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Research Article

A Mediterranean melting pot: native and non-indigenous sea slugs (Gastropoda, Heterobranchia) from Lebanese waters

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Abstract

The Lebanese coastal area (Eastern Mediterranean Sea), despite being only 220 km long, is a sentinel zone for detecting and monitoring thermophilic newcomers entering the Mediterranean Sea from the Red Sea and the Indo-Pacific province. This is due to its strategic geographical position near the Suez Canal and the high sea surface temperature. In the framework of the Blue Tyre Project – Local Partnership for Sustainable Marine and Coastal Development (AID 012314/01/6), the coastal area of the Tyre Municipality was investigated to update the existing list of the sea slug species (Mollusca, Heterobranchia) known from this area. A total of 24 species were observed and studied through snorkelling, scuba diving and fishing by-catch census during three scientific expeditions in June and November 2022, and June 2023, at depths ranging from 0.5 to 65 meters. Results allowed to increase from 35 to 55 the number of known Lebanese species of the orders Nudibranchia, Cephalaspidea, Pleurobranchida, Sacoglossa and Umbraculida. The Nudibranchia order had the highest increase, rising from 19 previously documented species to a total of 35 species now reported for Lebanon. Among the newly reported sea slugs, five were not identifiable as already known species and will require additional analysis, being potentially new species to science. Four species were new records for the entire Mediterranean fauna, and 42% of the total recorded taxa were non-indigenous species (NIS). These results highlight the area great potential as a hot spot for heterobranch diversity, particularly regarding cryptic and/or unknown diversity. They also confirm the paramount role of Lebanese waters in providing early warning of NIS reaching the Mediterranean basin.

Key words: checklist, Nudibranchia, biodiversity, Levantine basin, climate change, global warming

Introduction

The Mediterranean Sea is an important biodiversity hotspot, hosting a high level of endemism, with about 45% of its total species being unique to the region (Costello et al. 2017). This is due to its climatic and environmental

characteristics and its peculiar evolutionary history. Nowadays, the Mediterranean is currently facing several threats, mainly caused by human activities, which are weakening its species richness by significantly reducing the variety and good health of its natural habitats. The current decline of Mediterranean indigenous species is primarily driven by ever-increasing anthropic pressure (Mouillot et al. 2011; Coll et al. 2010, 2012; Micheli et al. 2013), climate change (Lejeusne et al. 2010; Albano et al. 2021), the continuous arrival of species from extra Mediterranean localities, and biological invasions (Zenetos et al. 2012; Katsanevakis et al. 2013; Albano et al. 2024). These factors make the Mediterranean basin one of the most impacted ecoregions globally (Halpern et al. 2008; Costello et al. 2010). Over recent decades, this decline has been exacerbated by the notable influx of non-indigenous species (hereafter NIS) into the Mediterranean basin, a direct consequence of both human activity and the effects of global warming (Katsanevakis et al. 2014; Bariche et al. 2015; Toso and Musco 2023). The introduction and invasiveness of NIS are manifested by their occupation and potential expansion into new ecological niches, often to the detriment of local species (Toso and Musco 2023). This has been enhanced since the opening of the Suez Canal and the damming of the Nile in 1964, making the eastern area, the most oligotrophic sector of the Mediterranean Sea (Sisma-Ventura et al. 2017) and a sentinel zone for newcomer species (Galil 2009; Zenetos et al. 2012). The Levantine basin of the Mediterranean Sea is characterized by its own specific oceanographic features (El-Geziry and Bryden 2010), ranging from the Mediterranean coast of Egypt to Turkey, including Cyprus. The afore mentioned macro-region is far from the nutrient-rich inflow of the Atlantic Ocean and it is characterized by a negative freshwater balance, where evaporation exceeds the supply of freshwater from rivers and rain (Bariche 2010; Sisma-Ventura et al. 2017). The rapid increase in surface water temperature and warmer winters have caused a reduction in convective mixing depth and vertical nutrient transport into the photic layer, resulting in lower primary productivity, high salinity, and elevated temperature in this region (Sisma-Ventura et al. 2017). For all these reasons, along with habitat degradation and loss, and the impact of fishing activities, the Levantine basin is the Mediterranean area most sensitive to climate change. Therefore, the abiotic conditions in this region favour the establishment of new thermophilic species coming from the Red Sea and the Indo-Pacific province via the Suez Canal and transported by both currents and vessels traffic (Bitar et al. 2007; Tzomos et al. 2012; Yokeş et al. 2012; Zenetos 2017; Galil et al. 2017, 2018; Bilecenoğlu and Yokeş 2022). The Lebanese region is characterized by a narrow continental shelf that hosts a variety of marine habitats (Shaban 2010; Talhouk et al. 2018; Badreddine et al. 2019; Bariche et al. 2021) and a high number of NIS (Bitar and Zibrowius 1997; Zibrowius and Bitar 2003; Harmelin-Vivien et al. 2005; Morri et al. 2009;

Kalogirou et al. 2012; Crocetta et al. 2013a, 2014; Bariche et al. 2020). Due to its importance as a key sentinel for the Mediterranean environmental changes, increasing efforts have been made to monitor potential shifts in the associated fauna of Lebanon, with particular attention to the Mollusca group (Bogi and Khairallah 1987; Bitar and Kouli-Bitar 1998; Crocetta et al. 2013a, 2014; Bitar 2014). Considering that Gastropoda is the most conspicuous class in terms of number of species (Geiger 2006), it is not surprising that scientific works on Lebanese fauna have focused on this group of molluscs (Schütt and Şeşen 1993; Neubert and Bariche 2013; Crocetta et al. 2020). However, within gastropods, the Heterobranchia subclass has only been partially considered, even though these specialized molluscs are considered very attractive in many fields of research (as detailed below). To date, only scattered records of singular or a few species have been reported from the area (Valdés and Templado 2002; Crocetta and Galil 2012; Orfanidis et al. 2021), except for one study specifically aimed at describing the Lebanese Heterobranchia fauna (Crocetta et al. 2013b).

