linensis*, (**D**) *Eubranchus viriola*, (**E**) *Eubranchus exiguus*, (**F**) *Edmundsella pedata*, (**G**) *Flabellina affinis*, (**H**) *Flabellina cavolini*, (**I**) *Cratena peregrina*, (**J**) *Facelina dubia*, (**K**) *Facelina rubrovittata*, (**L**) *Favorinus branchialis*, (**M**) *Antiopella cristata*, (**N**) *Tethys fimbria*, (**O**) *Trinchesia cuanensis*, (**P**) *Trinchesia diljuvia*.

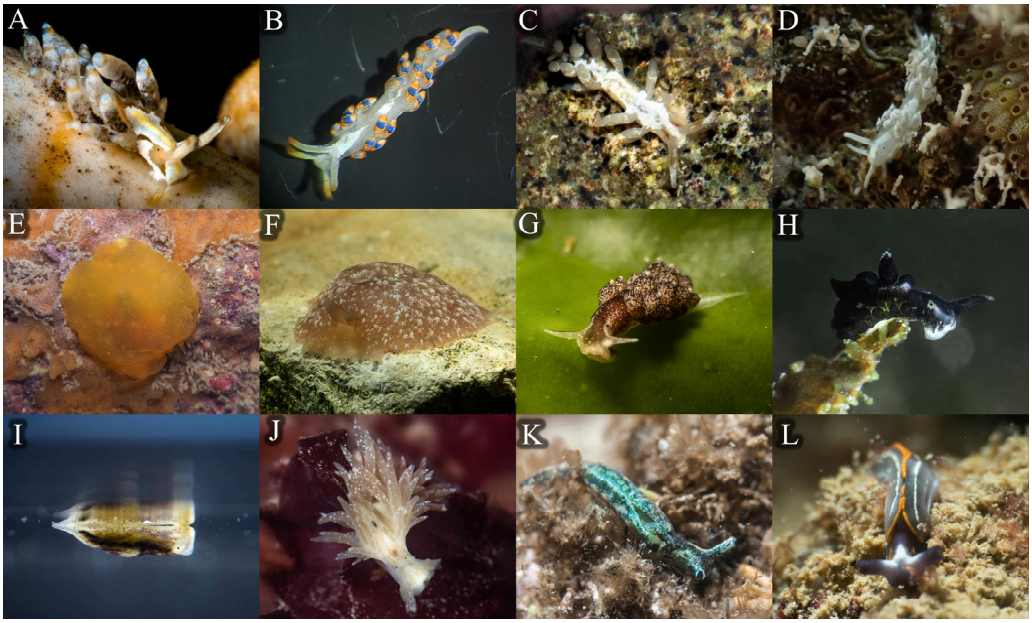

Figure 4. Heterobranchia found in the semi-closed rocky pool system at 'Passetto' (Ancona, Italy). (**A**) *Trinchesia genovae*, (**B**) *Trinchesia morrowae*, (**C**) *Trinchesia* sp. 1, (**D**) *Trinchesia* sp. 2, (**E**) *Berthella perforata*, (**F**) *Berthella stellata*, (**G**) *Calliopaea bellula*, (**H**) *Ercolania viridis*, (**I**) *Limapontia capitata*, (**J**) *Placida dendritica*, (**K**) *Elysia viridis*, (**L**) *Thuridilla hopei*.

Two *Trinchesia* species, here indicated as *Trinchesia* sp. 1 and *Trinchesia* sp. 2, have been photographed during this study. The examination of the external diagnostic features did not allow us to identify them as any known species since they showed morphological characteristics that, to our knowledge, have never been reported before in Trinchesiidae. Additional studies are required to determine if they belong to species that still need to be described or not.

4. Discussion

The in-depth study of the 'Passetto' semi-closed tide pools has more than doubled the number of species known in the studied area. Among these new findings, one was a new report for Sector 9 of the Italian Seas (according to [57]), two are new for Italian fauna, one is reported for the first time in the Mediterranean Sea, and two species are potentially new for science.

