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Two Seas for One Great Diversity: Checklist of the Marine Heterobranchia (Mollusca; Gastropoda) from the Salento Peninsula (South-East Italy)

Giulia Furfaro [1,*], Fabio Vitale [2,3], Cataldo Licchelli [2,4] and Paolo Mariottini [5]

1 Department of Biological and Environmental Sciences and Technologies—DiSTeBA, University of Salento, I-73100 Lecce, Italy
2 Salento Sommerso Association, I-73100 Lecce, Italy; fabio_vitale@tin.it (F.V.); cataldo.licchelli@gmail.com (C.L.)
3 Museum of Natural History of Salento, I-73021 Calimera-Lecce, Italy
4 Cooperativa Hydra, I-73100 Lecce, Italy
5 Department of Science, University of Roma Tre, I-00146 Rome, Italy; paolo.mariottini@uniroma3.it
* Correspondence: giulia.furfaro@unisalento.it

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Abstract: The Salento peninsula is a portion of the Italian mainland separating two distinct Mediterranean basins, the Ionian and the Adriatic seas. Several authors have studied the marine Heterobranchia (Mollusca, Gastropoda) fauna composition living in the Ionian Sea, but to date further knowledge regarding this interesting group of mollusks is still needed. Recent studies have corroborated the peculiarity of the Mediterranean Sea showing high levels of endemism and cryptic diversity. On the other hand, marine sea slugs have been revealed to be important indicators of the marine ecosystem's health, due to their species-specific diet that consist of a vast variety of sessile and benthic invertebrates. A baseline study of the marine Heterobranchia diversity is therefore a necessary step to reveal the hidden diversity and to monitor the possible presence of alien species. The present study shows results from approximately 600 scientific dives carried out during a nine-year period in all of the main submarine habitats of the studied area, while accounting for the marine Heterobranchia from both the Ionian and Adriatic Seas. With this contribution, the list of marine Heterobranchia inhabiting the Salento Peninsula rises to 160. Furthermore, it also reports, for the first time, the presence of one alien species and three new records for Italian waters. Ecological notes and geographical distribution for each added species are provided together with animal iconography, consisting mainly of in situ photographs, for species identification.

Keywords: diversity; sea slugs; nudibranchs; Mediterranean Sea; monitoring

1. Introduction

The Salento Peninsula (South-East Italy) in Puglia is a strip of land right on the border of the eastern Mediterranean Sea, made up of a large variety of habitats: rocky formations, open sandy beaches, marine caves, etc. The Salento Peninsula is a physically well-identified region, encompassing the innermost point of the Gulf of Taranto (40°31′17.8′′N 17°06′10.7′′E) and the beach of Torre Santa Sabina di Ostuni (40°45′52.3′′N 17°41′20.1′′E), with a length of 138 kilometers, calculated on the axis between Martina Franca (40°42′17.7′′N 17°20′20.9′′E) and the Cape of Santa Maria di Leuca (39°47′40.4′′N 18°22′05.0′′E). The maximum and minimum width are 54 and 33 kilometers, respectively, and the total coastal length is 365 kilometers [1–5]. This peninsula protrudes between two ecoregions of the Mediterranean Sea [6], the Northern Ionian, and the Southern Adriatic seas, which are conventionally separated by the dividing line passing through Otranto (according to the biogeographical zones described by Bianchi [7], i.e., zones 6 and 7, respectively). These two basins are characterised by peculiar and distinct main currents and submarine morphologies, resulting in very complex and dynamic ecosystems affected by seasonal fluctuations, which influences both the shallow and deep communities [8,9]. Therefore, this marine area could potentially host a high abundance of Heterobranchia species, due to their pelagic larval stage and diversified diet. Knowledge on diversity is a basic requisite to identify targets and to monitor species composition shifts over time, caused by natural or anthropogenic factors. In fact, the change in marine Heterobranchia fauna composition over time is considered a good ecological indicator of potential environmental modifications [10–14] and this peculiarity is particularly interesting in such a heterogeneous area of the Mediterranean. Furthermore, the capability of marine Heterobranchia to host biological compounds that are potentially interesting for biomedical applications, provides additional value to the study of this particular group of mollusks [14]. In fact, many species of marine Heterobranchia display aposematic vivacious warning colors to indicate that they contain defensive secondary metabolites that are sequestered, transformed from dietary sources, or synthesized de novo [15]. These compounds are used, mainly by nudibranchs, as a chemical defense from predation [16,17] and have revealed to be an important source of diverse bioactive products used as effective analgesic, anti-inflammatory, antiviral, and anticancer drugs [18,19]. The Mediterranean marine diversity is therefore revealed to be interesting, mainly due to the presence of cryptic or endemic species. In fact, several species once considered to be widespread across the Atlantic and the Mediterranean were shown to be a complex of cryptic species, many of which are endemic to the Mediterranean (e.g., [20–23]). This trend is also true for marine Heterobranchia, as demonstrated by recent molecular papers that helped to unravel such cryptic diversity [24–29]. In this context, studies focused on the production of species lists from different geographical areas are needed as an essential starting point to unveil this hidden diversity. In the past decade, the key works on marine Heterobranchia in the area under investigation have been published by Perrone [30–37], who mainly provided data regarding the species inhabiting the Ionian side of the Salento peninsula (Gulf of Taranto), and only provided ecological notes on a small number of species. Recently, Onorato and Belmonte [38] reviewed the biodiversity assessment of the marine submerged caves in the Salento peninsula, which includes some heterobranchs, while Micaroni et al. [39] published a check-list of Heterobranchia from the Ionian locality of Tricase, adding 20 species to Perrone's previous lists [30–37]. Finally, several published papers that did not have proper checklists, added new records of single species sampled from the Salento Peninsula [29,40–44], contributing to the increase of lists of species known from this Apulian area. However, to date, there is no published checklist of marine Heterobranchia fauna from the Adriatic side of the Salento peninsula. During the last ten years, there has been an increasing interest in marine observation by underwater photographers [45,46]. This has allowed the possibility to extend the research on fauna, to detect lesser known species, and to create a collaboration network between scientists and amateurs who are experts in this field. The so-called Citizen Science dedicated to the Heterobranchia is an important support in expanding the body of knowledge on this group of gastropods, by providing field observations on bathymetric distribution, seasonality [47], egg deposition, reproductive behavior and trophic niche, and by making the data immediately available to the scientific community through web-based social networks [48]. In the last decade, due to the combined efforts of professional underwater photographers and acknowledged systematic experts in sea slugs, many new records and ecological observations on the Salento Peninsula marine Heterobranchia have been collected. Taking all of these points into account, the present study had the following aims to: (i) contribute to the Salento Peninsula marine Heterobranchia checklist with new records from the sublittoral waters, considering both sides of this Peninsula, the Ionian and the Adriatic sides, for the first time; (ii) provide ecological notes and local distribution for each new recorded species; (iii) show in situ photographs of the live animals to document species identification.

2. Materials and Methods

The geographical area under investigation was a stretch of more than 270 km of coastline around the Salento Peninsula in Southern Italy (Figure 1). With 600 scuba dives in the past 9 years, different benthic habitats were surveyed, some of which were included in the European legislative context (EU WFD, EU Habitat Directive, EU MSFD): pre-coralligenous and coralligenous assemblages, soft-bottom substrates, *Posidonia oceanica* (Linnaeus) Delile, 1813 meadows, and algal biocoenosis on rocky substrates. All sampled sites were georeferenced (Table 1) to provide accurate data of the studied locations for future monitoring and comparison. When possible, ecological observation and in situ photographs of the individuals recorded were performed and catalogued for species identification. The Scuba dives (0–40 m depth) took place all year round, almost every week, during daylight and at night, between 2011 and 2019. Specimens between 2 and 5 mm in length were photographed alive in the laboratory, in Petri capsules illuminated by a series of low voltage LED lamps, with 6500 K bulbs. The camera used for filming was a tripod mounted Nikon D7100, with 60 mm micro Nikkor or 105 mm micro Nikkor optics, a series of extension rings and additional lenses. The underwater photographic or videography equipment (F.V.) was a Nikon D7000 body, 60 mm micro Nikkor, or 105 mm micro Nikkor optics, extension rings inside an Isotta housing, as well as additional wet lenses SubSee +10 diopter, two underwater flashes Inon z240, and two LED lamps of great luminous power. Or alternatively, (C.L.) a Canon 600D body in a Nauticam 600D housing, equipped with the following lenses—for macro photography and in some cases for micro subjects, the Canon 60 mm USM Macro, and Canon 100 mm USM Macro were used, in addition to wet lenses SubSee +5 and +10 diopter; for wide angle photography, the Tokina 10–17 mm fisheye lens was used. The light source was provided by a couple of Inon strobes z240 and a single focus light I-Torch Video Pro 3. The systematics and the validity of names were checked with the help of the Word Register of Marine Species [49]. Species identification was obtained by morphological investigation and a subsequent consultation of the existent literature ([29,41,44] and other references cited in the present work), guide books [50,51] and websites [48,52,53]. In the case of *Berthellina* cf. *edwardsii*, the shell from an individual (Voucher RM3_1865) was extracted and used to confirm the identification as this anatomical feature is commonly considered to be diagnostic for this species. The shell was removed and dissolved in a 10% NaOH solution, then rinsed in water, dried, and mounted for examination by optical microscopy, following the same protocol described by Furfaro et al. [54]. Voucher numbers were assigned to the collected individuals that were selected for future molecular analyses; samples were preserved in 95% alcohol and stored in the Department of Science at the Roma Tre University (Rome, Italy) (Table 2). Finally, a comparison between species as recorded by previous authors (Perrone [30–37] (**A**), Onorato and Belmonte [38] (**B**), Micaroni et al. [39] (**C**)) and the present study (**D**) was carried out and reported in Table 2 with new records highlighted in bold letters. An ethical approach in this research was also carried out by complying with the restrictions in term of collected sample size, environmental survey of the collection sites, use of hand-net picking of specimens (harmless and not destructive) as well as complying with local, regional, national, and international rules, and regulations for access to biodiversity, sustainable use, and benefit sharing (Convention on Biological Diversity and its Nagoya Protocol, national regulations).