Marine Heterobranchia are characterized by the reduction, internalization, or loss of the shell in the adult stage, an important evolutionary adaptation that coevolved with highly specialized alternative defensive strategies (Cimino and Ghiselin 1998; Wägele and Klussmann-Kolb 2005; Putz et al. 2010; Winters et al. 2018). These latter include the ability to collect and store exogenous structures, chemical compounds, and/or intracellular organelles obtained from their prey within their own tissues and cells (Jensen 1994; Carbone et al. 2013; Cheney et al. 2016; Avila et al. 2018; Winters et al. 2022). Such skills make marine sea slugs model species in several fields of basic and applied research, including biology, ecology, chemistry, neuroscience, biomedicine, and pharmaceutics (Cimino and Ghiselin 1999; Dean and Prinsep 2017; Gavagnin et al. 2019; Katz and Quinlan 2019; Zhuo et al. 2022). Body colours play a very important role in these molluscs, which can display bright aposematic colours to warn potential predators about their defensive mechanisms, or cryptic or camouflage colours to blend with the substrate on which they live. The body colour pattern is usually an important diagnostic feature for species identification, although this character could be affected by natural selection (due to Batesian and Mullerian mimicry), potentially hiding the real evolutionary relationships between species (Furfaro et al. 2018, 2023b). However, providing photographic data of the species reported from a specific area, together with the collection and storage of specimens, is fundamental for species recognition and for understanding intra- and inter-species morphological variability (Chow et al. 2022).

The Mediterranean Sea plays a crucial role in the Heterobranchia taxonomy, serving as the reference point for several species originally described from this basin. Improving our current knowledge of the Mediterranean fauna is of particular importance for monitoring possible shifts in species composition,

which can indicate changes in the environmental status (Nimbs and Smith 2016, 2017; Goddard et al. 2016, 2018; Furfaro et al. 2020; Chow et al. 2022). Despite significant recent efforts to investigate Heterobranchia in various Mediterranean localities (Trainito and Doneddu 2015, 2016; Furfaro et al. 2016, 2018, 2020, 2022b, 2023a; Artüz et al. 2018; Pola et al. 2019; Manousis et al. 2020; Renda et al. 2022; Ciriaco et al. 2023), some areas remain poorly investigated (Toso et al. 2024). In this context, knowledge on Heterobranchia from Lebanese waters is limited despite Lebanon's strategic position for early warning of NIS and for monitoring the effects of global change on Mediterranean biodiversity. Investigating the rate of endemic and non-indigenous Lebanese species over time could be particularly important for early detection of the hidden effects of climate change, positively influencing regional conservation and management policies.

Based on material collected within the framework of Blue Tyre Local Partnership For Sustainable Marine And Coastal Development (AID 012314/01/6), this study aims to provide i) a substantially updated list of Heterobranchia species observed in Lebanon ii) the first photographic and material collection of Lebanese sea slugs and iii) an in depth discussion on the rare, neglected and/or particularly interesting species recorded so far from this strategic Mediterranean area.

Materials and methods

Study area

The study was conducted along the peninsula of the natural reserve of Tyre Municipality (Lebanon), during the three expeditions carried out in the Blue Tyre Project. Three expeditions occurred in June and November 2022, and in June 2023. The investigation site is characterized by islets of rocks and vermetid platforms. On both sides of the peninsula, sandy beaches alternate with hard shorelines. The shallow sea bottom consists of vermetid platforms, rocks, and coralligenous concretions. Crevices and overhangs are common, with occasional pebble areas from surface to a depth of 20 meters (Bariche 2010; Bariche et al. 2021). The deeper area has coralligenous seabed, alternating with sand and mud substrates. At 35–40 meters depth, it is possible to find scattered freshwater springs and sulphur hot water springs. The current flow mainly goes northward, from the southern part of Lebanon to the northern part, forming gyres in correspondence with the bays (Lakkis and Novel-Lakkis 1981).

Collecting samples

Specimens belonging to the Heterobranchia subclass were collected by snorkelling, scuba diving and during the fishing sessions with local fishermen as by-catch products. To obtain a more reliable description of the Lebanese Heterobranchia diversity, different habitats were sampled in the three

expedition and using different methods. This sampling choice did not allow to speculate on the distribution in space (*e.g.* between different stations) and time (*e.g.* between different seasons) of the observed species due to the lack of a standardize protocol and replicas, but to date it is considered the best way to preliminarily explore the diversity of this heterogeneous group of gastropods (Kaligis et al. 2018; Undap et al. 2019; Furfaro et al. 2020; Chow et al. 2022).

Once sampled, the material was sorted at the laboratories of the Blue Tyre Project. All specimens were photographed alive with Olympus Tough TG-6 and Sony IL-CA 68 cameras. When possible, two individuals of each collected species, were catalogued, preserved in 98% EtOH, and stored as "the Blue Tyre Project inventory" in the RM3 Heterobranchia collection at the Department of Biological and Environmental Sciences and Technologies (DiSTeBA) of the "Pietro Parenzan" Marine Biology Museum of the University of Salento (Lecce, Italy). An exception was made for the specimen of *Goniobranchus annulatus* (Eliot, 1904) that was only photographed and not collected. This research complies with restrictions in terms of collected sample size, environmental survey of the collection sites, and the use of hand-net picking of specimens (harmless and not destructive), as well as 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).