These results highlight the importance of monitoring the biodiversity not only in well-known coastal areas but also in less-studied Mediterranean marine environments. In fact, the present study led to substantial improvements in knowledge on the diversity and biogeography of the heterobranch fauna associated with the rocky tide pools as demonstrated by the presence of species never reported before in the study area or of rare and particularly interesting taxa. Regarding this last point, the only cephalaspidean found in the 'Passetto' tide pools belongs to the *Haminoea* genus, which includes a group of very complicated-to-identify Mediterranean species recently investigated through an integrative taxonomy study [61] that revealed the importance of molecular analysis in achieving a correct species assessment, especially in this group. For this reason, the specimen recorded in the present study has been provisionally named *Haminoea* sp., until further molecular and anatomical investigations can be carried out. The same identification difficulties occurred in the case of *Flabellina cavolini* (Verany, 1846) and *Flabellina gaditana* (Cervera, García-Gómez & F. J. García, 1987), two sibling species that can be recognized only after DNA-barcoding analysis [27]. Considering the Mediterranean spreading of *F. cavolini* instead of *F. gaditana*, which indeed shows a greater affinity to the neighboring Atlantic areas [27], we provisionally named the specimens observed as *F. cavolini* until further molecular assessment. Other problematic groups included the two *Berthella* species. *Berthella perforata* (R. A. Philippi, 1844) was identified in a recent study by Ghanimi et al. [62], who revealed this species to be restricted to the Mediterranean Sea, while the morphologically indistinguishable *B. plumula* (Montagu, 1803) showed an Atlantic distribution [62]. However, it is very likely that the systematics of these species will be overturned after an overall revision of the entire genus; therefore, the identifications proposed here are to be considered with caution.

The 'Passetto' rocky tide pools were revealed to be an important source of taxa never reported before for the area as well as species that are considered rare and have only been characterized by a few other records. This is the case of the two sacoglossans, *Calliopaea bellula* d'Orbigny, 1837, and *Limapontia capitata* (O. F. Müller, 1774). The former is considered to occur in the European Atlantic Ocean [63] and in the Mediterranean Sea [59,64] with the present study as the second known record of this species in Sector 9 after that of Zenetos et al. [65]. The latter species has a similar distribution [33,65] and, including the present finding, has only been reported twice in Sector 9 (first report in Trieste by Zenetos et al. [65]). Another similar case is *Eubranchus linensis* (García-Gómez, Cervera & García, 1990), originally known in the Mediterranean Sea as *E. tricolor* Forbes, 1838, until Korshunova et al. [66] recognized it as a valid species and separated from the strictly Atlantic congeneric *E. tricolor*. *Eubranchus linensis* was reported in Croatia in 2018 and in the Italian Adriatic Sea by specimens photographed in Trieste Gulf before 2017 [67]. For this reason, the specimen photographed in the present study is the second for the Italian Adriatic and the third for the whole Adriatic Sea. However, we can assume that this species is much more common and that its distribution is much wider than reported so far due to the wrong attribution of observed individuals to *E. tricolor*.