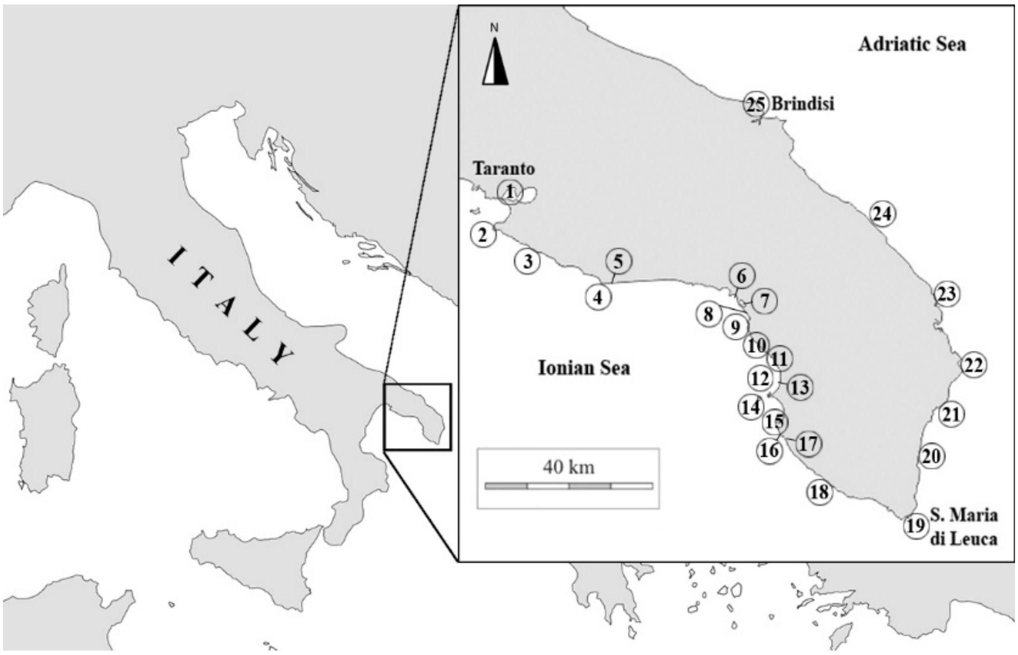

Figure 1. Map of the Salento Peninsula indicating the sampling localities; the box highlights the Salento Peninsula at a higher magnification. Numbers refer to sampling stations reported in Table 1.

Table 1. Number, name, geographic coordinates (latitude and longitude), and the depth range of the sampled stations.

N°	Station	Latitude	Longitude	Depth
1	Mar Piccolo, Taranto (Ionian Sea)	40°28′53.62″N	17°16′00.91″E	1–10 m
2	Capo S. Vito, Taranto (Ionian Sea)	40°24′35.85″N	17°12′06.07″E	1–20 m
3	Porto Pirrone, Taranto (Ionian Sea)	40°21′27.79″N	17°19′40.93″E	4–20 m
4	Torre Ovo, Taranto (Ionian Sea)	40°17′29.18″N	17°30′11.20″E	1–20 m
5	Campomarino, Taranto (Ionian Sea)	40°17′34.76″N	17°31′41.48″E	1–20 m
6	Porto Cesareo I. Conigli, Lecce (Ionian Sea)	40°15′32.15″N	17°52′57.59″E	1–15 m
7	Porto Cesareo, Lecce (Ionian Sea)	40°14′51.48″N	17°54′33.51″E	0–1 m
8	S. Isidoro, Lecce (Ionian Sea)	40°13′15.51″N	17°55′29.27″E	1–5 m
9	Torre Inserraglio, Lecce (Ionian Sea)	40°10′41.44″N	17°55′51.53″E	1–20 m
10	Santa Caterina di Nardò, Lecce (Ionian Sea)	40°08′05.87″N	17°59′15.53″E	1–15 m
11	Santa Maria al Bagno, Lecce (Ionian Sea)	40°07′30.58″N	17°59′46.43″E	0–6 m
12	O.R. Gallipoli, Lecce (Ionian Sea)	40°06′04.68″N	17°58′04.22″E	36 m
13	Gallipoli, Lecce (Ionian Sea)	40°04′34.70″N	17°59′50.25″E	1–20 m
14	Gallipoli - Isola S. Andrea, Lecce (Ionian Sea)	40°02′36.77″N	17°57′01.64″E	5–20 m
15	Gallipoli Pizzo, Lecce (Ionian Sea)	39°59′57.64″N	17°59′27.36″E	1–15 m
16	Marina Mancaversa, Lecce (Ionian Sea)	39°58′58.04″N	18°00′18.58″E	1–5 m
17	Torre Suda, Lecce (Ionian Sea)	39°56′27.95″N	18°02′17.36″E	1–5 m
18	Ugento, Lecce (Ionian Sea)	39°52′36.70″N	18°05′25.14″E	5–30 m
19	Santa Maria di Leuca, Lecce (Ionian Sea)	39°48′18.38″N	18°22′42.56″E	1–20 m
20	Tricase, Lecce (Adriatic Sea)	39°55′51.31″N	18°23′47.92″E	1–30 m
21	Porto Miggiano, Lecce (Adriatic Sea)	40°01′46.72″N	18°27′01.30″E	1–25 m
22	Otranto, Lecce (Adriatic Sea)	40°08′18.52″N	18°30′47.65″E	1–40 m
23	Roca, Lecce (Adriatic Sea)	40°17′50.18″N	18°24′49.69″E	1–6 m
24	Frigole, Lecce (Adriatic Sea)	40°26′20.52″N	18°14′46.56″E	0–1 m
25	Brindisi (Adriatic Sea)	40°39′54.11″N	17°57′34.81″E	1–8 m

Table 2. List of species of Heterobranchia occurring around the Salento peninsula; localities are numbered according to Figure 1, with new records in column D, highlighted in bold. Column **A** = Perrone [30–37]; **B** = Onorato and Belmonte [38]; **C** = Micaroni et al. [39]; and **D** = Present study.

Taxonomy	A	B	C	D	Vouchers
Pleurobranchida					
Family Pleurobranchidae Gray, 1827					
Pleurobranchus membranaceus (Montagu, 1803)	16				
***Pleurobranchus testudinarius* Cantraine, 1835**				**1,17**	
Berthellina* cf. *edwardsii				**1,4,6,10,13,15**	RM3_1865
Berthella aurantiaca (Risso, 1818)	8,9,19,23				
Berthella elongata (Cantraine, 1836)	16				
Berthella ocellata (delle Chiaje, 1830)	8			5,6	
Berthella plumula (Montagu, 1803)	16				
Berthella stellata (Risso, 1826)	9			22	
Berthellina citrina (Rüppell & Leuckart, 1828)	18				
Family Pleurobranchaeidae Pilsbry, 1896					
Pleurobranchaea meckeli (Blainville, 1825)	16		11	5,6	
Nudibranchia - Doridina					
Family Calycidorididae Roginskaya, 1972					
***Diaphorodoris luteocincta* (M. Sars, 1870)**				**6**	
Diaphorodoris papillata Portmann & Sandmeier, 1960			11	5,6,10	
Family Onchidorididae Gray, 1927					
Adalaria proxima (Alder & Hancock, 1854)	21				
Knoutsodonta albonigra (Pruvot-Fol, 1951)	20,24				
Family Goniodorididae H. & A. Adams, 1854					
Goniodoris castanea Alder & Hancock, 1845	20			1	
Okenia longiductis Pola M, Paz-Sedano S, Macali A, Minchin D, Marchini A, Vitale F, 2019 [41]				1,4	
Okenia mediterranea (von Ihering, 1886) [41]				6	
Okenia problematica Pola M, Paz-Sedano S, Macali A, Minchin D, Marchini A, Vitale F, 2019 [41]				7	
***Trapania lineata* Haefelfinger, 1960**				**4–6,10,13,15**	**RM3_1042, RM3_1048, RM3_1077**
***Trapania maculata* Haefelfinger, 1960**				**4–6,10,13,15**	**RM3_1076**
Family Polyceridae Alder & Hancock, 1845					
***Crimora papillata* Alder & Hancock, 1862**				**5,6,11**	
Kaloplocamus ramosus (Cantraine, 1835)			11		
***Polycera elegans* (Bergh, 1894)**				**1**	
***Polycera hedgpethi* Marcus, 1964**				**1**	
Polycera quadrilineata (O. F. Müller, 1776)	3,16		11	1,3–6,10,13,15	RM3_1065
Thecacera pennigera (Montagu, 1815)				1	
Family Aegiridae P. Fischer, 1883					
Aegires punctilucens (d'Orbigny, 1837)	4				
Family Cadlinidae Bergh, 1891					
Aldisa banyulensis Pruvot-Fol, 1951	23,24			6	
Family Chromodorididae Bergh, 1891					
***Felimare fontandraui* (Pruvot-Fol, 1951)**				**6,8**	**RM3_1039, RM3_1040, RM3_1099, RM3_1100**
***Felimare orsinii* (Vérany, 1846)**				**8,12,13,15**	
Felimare picta (Philippi, 1836)	19	13	11	1,4–6, 8,10,12,13,15	RM3_1041, RM3_1052, RM3_1053
Felimare tricolor (Cantraine, 1835)	5		11	1,4–6, 8,10,12,13,15	RM3_1074, RM3_1075
Felimare villafranca (Risso, 1818)	16		11	1,5,6	RM3_1231, RM3_1232