Species morphological identification

Species identification was done by careful observation of the morphological diagnostic features and subsequent consultation of existing literature (Cattaneo-Vietti et al. 1990; Zenetos et al. 2004; Yokeş and Rudman 2004; Debelius and Kuiter 2007; Yonow 2008; Trainito and Doneddu 2014; Gosliner et al. 2015). The nomenclature followed in the present study is in accordance with WoRMS website (2024). The list of the species recorded in this study is compared with the known Lebanese Heterobranchia diversity (Valdés and Templado 2002; Crocetta et al. 2013b, 2020). For each one of the species discussed in the present study, the Known distribution, morphological description of the observed specimen/s and some remarks are reported, except in the case of unidentifiable species for which only the morphological description and remarks are reported.

Results

The scientific expeditions involved 39 sampling sessions among 14 different stations located in the Tyre municipality in Lebanon (Figure 1, Table 1). A total of 65 specimens belonging to 24 sea slug species were recorded during this study (Table 1), raising up the total number of the species (belonging to the taxonomical orders here investigated) known for Lebanon from 35 to 55

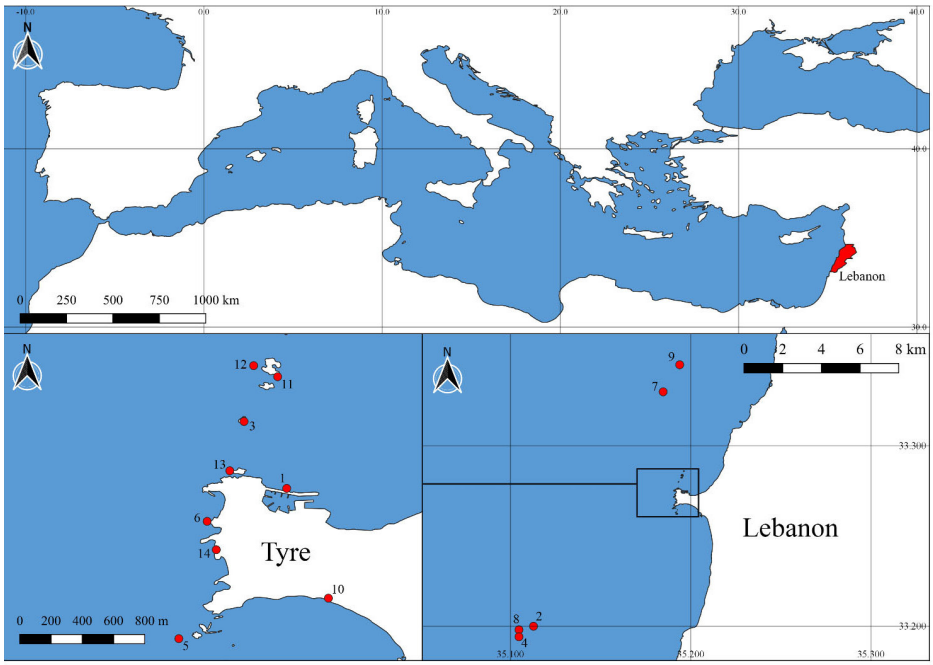

Figure 1. Map of the Mediterranean Sea with a focus on the study area and with the investigated stations highlighted by red dots. More information on each station is reported in Table 1 using the same station number.

and the NIS, of the investigated orders, reported so far in the study area from 13 to 20 (Figure 2A). Twenty out of the 24 species recorded in this study were firstly reported for Lebanon (Table 1). The total number of Nudibranchia was almost doubled with the addition of 16 species to the 19 already known for the area while only one species was added to each one of the other Heterobranchia orders investigated (*i.e.* Cephalaspidea, Pleurobranchida, Sacoglossa and Umbraculida) (Figure 2B). The list of the recorded species was added on the Ocean Biodiversity of Information System (OBIS, www.obis.org) and Global Biodiversity Information Facility (GBIF, www.gbif.org) platforms while the list of the specimens observed, photographed (Figures 3–6) and collected per each species is reported in Table 1 together with data on collecting station and depth. The seven NIS added in this study correspond to 35% of all the added species, updating the number of NIS (belonging to the orders object of this study) currently known for Lebanon to 20 (Table 2). Among the new reports for Lebanon, four species are new records for the whole Mediterranean Sea and three species can be cases of still not known diversity that will deserve future in-depth integrative taxonomic investigation (Tables 1, 2).

Rare, neglected or questioned Mediterranean Heterobranchia newly reported from Lebanon

Among the species observed during the scientific expeditions carried out in Lebanon, some are of particular interest with taxa considered rare in the Mediterranean Sea, that have been almost ignored so far or that are object of debate by experts and taxonomists.

Table 1. List of the 24 sea slug species recorded from the Tyre Municipality, Lebanon. Order, suborder, species name, voucher, number and geographic coordinates of each sampling station and depth (in meters) of collection of each specimen observed during this study are here reported. In bold are the species newly reported for Lebanon. Highlighted with the # symbol are the NIS species while with an asterisk (*) are those specimens for which a photograph is provided. With the letters (D), (T) and (A) are indicated the superfamilies Dendronotoidea, Tritonioidea and Aeolidioidea respectively.