The genus *Eubranchus* has a troubled systematics that has not been fully clarified yet. In fact, some authors accepted the validity of the genus *Amphorina* after a recent paper [66] that moved five European *Eubranchus* species into this newly resurrected genus. However, the validity of the *Amphorina* genus was proposed in a paper written in Russian [68] and thus not available to most of the scientific community. Here, we prefer to maintain the traditionally accepted nomenclature until a comprehensive review of the genus *Eubranchus* is conducted. Moreover, two new *Eubranchus* species were recently described [6], *E. viriola* and *E. andra;* however, they should be re-evaluated due to the weak diagnostic characters used by the authors to separate these two species. In fact, *E. viriola* and *E. andra* show a 0.15% genetic distance at the Cytochrome Oxidase subunit I (COI) [66], a value that perfectly falls within the range of intraspecific variability accepted for mollusks in general and already demonstrated for nudibranchs [27,69]. The different ecological niches (shallower brackish water for *E. viriola* and deeper layers characterized by normal oceanic salinity for *E. andra* [66]) and the distinct color patterns ("the brackish water-living *A. viriola* sp. nov. differs from the closely related *A. andra* sp. nov. by the presence of light pinkish subapical rings on the cerata, the absence of forms with non-transparent blackish pigmentation, or any forms with uniform orange colour" [66]) are the two key features used by the authors to support the validity of these species. However, our findings constitute additional proof of the unfoundedness of the separation proposed by the authors of [66] since the 'Passetto' rocky tide pools hosted specimens showing the color pattern assigned to the *E. andra* morphotype but sharing the brackish water environment typical of *E. viriola* (Figure 5). For all these reasons, we propose considering *Eubranchus andra* as a junior synonym of *Eubranchus viriola* and we confirm that the specimen found in the 'Passetto' tide pools belongs to *E. viriola*.

Figure 5. In situ pictures of *Eubranchus viriola* specimens showing a uniform orange body color pattern. (**A**) Dorsal, (**B**) lateral, and (**C**) frontal views of the specimens observed in the 'Passetto' rocky tide pools.

The Mediterranean *Trinchesia genovae* (O'Donoghue, 1926), described in the Ligurian Sea, is known from the Atlantic Ocean [70] to the Levantine Sea [71], and in the Adriatic and Tyrrhenian Seas [72,73]. It was first reported for Sector 9 in 2023 [67], and the present finding is the second for the same Sector and the third for the whole Adriatic Sea. The finding of the congeneric species *T. diljuvia* in the 'Passetto' rocky tide pools is also particularly interesting from another point of view. In fact, all the known specimens were found in environments characterized by similar depths and salinity. The topotypical specimens from the Black Sea (mean salinity 21‰ [74]) were found at a depth of around 1 m, while the specimens from 'Passetto' tide pools were found at a depth of less than 0.5 m in February 2018, and February, March, and April 2021 (mean T = 9.9 °C, mainly in the days after rain. windyapp.it). For this reason, it is possible to speculate that the nudibranch *T. diljuvia* is restricted to hyposaline marine environments, especially in shallow hard substrates.

The results from the present study highlight how continuous monitoring of tide pool environments is crucial to better understand the complexity of heterobranch communities in this poorly studied ecosystem, and they can help shed light on important ecological aspects of the species inhabiting these highly variable habitats. Furthermore, the large quantity of species added thanks to this study and the local variable conditions, followed

by the constant shifting in sessile invertebrate communities, could suggest a constantly increasing number of Heterobranchia recorded in the future, suggesting the need for continuous monitoring. These semi-closed basins can play a key role as a hot spot for local biodiversity and can act as a natural laboratory for investigating poorly known ecological dynamics to unravel cases of cryptic and neglected species and provide early warning of Non-Indigenous Species that can find good conditions to survive and reproduce in this heterogeneous environment. Moreover, the high trophic and environmental selectivity of marine Heterobranchia makes their community very variable in space and time, and this is especially true in extremely variable environments like tide pools. Finally, this study provides important data on the seasonality of the species and their sensitivity to specific environmental factors that could be very important from a conservation perspective. In fact, knowing how heterobranch communities can change over time could be useful to highlight significant trends of environmental variation, helping promote more efficient conservation strategies.

In conclusion, a more representative assessment of the Heterobranchia fauna associated with this peculiar habitat was obtained, also shedding light on the geographical ranges of distribution, which may be useful for future comparative analyses in other similar environments. Furthermore, filling some gaps in the knowledge on this susceptible environment can constitute an opportunity to value this easily accessible habitat, promoting biodiversity management and protection.

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