Table 2. *Cont.*

Taxonomy	A	B	C	D	Vouchers
***Felimida binza* (Ev. Marcus & Er. Marcus, 1963)**				**15**	
Felimida krohni (Vérany, 1846)		13	11	4–6,13	RM3_1061, RM3_1068
Felimida luteorosea (Rapp, 1827)	16		11	1,6	
Felimida purpurea (Risso, 1831)	4			6	
Family Dorididae Rafinesque, 1815					
Doris ocelligera (Bergh, 1881)			11	3	
Doris pseudoargus Rapp, 1827	19				
Doris verrucosa Linnaeus, 1758	19			1	
Family Discodorididae Bergh, 1891					
Atagema rugosa Pruvot-Fol, 1951	19				
Baptodoris cinnabarina Bergh, 1884	8,20			22, 6	
Discodoris stellifera (Vayssière, 1903)	9,19			15	
Gargamella perezi (Llera & Ortea, 1982)	19,24				
Geitodoris bonosi Ortea & Ballesteros, 1981	3				
Geitodoris portmanni (Schmekel, 1972)	20				
Jorunna tomentosa (Cuvier, 1804)	3,10,20			1, 13	
Paradoris indecora (Bergh, 1881)	3,24				
Peltodoris atromaculata Bergh, 1880	8		11	ALL	RM3_1054, RM3_1056, RM3_1057
Peltodoris sordii Perrone, 1989	3				
Platydoris argo (Linnaeus, 1767)	8		11	5,6,10,13	
Rostanga anthelia Perrone, 1991	4				
Rostanga rubra (Risso, 1818)	20,23				
Taringa armata Swennen, 1961	22				
Taringa pinoi Perrone, 1985	20,24				
Tayuva lilacina (Gould, 1852)	3			1,22	
Family Phyllidiidae Rafinesque, 1814					
Phyllidia flava Aradas, 1847	19		11	4–6,9,10,13	RM3_1049, RM3_1055, RM3_1058
Family Dendrodorididae O'Donoghue, 1924					
Dendrodoris grandiflora (Rapp, 1827)	16		11	1,3,5,6	
Dendrodoris limbata (Cuvier, 1804)	1,16, 19			1,3,5,6	
Doriopsilla areolata Bergh, 1880	17,23			3,4,6	
Nudibranchia - Cladobranchia					
Family Tritoniidae Lamarck, 1809					
Marionia blainvillea (Risso, 1818)	9			19	
Tritonia manicata Deshayes, 1853	8,16		11	5,6	
***Tritonia nilsodhneri* Marcus Ev., 1983**				**9**	
Tritonia striata Haefelfinger, 1963	16			5,6	
Family Hancockiidae MacFarland, 1923					
Hancockia uncinata (Hesse, 1872)	16				
Family Scyllaeidae Alder & Hancock, 1855					
Scyllaea pelagica Linnaeus, 1758	19				
Family Tethydidae Rafinesque, 1815					
Melibe viridis Kelaart, 1858 [55]				1,5,6	
Tethys fimbria Linnaeus, 1767	16			6,13	
Family Dotidae Gray, 1853					
Doto acuta Schmekel & Kress, 1977			11		
***Doto cervicenigra* Ortea & Bouchet, 1989**				**3,6**	
***Doto floridicola* Simroth, 1888**				**13**	
Doto fragaria Ortea & Bouchet, 1989 [56]				6	
Doto koenneckeri Lemche, 1976			11	13	
Doto paulinae Trinchese, 1881			11	6	
Doto pygmaea Bergh, 1871			11		

Table 2. *Cont.*

Taxonomy	A	B	C	D	Vouchers
Family Proctonotidae Gray, 1853					
Antiopella cristata (Delle Chiaje, 1841)	9			1,3,5,6	
Family Arminidae Iredale & O'Donoghue, 1841					
Armina tigrina Rafinesque, 1814	18			5	
Dermatobranchus cf. *rubidus* (Gould, 1852) [40]				2	
Family Coryphellidae Bergh, 1889					
Fjordia lineata (Lovén, 1846)	16			6,7	
Family Flabellinidae Bergh, 1889					
Calmella cavolini (Vérany, 1846)	8		11	5,6,13,15	RM3_354, RM3_481, RM3_482, RM3_484, RM3_1079, RM3_1080, RM3_1081, RM3_1082, RM3_1083, RM3_1084, RM3_1085, RM3_1086, RM3_1087, RM3_1088, RM3_1089, RM3_1090, RM3_1091, RM3_1092, RM3_1093, RM3_1095
Edmundsella pedata (Montagu, 181)	8	13		ALL	RM3_1046, RM3_1047, RM3_1059, RM3_1067
Flabellina affinis (Gmelin, 1791)	8	13	11	ALL	RM3_1050, RM3_1043, RM3_1060, RM3_1063, RM3_1064, RM3_1070, RM3_1071, RM3_1073
***Paraflabellina gabinierei* (Vicente, 1975)**				**1,4,5,13**	
Paraflabellina ischitana (Hirano & T. E. Thompson, 1990)		13		2,4–6,9,10,13,15	RM3_345, RM3_346, RM3_532, RM3_533
Family Samlidae Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl & Picton, 2017					
Luisella babai (Schmekel, 1972)		13	11	13	RM3_1069
Family Piseinotecidae Edmunds, 1970					
Piseinotecus soussi Tamsouri, Carmona, Moukrim & Cervera, 2014 [57]				6	RM3_862, RM3_863, RM3_1236
Family Aeolidiidae Gray, 1827					
Aeolidiella alderi (Cocks, 1852)	16,20			1,3	
Aeolidiella glauca (Alder & Hancock, 1845)	16				
Berghia coerulescens (Laurillard, 1832)	16		11	5,6,13,15,18	
Berghia verrucicornis (A. Costa, 1867)	19			13,6	
***Limenandra nodosa* Haefelfinger & Stamm, 1958**				**2**	
Spurilla neapolitana (Delle Chiaje, 1841)	16		11	1,3,4	
Family Facelinidae Berg, 1889					
***Caloria elegans* (Alder & Hancock, 1845)**				**4,5,6**	RM3_1051

Table 2. *Cont.*

Taxonomy	A	B	C	D	Vouchers
Cratena peregrina (Gmelin, 1791)			11	ALL	RM3_1038, RM3_1045, RM3_1062, RM3_1066, RM3_1072, RM3_1078, RM3_1094
***Dicata odhneri* (Schmekel, 1967)**				**1,4,6**	
***Dondice banyulensis* Portmann & Sandmeier, 1960**				**6,8,13,19**	
Facelina annulicornis (Chamisso & Eysenhardt, 1821)			11	5,6,13–15	
***Facelina fusca* Schmekel, 1966**				**3**	**RM3_1201, RM3_1235**
***Facelina rubrovittata* (A. Costa, 1866)**				**2,4–6,12,13,15**	
***Facelina vicina* (Bergh, 1882)**				**6**	**RM3_1202**
Favorinus branchialis (Rathke, 1806)	16,20			1,3,5,6,13,15	
Family Eubranchidae Odhner, 1934					
Eubranchus andra (Korshunova, Malmberg, Prkić, Petani, Fletcher, Lundin, Martynov, 2020) [44]				1, 3, 6	
***Eubranchus* cf. *farrani* (Alder & Hancock, 1844)**				**1,3,10,13,15,21**	
***Eubranchus* cf. *linensis* Garcia-Gomez, Cervera & Garcia, 1990**				**1,6**	
Eubranchus cf. *exiguus* (Alder & Hancock, 1848)			11	10,6	
Family Fionidae Gray, 1857					
Fiona pinnata (Eschscholtz, 1831)			11		RM3_1097, RM3_1098
Family Trinchesiidae F. Nordsieck, 1972					
***Catriona maua* Ev. Marcus & Er. Marcus, 1960**				**4**	
***Rubramoena amoena* (Alder & Hancock, 1845)**				**13**	
***Trinchesia genovae* (O'Donoghue, 1926)**				**3,5,6,13**	
***Trinchesia morrowae* Korshunova, Picton, Furfaro, Mariottini, Pontes, Prkić, Fletcher, Malmberg, Lundin & Martynov, 2019**				**6**	
***Trinchesia* cf. *miniostriata* Schmekel, 1968**				**13**	
Trinchesia ocellata Schmekel, 1966	5				
Umbraculida					
Family Tylodinidae Gray, 1847					
Tylodina perversa (Gmelin, 1791)	4,16			5,6	
Family Umbraculidae Dall, 1889					
Umbraculum umbraculum (Lightfoot, 1786)	16	13	11	3,5,6,13,15	RM3_1037
Runcinida					
Family Runcinidae H. & A. Adams, 1854					
Runcina adriatica T. E. Thompson, 1980 [42]			11	3,5,6,13	
Runcina brenkoae T. E. Thompson, 1980			11		
***Runcina* cf. *ferruginea* Kress, 1977**				**13**	
Runcina cf. *ornata* (Quatrefages, 1844)			11		
Cephalaspidea					
Family Philinidae Gray, 1850					
Philine punctata **(J. Adams, 1800)**				**4**	
***Philine quadripartita* Ascanius, 1772**				**1**	
Family Aglajidae Pilsbry, 1895					
Aglaja tricolorata Renier, 1807			11	5,6	
***Camachoaglaja africana* (Pruvot-Fol, 1953)**				**3,5,6,13**	
***Philinopsis depicta* (Renier, 1807)**				**5,6**	
***Melanochlamys wildpretii* Ortea, Bacallado & Moro, 2003**				**3**	
Family Bullidae Gray, 1827					
Bulla striata Bruguière, 1792			11	1, 4	
Family Haminoeidae Pilsbry, 1895					