Order	Suborder	Species	Voucher	Station	Geographic Coordinates	Depth
Nudibranchia	Cladobranchia (D)	***Doto* cf. *coronata***	RM3_2985*	8	32.2°N, 35.1128°E	38
		***Doto* sp.**	RM3_2988*	8	33.2°N, 35.1128°E	38
		***Lomanotus vermiformis*#**	RM3_2987	12	33.2836°N, 35.1936°E	9
			RM3_2992, RM3_2993	1	33.2753°N, 35.1953°E	4
			RM3_2990*, RM3_2994, RM3_2995	11	33.2831°N, 35.1958°E	6
	Cladobranchia (T)	***Marionia blainvillea***	RM3_2564*	3	33.2797°N, 35.1933°E	6
	Cladobranchia (A)	***Anteaeolidiella lurana*#**	RM3_2641*	8	33.2°N, 35.1128°E	38
			RM3_2651	3	33.2798°N, 35.1933°E	3
		Eubranchus capellinii	RM3_3000, RM3_3001*, RM3_3002	14	33.2708°N, 35.1908°E	2
		***Noumeaella* sp.**	RM3_2643*, RM3_2644	8	33.2°N, 35.1128°E	35
		***Phidiana anulifera*#**	RM3_2638*, RM3_2639	8	33.2°N, 35.1128°E	15-20
		Flabellina ischitana	RM3_2646*	8	33.2°N, 35.1128°E	35
		Edmundsella pedata	RM3_2556*	2	33.1980°N, 35.1047°E	38
		Trinchesia genovae	RM3_2986*	8	33.2°N, 35.1128°E	38
	Doridina	*Goniobranchus annulatus*#	LB_44*	1	33.2753°N, 35.1953°E	2
			LB_44b	13	33.2764°N, 35.1925°E	–
		***Goniobranchus obsoletus*#**	RM3_2549, RM3_2550	4	33.1942°N, 35.1047°E	20
			RM3_2560, RM3_2561	3	33.2797°N, 35.1933°E	5
			RM3_2543, RM3_2544	3	33.2797°N, 35.1933°E	6
			RM3_2648, RM3_2649	8	33.2°N, 35.1128°E	35
			RM3_2984*	1	33.2753°N, 35.1953°E	2
		Hypselodoris infucata#	RM3_2548	4	33.1942°N, 35.1047°E	20
			RM3_2555, RM3_2613	3	33.2797°N, 35.1933°E	5
			RM3_2983	1	33.2753°N, 35.1953°E	2
			RM3_2999*	14	33.2708°N, 35.1908°E	2
		***Verconia simplex*#**	RM3_2539	12	33.2836°N, 35.1936°E	6
			RM3_2540	1	33.2753°N, 35.1953°E	6
			RM3_2542	3	33.2797°N, 35.1933°E	6
			RM3_2553, RM3_2711*	4	33.1942°N, 35.1047°E	15-20
			RM3_2557	5	33.265°N, 35.1889°E	5
			RM3_2647	8	33.2°N, 35.1128°E	35
		***Dendrodoris fumata*#**	RM3_2563*	6	33.2731°N, 35.1908°E	3
		Doriopsilla rarispinosa	RM3_2650*	6	33.2731°N, 35.1908°E	3
		Discodorididae sp.	RM3_2652*	2	33.1980°N, 35.1047°E	38
Cephalaspidea		***Niparaya* cf. *regiscorona*#**	RM3_2989*	1	33.2753°N, 35.1953°E	4
Pleurobranchida		***Pleurobranchaea* sp.**	RM3_2536*, RM3_2541, RM3_2547	9	33.345°N, 35.1939°E	65
Sacoglossa		***Elysia flava***	RM3_2554	1	33.2753°N, 35.1953°E	5
			RM3_2537*	5	33.265°N, 35.1889°E	6
			RM3_2538	6	33.2731°N, 35.1908°E	6
		Elysia grandifolia#	RM3_2558*	1	33.2753°N, 35.1953°E	5
			RM3_2559	5	33.265°N, 35.1889°E	5
			RM3_2640	6	33.2731°N, 35.1908°E	3
		Elysia timida	RM3_2997	13	33.2764°N, 35.1925°E	2
			RM3_2998*	14	33.2708°N, 35.1908°E	2
Umbraculida		***Tylodina perversa***	RM3_2545*, RM3_2551	4	33.1942°N, 35.1047°E	20
			RM3_2546, RM3_2552	7	33.33°N, 35.1847°E	20
			RM3_2562	3	33.2797°N, 35.1933°E	5

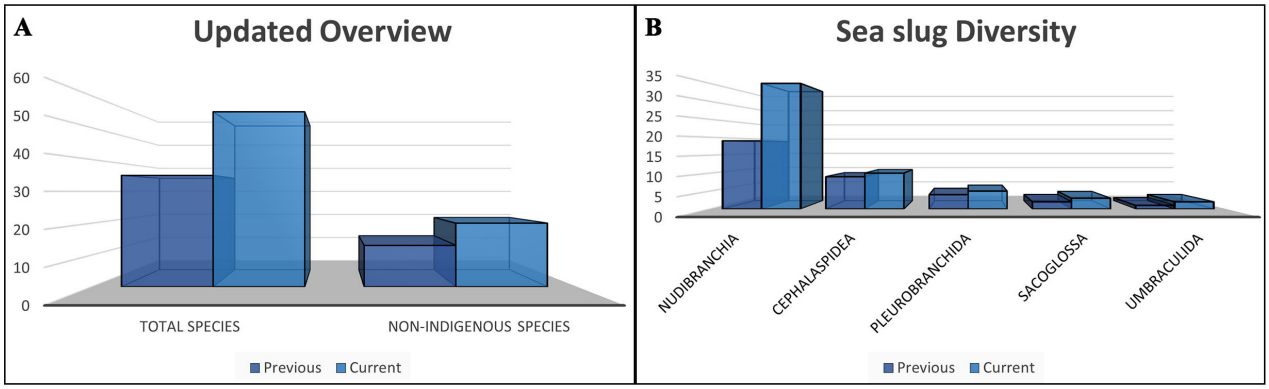

Figure 2. (A) Histogram showing an updated overview on the total number of sea slug species, belonging to the orders here investigated, previously known for Lebanon (in dark blue) and the total amount of species known after the present study (in light blue); (B) A detailed histogram showing the increase in species for each order (Nudibranchia, Cephalaspidea, Pleurobranchida, Sacoglossa and Umbraculida) following this work.

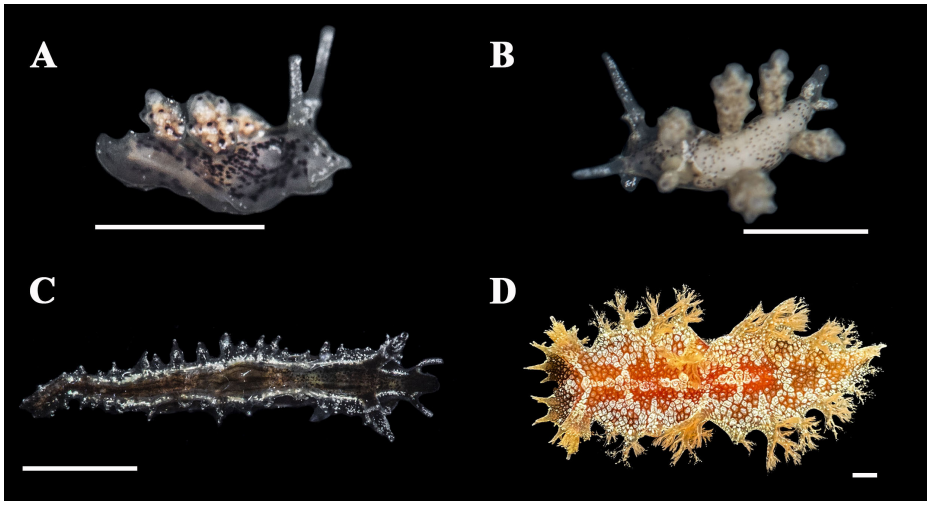

Figure 3. Photographic catalogue of the Nudibranchia suborder Cladobranchia belonging to the superfamilies Dendronotoidea (A–C) and Tritonioidea (D); (A) *Doto* cf. *coronata,* specimen with voucher RM3_2985 (3 mm); (B) *Doto* sp. specimen with voucher RM3_2988 (4 mm); (C) *Lomanotus vermiformis* specimen with voucher RM3_2990 (7 mm); (D) *Marionia blainvillea* specimen with voucher RM3_2564 (30 mm). Scale bar = 2 mm.

Eubranchus capellinii (Trinchese, 1879)

Known distribution: this species is known in the Atlantic Ocean from the British Islands (Garstang 1890: as *Galvina cingulata*; Edmunds and Kress 1969: as *Eubranchus doriae* and Thompson and Brown 1984: as *E. doriae*) to the south of the Iberian Peninsula and in the Mediterranean Sea only in Italy from Genoa (the type locality; Trinchese 1879: as *Tergipes capellinii*) and the Gulf of Naples (Schmekel and Portmann 1982: as *Eubranchus cingulatus*).

Morphological description: Specimens size ranged between 2–5 mm (Voucher RM3_3001, Figure 4B). Body was elongated and cream coloured, with light to dark brown spots and white dots on the dorsal side of the mantle, rhinophores, oral tentacles and head. Bigger and dark brown stains run horizontally on each side of the mantle while a trapezoid and characteristic dark brown spot was present on the head right behind rhinophores. An evident yellow spot occurred on the distal posterior part of the foot. Club-