Table 2. *Cont.*

Taxonomy	A	B	C	D	Vouchers
***Haminoea* cf. *orteai* Talavera, Murillo & Templado, 1987**				**3,13**	
Weinkauffia turgidula (Forbes, 1844)			11	**4**	
Aplysiida					
Family Aplysiidae Lamarck, 1809					
Aplysia dactylomela Rang, 1828 [58]				5,6,13	
Aplysia depilans Gmelin, 1791	16	13	11	ALL	
Aplysia fasciata Poiret, 1789	16		11	ALL	
Aplysia parvula Mörch, 1863	16		11	ALL	
Aplysia punctata (Cuvier, 1803)	16	13		ALL	
Bursatella leachii Blainville, 1817	2,16,19			1	
Notarchus punctatus Philippi, 1836	16				
Petalifera petalifera (Rang, 1828)	16,23			5,13	
Phyllaplysia lafonti (P. Fischer, 1870)	19				
Sacoglossa					
Family Oxynoida Stoliczka, 1868					
Lobiger serradifalci (Calcara, 1840)	2				
Oxynoe olivacea Rafinesque, 1814	2				
Family Plakobranchidae Gray, 1840					
Bosellia mimetica Trinchese, 1891	8,16,19		11	3,6,10	
Elysia flava Verrill, 1901	19,20			6	
***Elysia gordanae* T. E. Thompson & Jaklin, 1988**				**3,6,10,13,15**	
Elysia hetta Perrone, 1990	9,19			15	
***Elysia margaritae* Fez, 1962**				**6**	
Elysia timida (Risso, 1818)	16		11	ALL	
Elysia translucens Pruvot-Fol, 1957	19				
Elysia viridis (Montagu, 1804)	16		11	1,22	RM3_1096
Elysia rubeni Martín-Hervás, Carmona, Jensen, Licchelli, Vitale & Cervera, 2019 [29]				5,6,13	
Thuridilla hopei (Vérany, 1853)	16,19,20		11	ALL	RM3_1044
Family Hermaeidae H. & A. Adams, 1854					
***Aplysiopsis elegans* Deshayes, 1853**				**1**	
Cyerce cristallina (Trinchese, 1881)	19			2, 13	
***Cyerce graeca* T. E. Thompson, 1988**				**6**	
***Hermaea bifida* (Montagu, 1816)**				**1**	**RM3_1165, RM3_1166, RM3_1167**
***Hermaea paucicirra* Pruvot-Fol, 1953**				**3,6**	
Hermaea variopicta (A. Costa, 1869)	24			6	
Family Limapontiidae Gray, 1847					
Calliopaea bellula d'Orbigny, 1837	16,19				
***Ercolania coerulea* Trinchese, 1892**				**3**	
Ercolania viridis (A. Costa, 1866)			11	3,14	
***Limapontia capitata* (O. F. Müller, 1774)**				**25**	
***Placida cremoniana* (Trinchese, 1892)**				**1,6**	
Placida dendritica (Alder & Hancock, 1843)	9			3	

Abbreviation cf. is from the Latin *confer/conferatur*, both meaning compare.

3. Results

The present checklist reports 160 marine Heterobranchia species from the Salento Peninsula (Table 2) consisting of: 10 Pleurobranchida, 9 Cephalaspidea, 4 Runcinida, 2 Umbraculida, 9 Aplisiida, 24 Sacoglossa, and 102 Nudibranchia (50 Doridina, 52 Cladobranchia). This contribution added 45 species (Tables 2 and 3) to the marine heterobranchs fauna known from this area, so far, and in particular, 2 species belonging to Pleurobranchida, 6 Cephalaspidea, 1 Runcinida, 9 Sacoglossa, and 27 Nudibranchia (9 Doridina, 18 Cladobranchia). One alien species, *Polycera hedgpethi* Er. (Marcus, 1964), was reported for the first time in the studied area. This work reported for the first time the presence of *Elysia margaritae* (Fez, 1962), *Haminoea* cf. *orteai* (Talavera, Murillo and Templado, 1987), and *Rubramoena amoena* (Alder & Hancock, 1845), in Italian waters. Table 3 provides the species list with ecological remarks of all added species. Furthermore, an extensive photographic catalogue mainly consisting of pictures taken in situ is provided in Figures 2–9.

Table 3. List of the newly recorded Heterobranchia from the Salento peninsula; species names, relative figure numbers, ecological notes, and phenotypical variability for each added species are reported. Abundance is indicated as number of specimens.

	Species Name	Figure	Ecological Notes	Phenotypical Variability	Abundance
1	*Pleurobranchus testudinarius* Cantraine, 1835	Figure 2A	On soft bottom during diurnal dive. Depth: 25 m	-	1–2
2	*Berthellina* cf. *edwardsii* (Vayssière, 1897)	Figure 2B,C	Usually living singularly or in groups under stones, in small shaded crevices or in dark caves. Depth: 0–15 m	Body color ranging from light creamy-yellow to reddish-orange	>100
3	*Diaphorodoris luteocincta* (M. Sars, 1870)	Figure 2D	This sedentary species usually lives near bryozoan colonies (cf. *Nolella stipata* Gosse, 1855). Depth: 0–3 m	The specimens of this species show the red spot on the *dorsum* which varies in shape and size	3–10
4	*Trapania lineata* Haefelfinger, 1960	Figure 2E	Often in clusters, feeding on *Entoprocta* spp. covering black sponges. Recorded all year long. Depth: 0–30 m	-	>100
5	*Trapania maculata* Haefelfinger, 1960	Figure 2F	Often sympatric with the congeneric *T. lineata*. Depth: 0–30 m	-	>100
6	*Crimora papillata* Alder & Hancock, 1862	Figure 2G,H	Found all year long, in shady pre-coralligenous shallow waters or coastal caves. Often in association with encrusting bryozoans on which their egg masses are laid. Depth: 0–10 m	Color of the notum variable from light yellow to ochre	>100
7	*Polycera elegans* (Bergh, 1894)	Figure 3A,B	Observed during the winter months, in shallow waters. Depth: 5 m	Very typical body color pattern, showing blue spots differing in size and number between individuals	11–30
8	*Polycera hedgpethi* Er. Marcus, 1964	Figure 3C,D	Observed during winter season, in shallow waters. Depth: 5 m	-	3–10
9	*Felimare fontandraui* (Pruvot-Fol, 1951)	Figure 3E,F	Observed in large assemblages at the end of spring, associated with the sponge *Dysidea avara* (Schmidt, 1862). Depth: 7 m	Even if this species shows a variable phenotype [26], the Salentine specimens have constant body color pattern	31–100
10	*Felimare orsinii* (Vérany, 1846)	Figure 3G	In large groups mating and feeding on black sponges. Found between April and July. Depth: 0–15 m	-	31–100
11	*Felimida binza* (Ev. Marcus & Er. Marcus, 1963)	Figure 3H	On a rocky substrates. Found during September. Depth: 5–7 m	-	1–2
12	*Tritonia nilsodhneri* Marcus Ev., 1983	Figure 4A	Found on the yellow gorgonian *Eunicella cavolinii* Koch, 1887. Depth: 30 m	Very mimetic morphotype that can consistently vary from dark brown to pale yellow or white	1–2
13	*Doto cervicenigra* Ortea & Bouchet, 1989	Figure 4B	This small species (few millimetres) is found from winter to early spring on hydrozoans colonies of *Aglaophenia* Lamouroux, 1812. Depth: 0–3 m	-	11–30

Table 3. *Cont.*

	Species Name	Figure	Ecological Notes	Phenotypical Variability	Abundance
14	*Doto floridicola* Simroth, 1888	Figure 4C,D	The average size of specimens observed is ca. 5 mm. Its host hydrozoan colonies belonging to *Aglaophenia*. Depth: 8–12 m	-	11–30
15	*Paraflabellina gabinierei* (Vicente, 1975)	Figure 4E	On hard substrates. Depth: 0–25 m	The body colors vary from white to opaque pinkish	3–10
16	*Limenandra nodosa* Haefelfinger & Stamm, 1958	Figure 4F	Found on *Padina pavonica* (Linnaeus) Thivy, 1960 in summer. Depth 6 m	-	1–2
17	*Caloria elegans* (Alder & Hancock, 1845)	Figure 4G	Usually observed on hard substrata. Depth: 0–15 m	Body pattern with cerata brightly colored from white to light orange	11–30
18	*Dicata odhneri* Schmekel, 1967	Figure 4H	Found in shallow waters. Depth: 0–15 m	-	11–30
19	*Dondice banyulensis* Portmann & Sandmeier, 1960	Figure 5A	Conspicuous in size and brightly coloured. Depth: 0- to more than 30 m	-	11–30
20	*Facelina fusca* Schmekel, 1966	Figure 5B	Found in a tidal pool in association with the green algae *Anadyomene stellata* (Wulfen) C. Agardh, 1823. Depth: 0.5 m	-	1–2
21	*Facelina rubrovittata* (A. Costa, 1866)	Figure 5C	Observed all year long in shady pre-coralligenous habitats. Depth: 0–15 m.	-	31–100
22	*Facelina vicina* (Bergh, 1882)	Figure 5D,E	Depth: 0–15 m	The color of the digestive gland visible through cerata vary from light orange/pink to dark violet	3–10
23	*Eubranchus* cf. *farrani* (Alder & Hancock, 1844)	Figure 5F	Found at shallow depth on hydrozoans colonies. Depth: 0–15 m	This species shows differences in the shape and the number of the dorsal yellow spots	11–30
24	*Eubranchus* cf. *linensis* Garcia-Gomez, Cervera & Garcia, 1990	Figure 5G,H	Depth: 0–5 m.	-	11–30
25	*Catriona maua* Ev. Marcus & Er. Marcus, 1960	Figure 6A	Recorded in shallow water on hydrozoans colonies. Depth: 0.5 m	-	1–2
26	*Rubramoena amoena* (Alder & Hancock, 1845)	Figure 6B	Found in April in shallow water. Temperature 15 °C. Depth: 8 m	-	1–2
27	*Trinchesia genovae* (O'Donoghue, 1926)	Figure 6C,D	Occurs in shallow water on algae, bryozoan, and hydrozoans substrates. Depth: 0–20 m	-	11–30
28	*Trinchesia* cf. *miniostriata* Schmekel, 1968	Figure 6E	On rocky substrates. Depth: 9 m	-	1–2
29	*Trinchesia morrowae* Korshunova, Picton, Furfaro, Mariottini, Pontes, Prkić, Fletcher, Malmberg, Lundin & Martynov, 2019	Figure 6F	Common in March-April and in July when it is possible to observe several specimens living and laying eggs on hydroid of the genus *Sertularella* Gray, 1848. Depth: 0–15 m	The typical color of the apical portion of the rhinophores and the oral tentacles varies from orange to yellow or it could completely lack.	>100