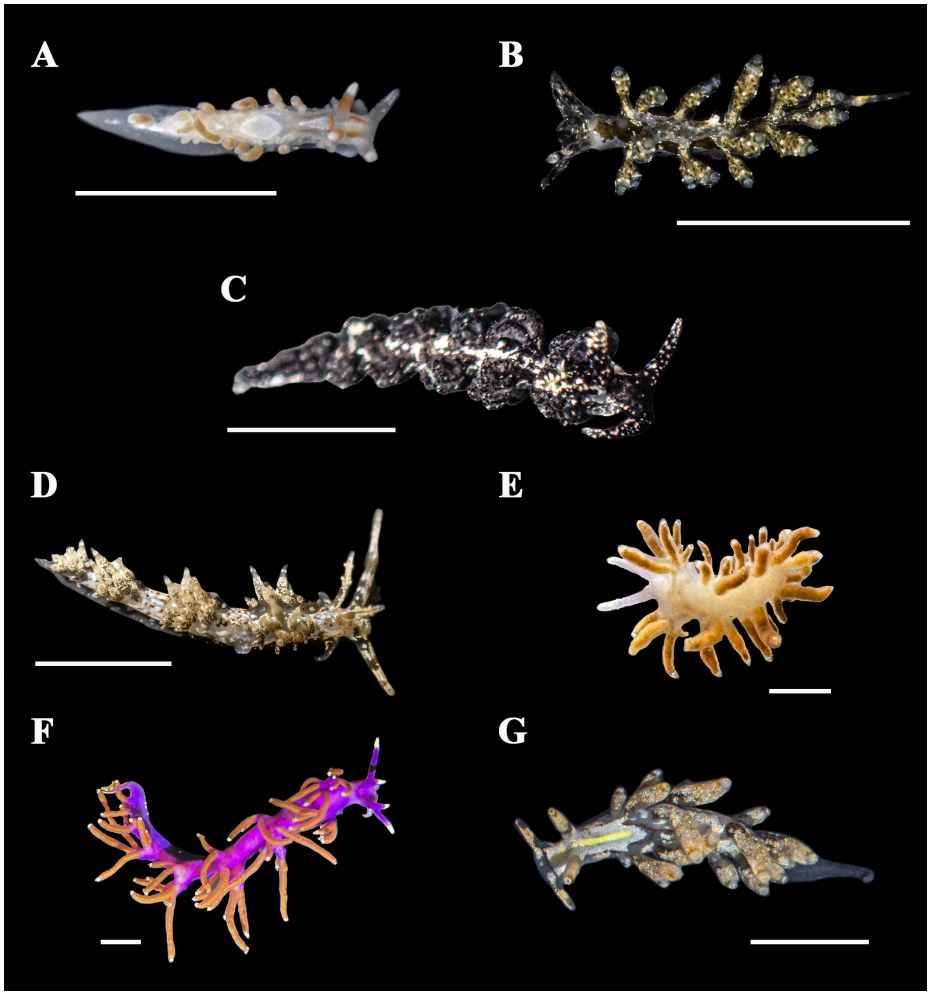

Figure 4. Photographic catalogue of the Nudibranchia suborder Cladobranchia of the superfamily Aeolidioidea observed during this study from Lebanon; (A) *Antaeolidiella lurana* specimen with voucher RM3_2641 (3 mm); (B) *Eubranchus capellinii* specimen with voucher RM3_3001 (3 mm); (C) *Noumeaella* sp. specimen with voucher RM3_2643 (7 mm); (D) *Phidiana anulifera* specimen with voucher RM3_2638 (5 mm); (E) *Edmundsella pedata* specimen with voucher RM3_2556 (8 mm); (F) *Flabellina ischitana* specimen with voucher RM3_2646 (20 mm); (G) *Trinchesia genovae* specimen with voucher RM3_2986 (6 mm). Scale bar = 2 mm.

shaped cerata were creamish with scattered brown and white dots. The top of each ceras was colourless except for a brown ring. The characteristic elevated white anus occurred on the right side of the body between the second and the third clade of cerata. These characters well match with the original description made by Trinchese as *Tergipes capellini* and with the description of other Italian specimens (Schmekel and Portmann 1982).

Remarks: It was found in intertidal and shallow subtidal zone on hard substrates colonized by the biocoenosis of photophilic algae. Even if it shows a widespread distribution, this species can be considered rare, being the present record the third ever known for the whole Mediterranean Sea.

***Doriopsilla rarispinosa* Pruvot-Fol, 1951**

Known distribution: this species was originally described from Banyuls-sur-Mer, France, Mediterranean Sea (Pruvot-Fol 1951), and nowadays is known from the Western to the Central Mediterranean basins (Furfaro et al. 2022a).

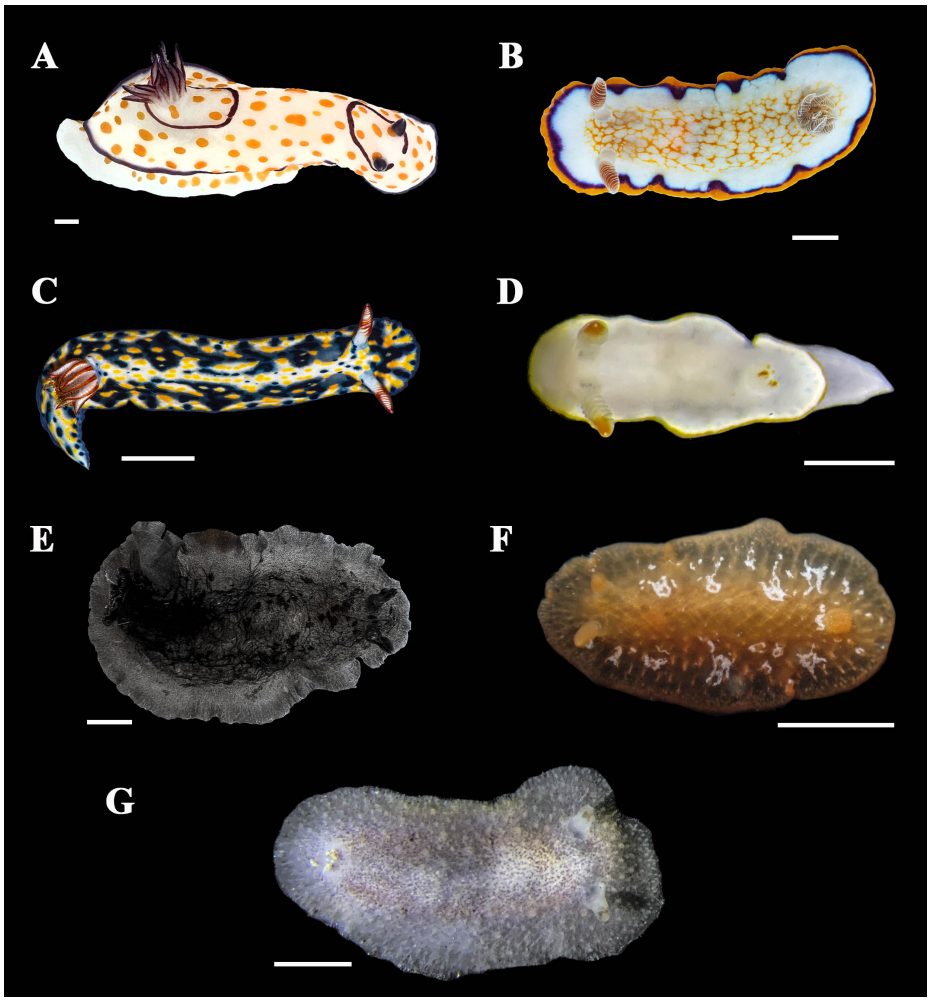

Figure 5. Photographic catalogue of the Nudibranchia suborder Doridina observed during this study from Lebanon; (A) *Goniobranchus annulatus* specimen with voucher LB_44 (40 mm); (B) *Goniobranchus obsoletus* specimen with voucher RM3_2984 (35 mm); (C) *Hypselodoris infucata* specimen with voucher RM3_2999 (30 mm); (D) *Verconia simplex* specimen with voucher RM3_2711 (20 mm); (E) *Dendrodoris fumata* specimen with voucher RM3_2563 (35 mm); (F) *Doriopsilla rarispinosa* specimen with voucher RM3_2650 (15 mm); (G) *Discodorididae* sp. specimen with voucher RM3_2652 (25 mm). Scale bar = 5 mm.

Morphological description: The specimen with voucher RM3_2650 was 15 mm in length (Figure 5F). It showed the coloration intensity of the tubercles and lines on the dorsum and their texture perfectly matching those reported for *D. rarispinosa* species, as the yellow-orange background colour perfectly recalling the morphotype reported for Western Mediterranean populations (Furfaro et al. 2022a).