Table 3. *Cont.*

	Species Name	Figure	Ecological Notes	Phenotypical Variability	Abundance
30	*Runcina* cf. *ferruginea* Kress, 1977	Figure 6G	Very small species 1.5–2 mm, as expected for *Runcina* species. Depth: 8 m.	-	3–10
31	*Philine punctata* (J. Adams, 1800)	Figure 6H	Found only as single and very small specimen, ca. 1 mm, on *Posidonia oceanica* rhizomes.	-	1–2
32	*Philine quadripartita* Ascanius, 1772	Figure 7A	Recorded on soft bottoms. Depth: 8 m	-	1–2
33	*Camachoaglaja africana* (Pruvot-Fol, 1953)	Figure 7B,C	Recorded on soft bottoms or on algae. Depth: 0–20 m	Its body color pattern varies from a dark form to another much lighter and densely covered by whitish tiny dots	11–30
34	*Philinopsis depicta* (Renier, 1807)	Figure 7D	Found on soft bottoms or on algae mainly during winter season and in shallow water. Depth: 0–15 m	-	11–30
35	*Melanochlamys wildpretii* Ortea, Bacallado & Moro, 2003	Figure 7E	Found in winter in shallow water. Depth: 0–3 m	-	1–2
36	*Haminoea* cf. *orteai* Talavera, Murillo & Templado, 1987	Figure 7F	Found on green algae. Depth: 0–3 m	-	31–100
37	*Elysia gordanae* T. E. Thompson & Jaklin, 1988	Figure 7G,H and Figure 8A	Very mimetic species, usually in association with the green alga *Flabellia petiolata* (Turra) Nizamuddin, 1987. Depth: 0–15 m	-	>100
38	*Elysia margaritae* Fez, 1962	Figure 8B	On *Dictyota dichotoma* (Hudson) J.V. Lamouroux, 1809. Depth: 0–2 m	-	1–2
39	*Aplysiopsis elegans* Deshayes, 1853	Figure 8C	Depth: 0–12 m	-	3–10
40	*Cyerce graeca* T. E. Thompson, 1988	Figure 8D	Found on April. 18 °C water temperature. Depth: 3 m	The body color patter varies from withe to light yellow	1–2
41	*Hermaea bifida*(Montagu, 1816)	Figure 8E	Found mainly in spring and summer on green algae. Depth: 5–6 m	-	3–10
42	*Hermaea paucicirra* Pruvot-Fol, 1953	Figure 8F	Found on algae in shallow waters. Depth: 0–1 m	-	1–2
43	*Ercolania coerulea* Trinchese, 1892	Figure 8G,H	Found on algae in shallow waters. Depth: 0–5 m	-	3–10
44	*Limapontia capitata* (O. F. Müller, 1774)	Figure 9A	Found on algae in shallow waters. Depth: 0.5 m	-	11–30
45	*Placida cremoniana* (Trinchese, 1892)	Figure 9B	Depth: 0–12 m	-	11–30

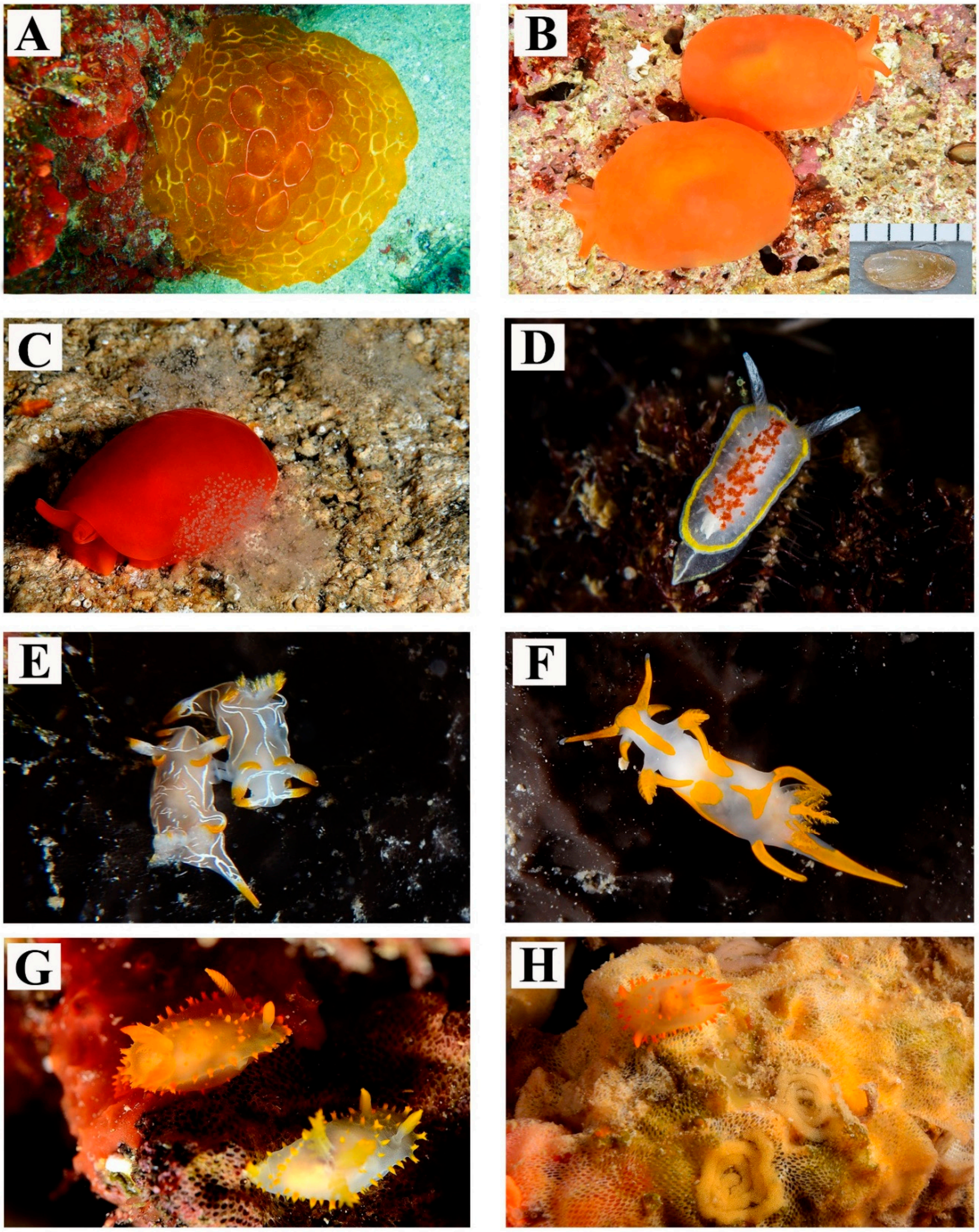

Figure 2. (**A**) *Pleurobranchus testudinarius.* St. 3 (**B**) *Berthellina* cf. *edwardsii*. St. 11. In the right-low corner, the internal shell (length: 4.2 mm). (**C**) *Berthellina* cf. *edwardsii*. St. 11 (**D**) *Diaphorodoris luteocincta.* St. 11 (**E**) *Trapania lineata*, mating individuals. St. 11 (**F**) *Trapania maculata.* St. 11 (**G**) *Crimora papillata.* St. 11 (**H**) *Crimora papillata*, with spawn. St. 11.

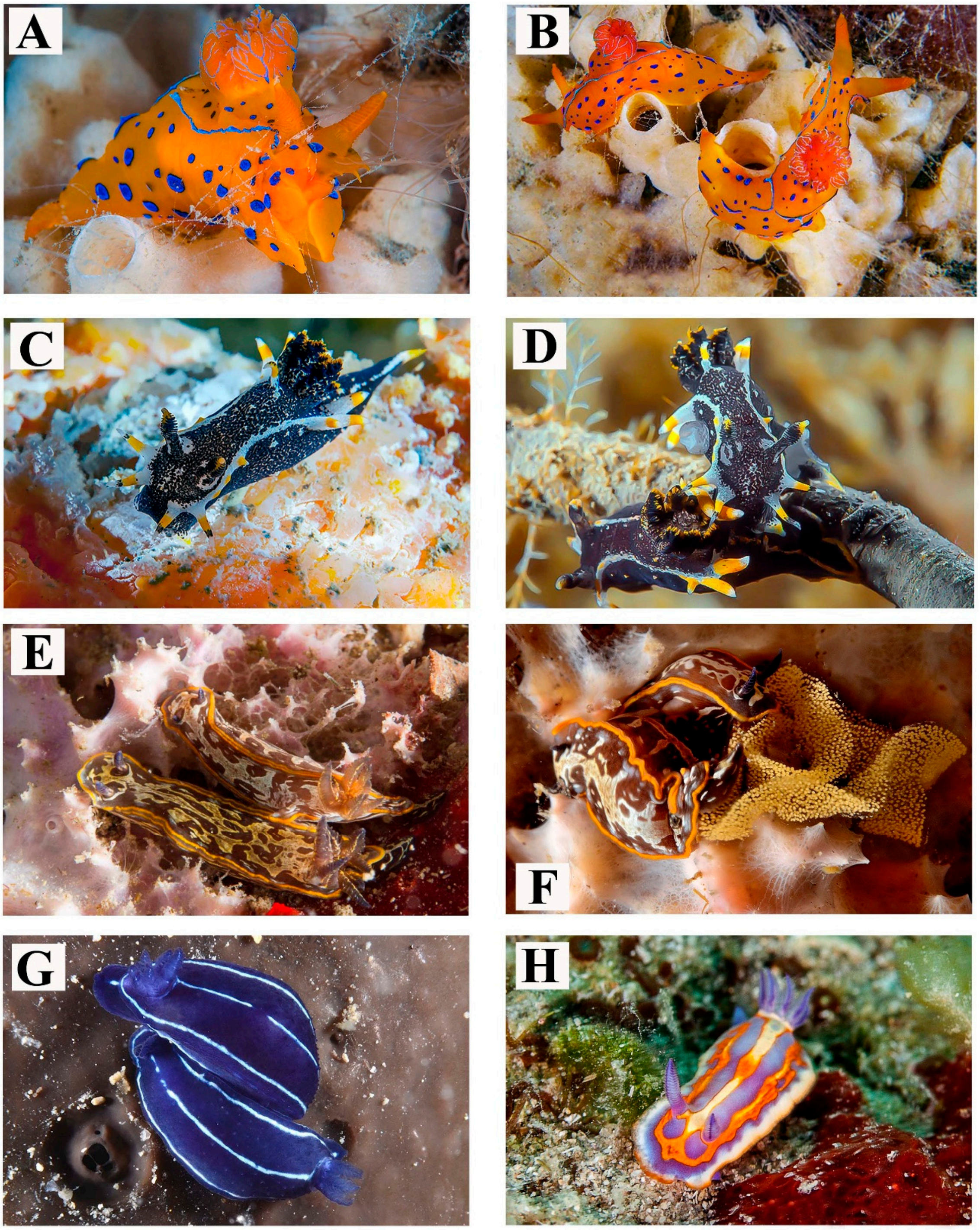

Figure 3. (**A**) *Polycera elegans.* St. 1 (**B**) *Polycera elegans.* St. 1 (**C**) *Polycera hedgpethi.* St. 1 (**D**) *Polycera hedgpethi*. Two individuals after mating. The reproductive openings are clearly visible in the specimen on the upper portion. St. 1 (**E**) *Felimare fontandraui*. St. 11 (**F**) *Felimare fontandraui*, mating individuals with spawn. St. 11 (**G**) *Felimare orsinii*, mating individuals. St. 14 (**H**) *Felimida binza.* St. 25.

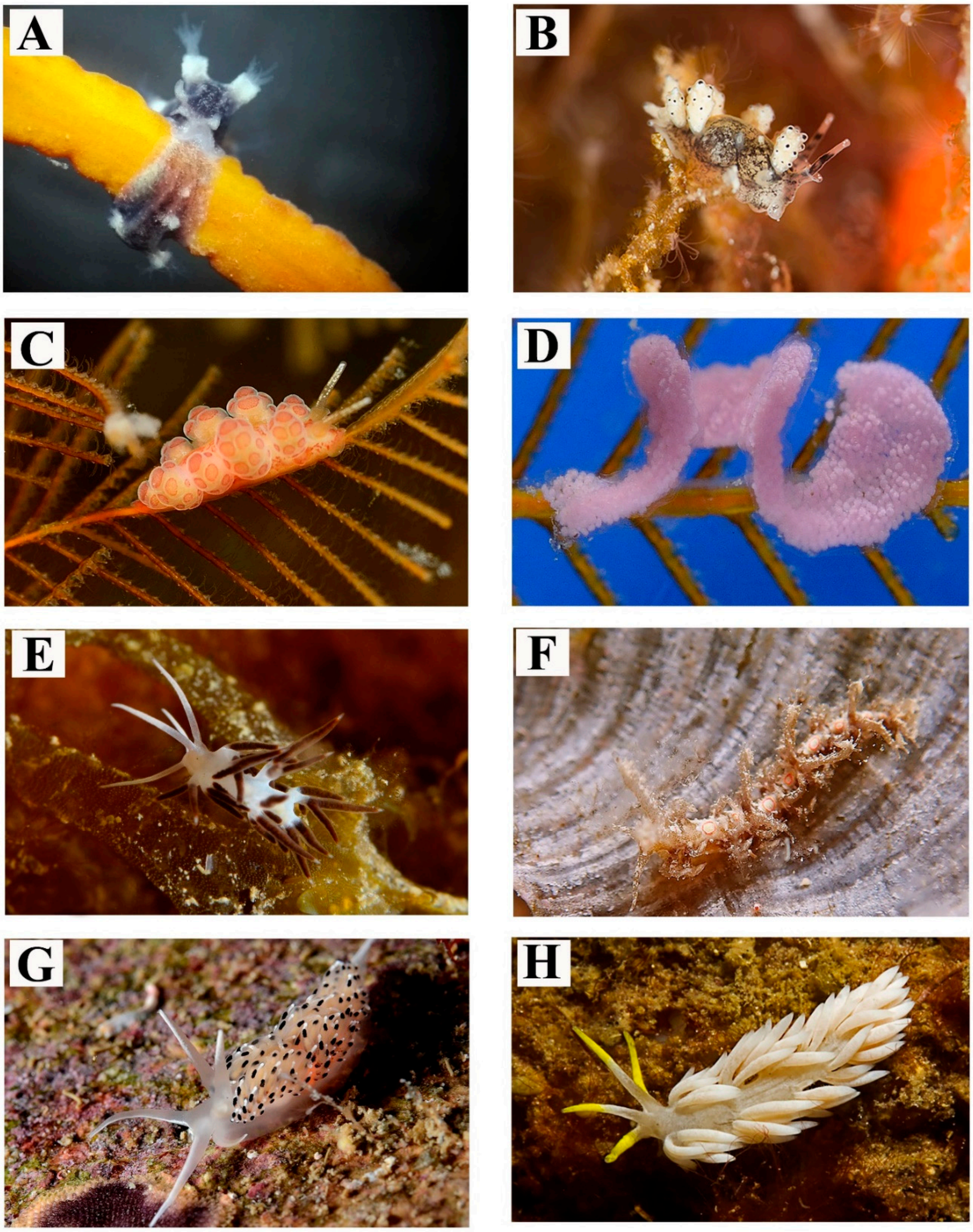

Figure 4. (**A**) *Tritonia nilsodhneri*. St. 18 (**B**) *Doto cervicenigra*. St. 11 (**C**) *Doto floridicola*. St. 22 (**D**) Spawn of *Doto floridicola*. St. 22 (**E**) *Paraflabellina gabinierei*. St. 11 (**F**) *Limenandra nodosa*. St. 3 (**G**) *Caloria elegans*. St. 11 (**H**) *Dicata odhneri* St. 11.

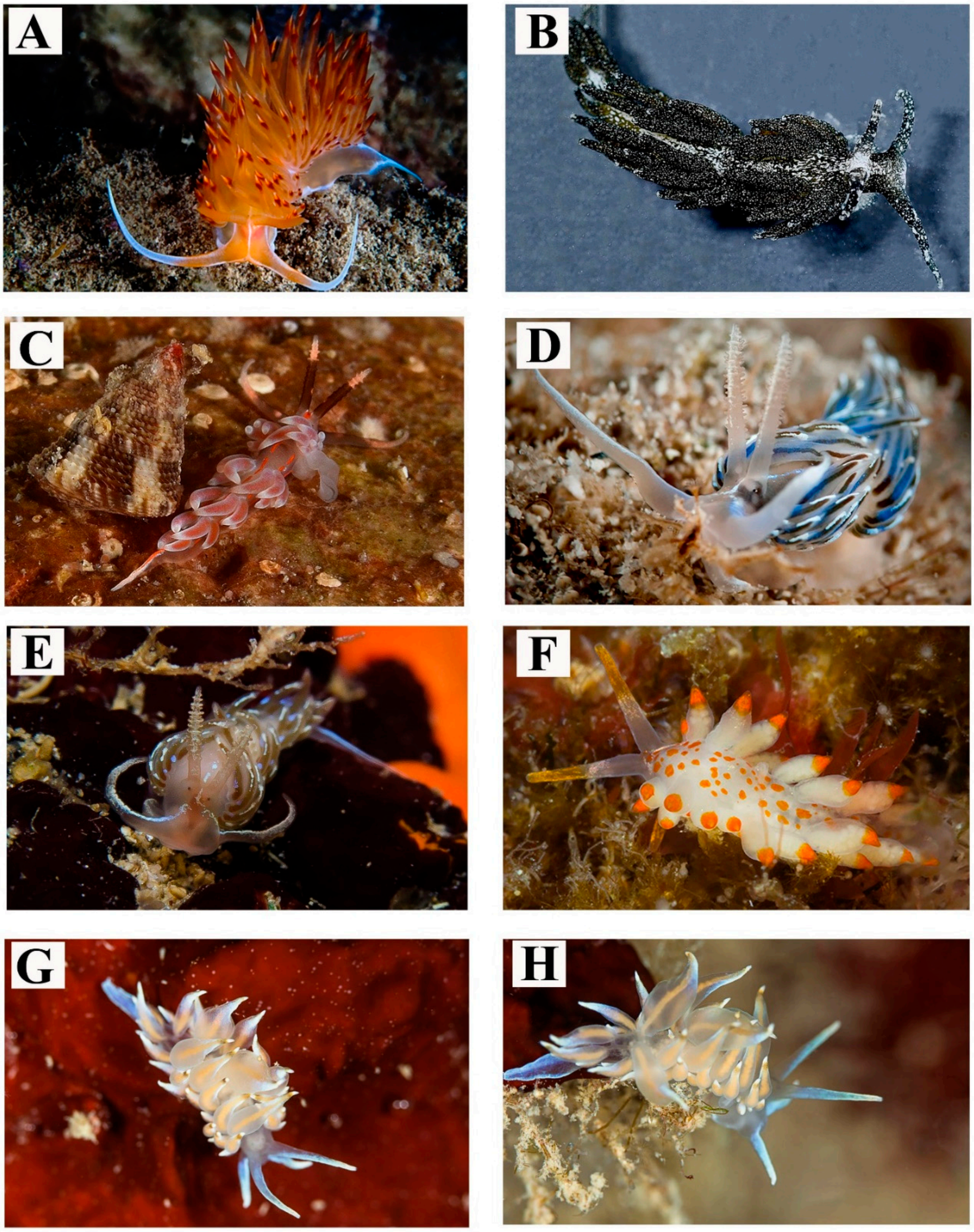

Figure 5. (**A**) *Dondice banyulensis*. St. 13 (**B**) *Facelina fusca*. St. 7 (**C**) *Facelina rubrovittata*. St. 11 (**D**,**E**) *Facelina vicina*. St. 11 (**F**) *Eubranchus* cf. *farrani*. St. 11 (**G**,**H**) *Eubranchus* cf. *linensis* St. 11.