Remarks: this species was recently resurrected as valid species and well separated from the congeneric *D. areolata* by both molecular and morphological characters (Furfaro et al. 2022a). The Lebanese individual was observed at 3 m of depth from station 6 (Table 1). The present record is the first for Lebanon and the easternmost in the Mediterranean Sea, expanding the range of distribution known for this species.

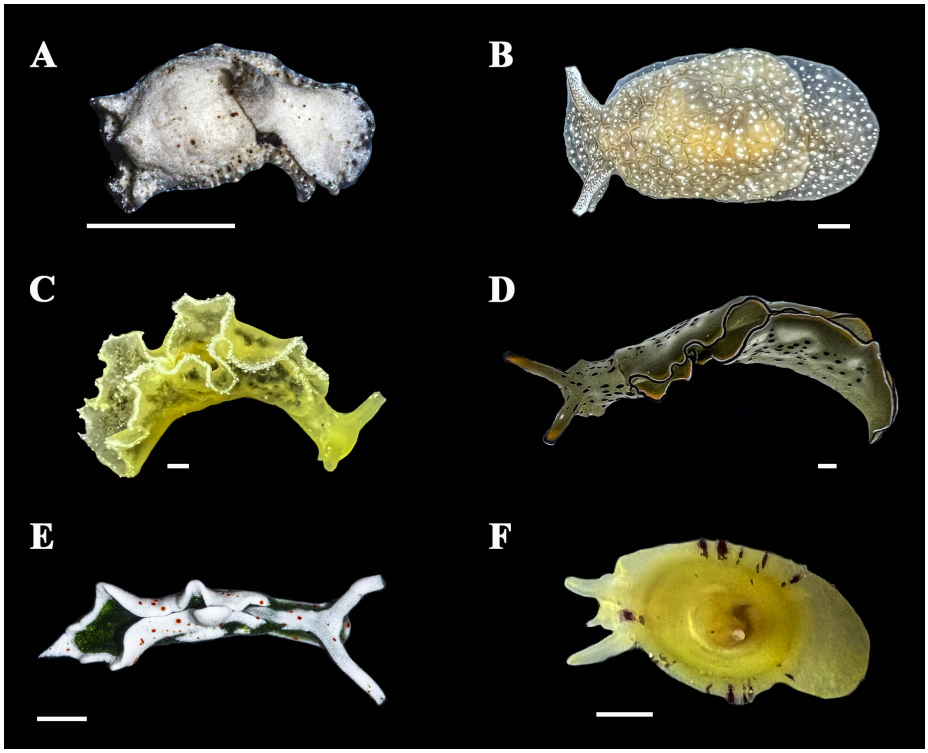

Figure 6. Photographic catalogue of the order Cephalaspidea, Pleurobranchida, Sacoglossa and Umbraculida observed during this study from Lebanon; (A) *Niparaya* cf. *regiscorona* specimen with voucher RM3_2989 (4 mm); (B) *Pleurobranchaea* sp. specimen with voucher RM3_2536 (20 mm); (C) *Elysia flava* specimen with voucher RM3_2537 (30 mm); (D) *Elysia grandifolia* specimen with voucher RM3_2558 (40 mm); (E) *Elysia timida* specimen with voucher RM3_2998 (12 mm); (F) *Tylodina perversa* specimen with voucher RM3_2545 (10 mm). Scale bar = 2 mm.

Elysia flava A. E. Verrill, 1901

Known distribution: originally reported from Bermuda (Verrill 1901) this species is currently known from the Western Atlantic Ocean (Belize, Cuba, Caribbean Sea, Venezuela), to the North and the East Atlantic Ocean (Cape Verde) and in the Mediterranean Sea reported from the Western basin in Spain, in the North-Eastern Sardinia, in Italy (Trainito and Doneddu 2016), and in the Eastern basin from the Aegean Sea (MolluscaBase 2024a).

Morphological description: The specimen observed in Lebanon was around 20–30 mm long and showed an elongated and yellow body with white marginal sides of the parapodia (Voucher RM3_2537, Figure 5C).

Remarks: the genus *Elysia* Risso, 1818 is the richest in species within the Sacoglossa order with a total of about 102 accepted species (MolluscaBase 2024a; Trainito et al. 2022; Martín-Hervás et al. 2024). It was the subject of a recent systematic revision which focused mainly on the European *Elysia* species (Martín-Hervás et al. 2024), but an additional in-depth revision of the Mediterranean species should be carried out to resolve some other cases of difficult identification still existing. The Lebanese individual was found in the intertidal and shallow subtidal zones on hard substrates colonized by the biocoenosis of photophilic algae (Table 1). The present finding constitutes the first report of this species for Lebanon (Table 2).

Table 2. Updated list of the species belonging to the orders Nudibranchia, Cephalaspidea, Pleurobranchida, Sacoglossa and Umbraculida currently known for the Lebanese waters, their indigenous or non-indigenous status and notes on their geographical distribution. In bold are the species reported for the first time in Lebanon after this study.