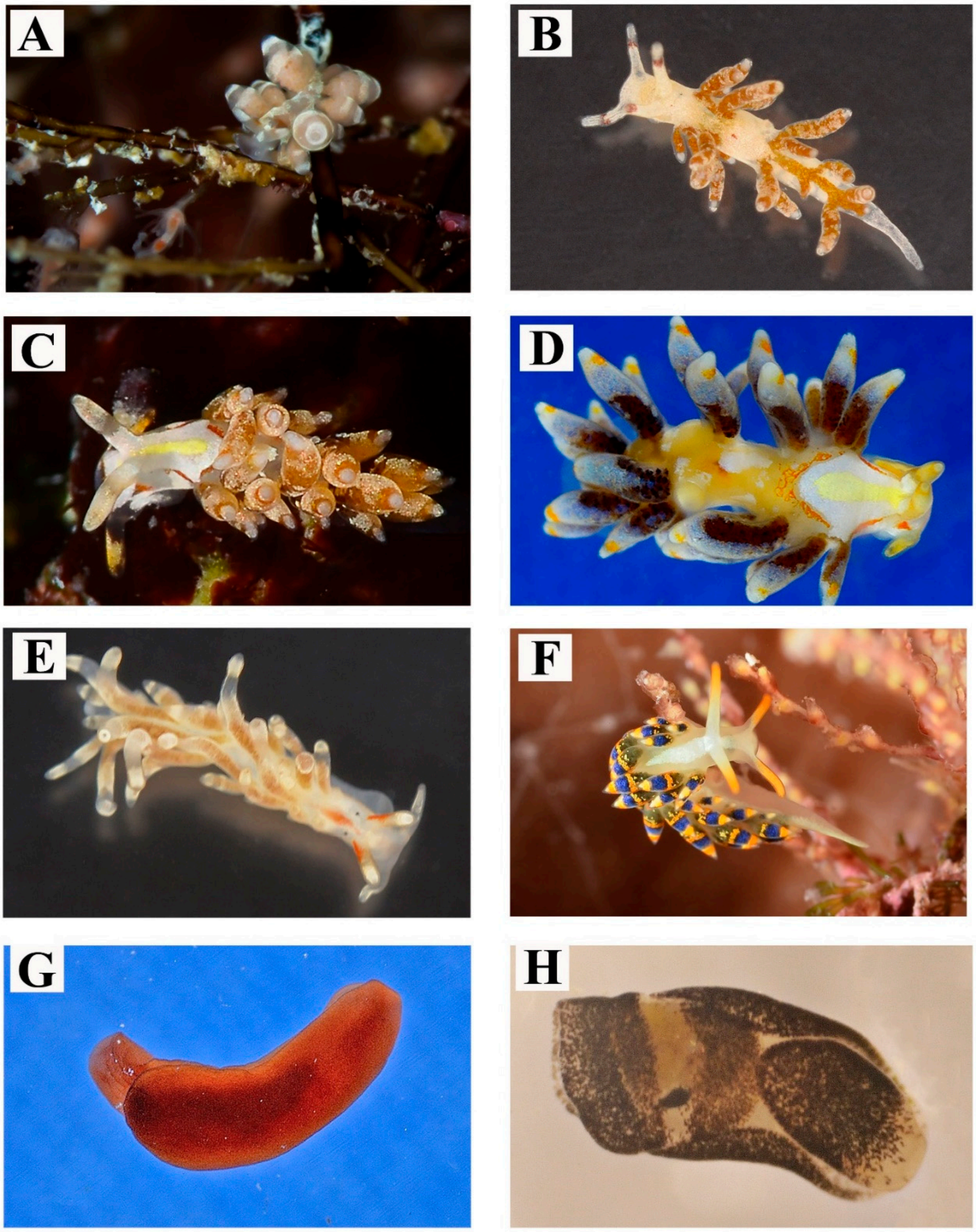

Figure 6. (**A**) *Catriona maua*. St. 9 (**B**) *Rubramoena amoena*. St. 22 (**C**) *Trinchesia genovae*. St. 11 (**D**) *Trinchesia genovae*. St. 7 (**E**) *Trinchesia* cf. *miniostriata* St. 22 (**F**) *Trinchesia morrowae*. St. 11 (**G**) *Runcina* cf. *ferruginea*. St. 22 (**H**) *Philine punctata* St. 9.

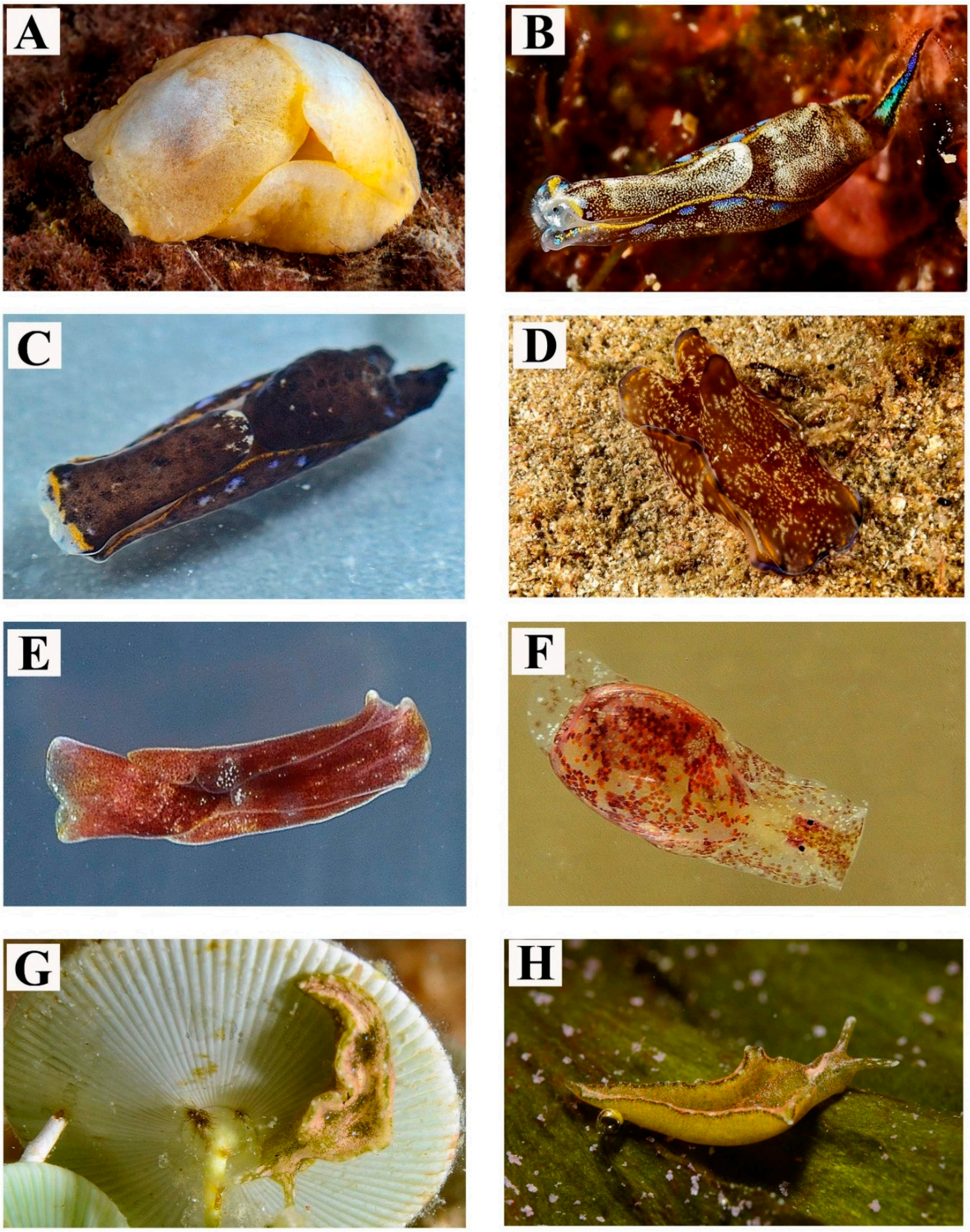

Figure 7. (**A**) *Philine quadripartita*. St. 1 (**B**) *Camachoaglaja africana*. St. 7 (**C**) *Camachoaglaja africana*. St. 22 (**D**) *Philinopsis depicta*. St. 10 (**E**) *Melanochlamys wildpretii*. St. 7 (**F**) *Haminoea* cf. orteai. St. 22 (**G**) *Elysia gordanae*. St. 11 (**H**) *Elysia gordanae*. St. 11.

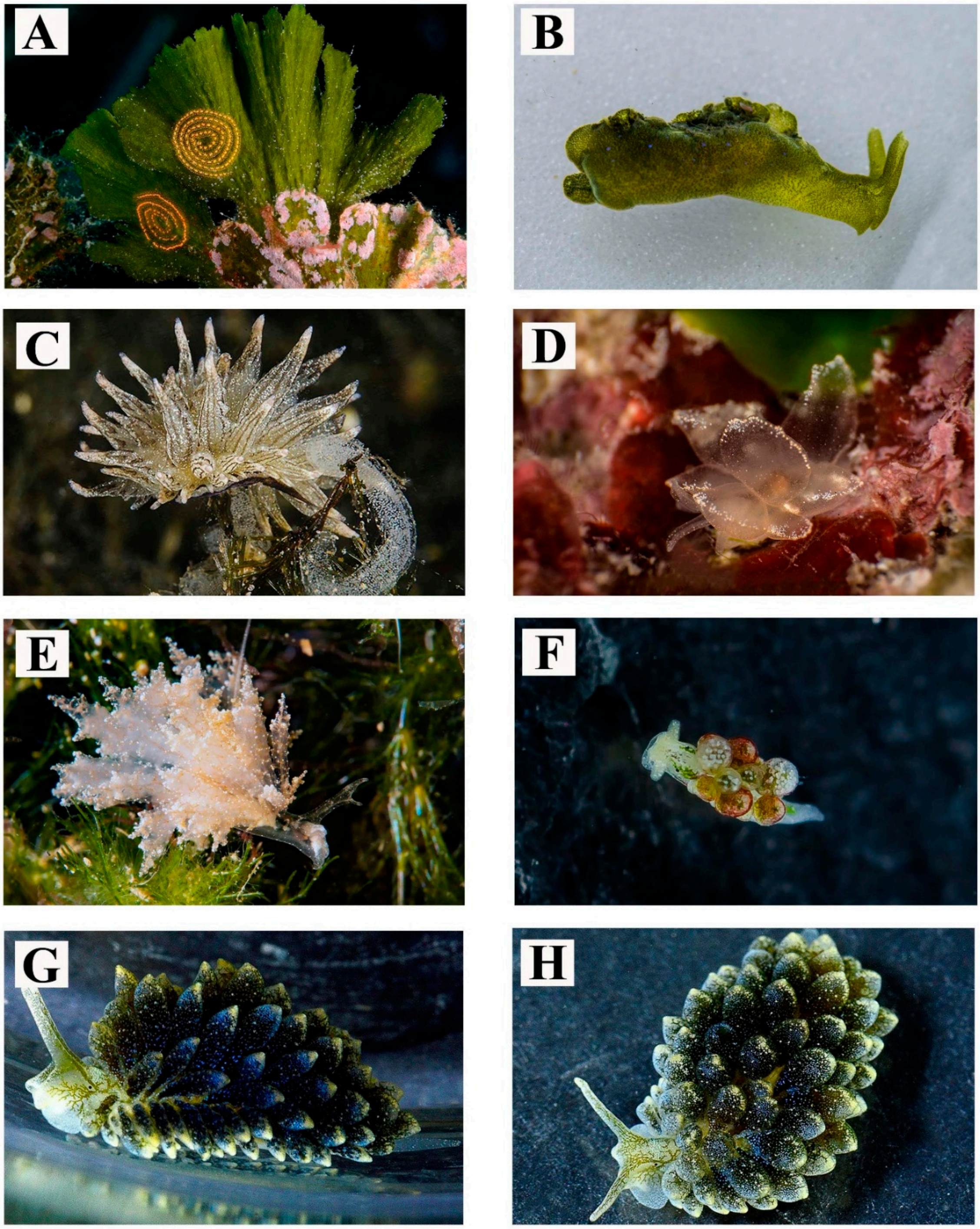

Figure 8. (**A**) Spawn of *Elysia gordanae*. St. 22 (**B**) *Elysia margaritae*. St. 11 (**C**) *Aplysiopsis elegans*. St. 1 (**D**) *Cyerce graeca*. St. 11 (**E**) *Hermaea bifida*. St. 1 (**F**) *Hermaea paucicirra*. St. 7 (**G**,**H**) *Ercolania coerulea*. St. 7.

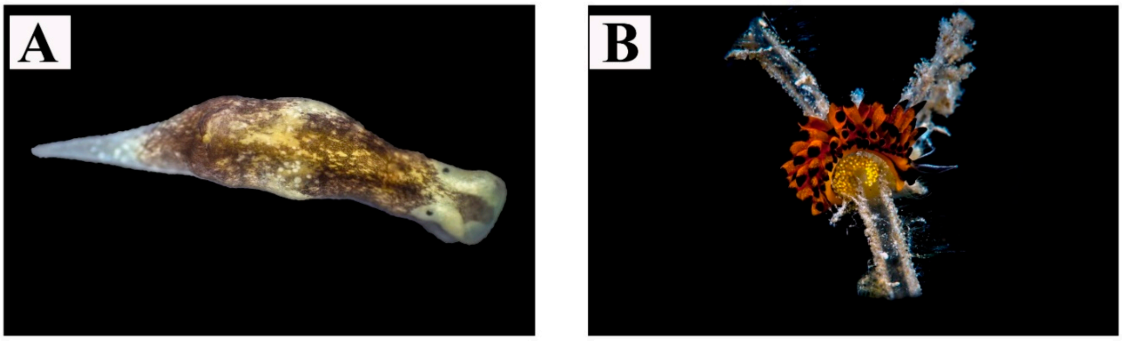

Figure 9. (**A**) *Limapontia capitata*. St. 24 (**B**) *Placida cremoniana*. St. 1.

4. Discussion

This study reported 160 marine Heterobranchia species from the Salento Peninsula giving the most up to date list of marine heterobranchs inhabiting this area of the Apulian coasts. This was particularly remarkable considering that the total number of registered species belonging to this mollusks group for the whole Mediterranean Sea was approximately 550 [51]. The composition of the main groups of marine heterobranchs living in the Salento Peninsula reported in this work is shown in Figure 10. The groups with the largest numbers of species added were Cladobranchia (18) and Doridina (9), as expected, these nudibranchs belonged to the groups with the richest variety of taxa. Surprisingly, with the present contribution, 6 species of Cephalaspidea were added to the ones reported previously. Another interesting consideration was concerning the superorder Sacoglossa, which almost doubled after this study. Finally, some species were noteworthy because they are currently under studied or because the validity of the species is still in doubt. This was the case for *Berthellina* cf. *edwardsii*, *Eubranchus* cf. *exiguus*, *E.* cf. *farrani*, *E.* cf. *linensis*, *Facelina fusca*, *Haminoea* cf. *orteai*, *Runcina* cf. *ferruginea* and *Trinchesia* cf. *miniostriata*. In particular, in the case of *Berthellina* cf. *edwardsii* we depicted (Figure 2B) the internal shell (4.2 mm long), since it fits the standard average length of the shell commonly used as diagnostic for this species, albeit we preferred to keep an uncertainty (cf.) before a further molecular study would allow a clear-cut identification. The studied marine area, deeply influenced by two different seas and characterized by a variety of submarine habitats, hosts a high variety of species, which makes it an important geographical area for this sea slug diversity. In fact, with this work we report that the total number of marine Heterobranchia living in the Salento Peninsula is 160, which is about a third of the total number of currently accepted species reported for the whole Mediterranean Sea. This is quite an important finding, also considering the fact that a high diversity in heterobranchs composition indirectly reflects a high structuring and diversification of the habitats involved, and consequently, of the biodiversity that they contribute to maintain [14]. Studying and monitoring the marine Heterobranchia diversity in the Salento Peninsula is valuable for highlighting the consequences of the global marine changes reported in the last decade [10–12,59,60], such as warming and acidification of waters or invasion of alien species. An additional detailed and constantly updated iconography is available on the website of the Salento Sommerso group (http://www.salentosommerso.it/index_opi.php), a non-profit association devoted to the preservation and documentation of the underwater biodiversity of the Salento Peninsula.

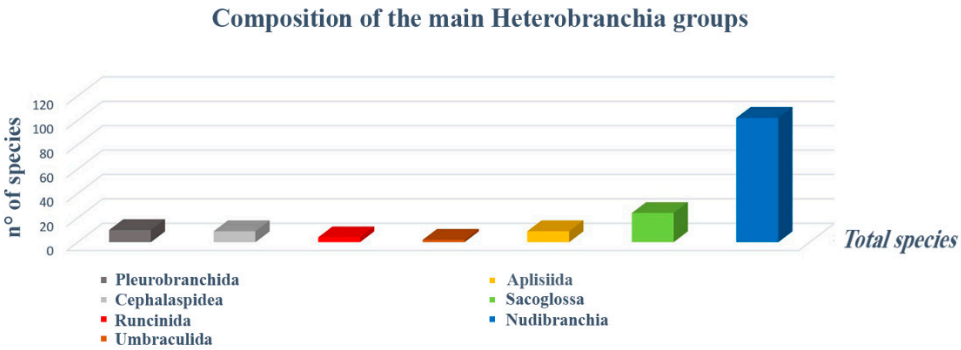

Figure 10. Comparison between the main heterobranch groups; the graph shows the comparison between the total species of the Salento Peninsula, clustered into the main groups.

5. Conclusions

The study of marine diversity is a fundamental topic especially if focused on areas like Mediterranean Sea, which is characterised by a high rate of endemic and cryptic species. In this study we have investigated the presence of marine Heterobranchia in the Salento Peninsula (Apulia, South-Italy) considering for the first time both Ionian and Adriatic coasts. Results of a nine-year study reports 160 species inhabiting the studied area with new records from the sublittoral waters, ecological notes, local distribution and systematic remarks. This inventory of marine Heterobranchia encountered in the Salento Peninsula adds 45 species to the previously known for this area. For all the added species, we have figured live animals mostly by in situ photographs to document species identification and reported data on ecology, phenotypical variability and abundance. This inventory of marine Heterobranchia provides a baseline for future monitoring of both coastal sides and could serve as a starting point for further molecular studies aiming to unveil Mediterranean cryptic diversity.

Author Contributions: F.V. and C.L. conceived the project and performed most of the SCUBA diving and took in situ pictures. G.F. and P.M. analysed the data and carried out the SCUBA diving. F.V., C.L., G.F., and P.M. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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