Order	Family	Species	NIS	Notes on distribution
Nudibranchia	Dotidae	***Doto* cf. *coronata* (Gmelin, 1791)**		First record for Lebanon
		***Doto* sp.**		First record for Lebanon
	Lomanotidae	***Lomanotus vermiformis* Eliot, 1908**	X	First record for the Mediterranean Sea
	Tethydidae	*Melibe viridis* (Kelaart, 1858)	X	
	Tritoniidae	***Marionia blainvillea* (Risso, 1818)**		First record for Lebanon
	Aeolidiidae	*Aeolidiella alderi* (Cocks, 1852)		
		***Anteaeolidiella lurana* (Ev. Marcus & Er. Marcus, 1967)**	X	First record for Lebanon
		Spurilla neapolitana (delle Chiaje, 1841)		
	Eubranchidae	***Eubranchus capellinii* (Trinchese, 1879)**		First record for Lebanon
	Facelinidae	*Caloria indica* (Bergh, 1896)	X	
		Cratena peregrina (Gmelin, 1791)		
		***Noumeaella* sp.**		Possible new to science
		***Phidiana anulifera* (Baba, 1949)**	X	First record for the Mediterranean Sea
	Flabellinidae	*Flabellina affinis* (Gmelin, 1791)		
		***Flabellina ischitana* Hirano & T. E. Thompson, 1990**		First record for Lebanon
		***Edmundsella pedata* (Montagu, 1816)**		First record for Lebanon
		Flabellina rubrolineata (O'Donoghue, 1929)	X	
	Myrrhinidae	*Nemesignis banyulensis* (Portmann & Sandmeier, 1960)		
	Trinchesiidae	***Trinchesia genovae* (O'Donoghue, 1926)**		First record for Lebanon
	Chromodorididae	*Felimare picta* (Schultz in Philippi, 1836)		
		Felimida britoi (Ortea & Perez, 1983)		
		Felimida luteorosea (Rapp, 1827)		
		Felimida purpurea (Risso in Guérin, 1831)		
		Goniobranchus annulatus (Eliot, 1904)	X	
		***Goniobranchus obsoletus* (Rüppell & Leuckart, 1830)**	X	First record for Lebanon
		Hypselodoris infucata (Rüppell & Leuckart, 1830)	X	
		***Verconia simplex* (Pease, 1871)**	X	First record for the Mediterranean Sea
	Dendrodorididae	***Dendrodoris fumata* (Rüppell & Leuckart, 1830)**	X	First record for Lebanon
		Dendrodoris grandiflora (Rapp, 1827)		
		Dendrodoris limbata (Cuvier, 1804)		
		***Doriopsilla rarispinosa* Pruvot-Fol, 1951**		First record for Lebanon
	Discodorididae	*Tayuva lilacina* (Gould, 1852)	X	
		Discodorididae sp.		Possible new to science
	Phyllidiidae	*Phyllidia flava* Aradas, 1847		
	Polyceridae	*Plocamopherus ocellatus* (Rüppell & Leuckart, 1831)	X	
Cephalaspidea	Aglajidae	***Niparaya* cf. *regiscorona* (Bertsch, 1972)**	X	First record for the Mediterranean Sea
	Bullidae	*Bulla striata* Bruguière, 1792		
	Cylichnidae	*Cylichna cylindracea* (Pennant, 1777)		
	Haminoeidae	*Haminoea hydatis* (Linnaeus, 1758)		
	Mnestiidae	*Mnestia girardi* (Audouin, 1826)	X	
	Retusidae	*Pyrunculus fourierii* (Audouin, 1826)	X	
		Retusa mammillata (Philippi, 1836)		
		Retusa truncatula (Bruguière, 1792)		
	Rhizoridae	*Volvulella acuminata* (Bruguière, 1792)		
	Tornatinidae	*Acteocina mucronata* (Philippi, 1849)	X	
Pleurobranchida	Pleurobranchaeidae	*Berthella aurantiaca* (Risso, 1818)		
		Berthella ocellata (delle Chiaje, 1830)		
		Berthellina citrina (Rüppell & Leuckart, 1828)	X	
		Pleurobranchus forskalii (Rüppell & Leuckart, 1831)	X	
		***Pleurobranchaea* sp.**		Possible new to science
Sacoglossa	Plakobranchidae	***Elysia flava* A. E. Verrill, 1901**		First record for Lebanon
		Elysia grandifolia Kelaart, 1858	X	
		Elysia timida (Risso, 1818)		
Umbraculida	Tylodinidae	***Tylodina perversa* (Gmelin, 1791)**		First record for Lebanon
		Umbraculum umbraculum (Lightfoot, 1786)		

Non-Indigenous Heterobranchia newly reported from Lebanon

Investigations and broad bibliographic research confirmed the presence of 20 NIS in the studied area of which seven were recorded for the first time while the other 13 were confirmed from the same area. Among the seven new taxa for Lebanon, four species, namely *Lomanotus vermiformis* Eliot, 1908, *Phidiana anulifera* (Baba, 1949), *Verconia simplex* (Pease, 1871) and *Niparaya* cf. *regiscorona* (Bertsch, 1972), are also reported for the first time for the whole Mediterranean basin (Figure 2, Table 2).

***Lomanotus vermiformis* Eliot, 1908**

Known distribution: Red Sea, Indo-Pacific province (Yonow 2008), East and West Pacific (Gosliner and Bertsch 1985; Gosliner et al. 2015).

Morphological description: specimens measured from 5 to 15 mm in length (Voucher RM3_2990, Figure 3C). Body is long and narrow, with a typical dark brown coloration with pale whitish brims on the lateral corners of the mantle.

Remarks: *Lomanotus stauberi* K. Clark & Goetzfried, 1976 from the West Pacific is considered a synonym, also considering its hydrozoan prey. In fact, this nudibranch is strongly associated to the hydrozoan *Macrorhynchia philippina* Kirchenpauer, 1872 on which it was exclusively found. The specimens here observed were found on *M. philippina* colonies on shallow rocky substrates characterized by photophilic biocenosis. It is noteworthy that *M. philippina* is a lessepsian species (Bitar and Bitar-Kouli 1995) making the present finding another case of lessepsian trophic chain (*i.e.* a trophic chain characterized by both lessepsian prey and predator) newly reported from the Mediterranean Sea (Mioni and Furfaro 2022). This is also the first record of *L. vermiformis* for the whole Mediterranean Sea.

***Anteaeolidiella lurana* (Ev. Marcus & Er. Marcus, 1967)**

Known distribution: distributed in the Atlantic Ocean and Queensland in Australia but already reported as NIS in the Mediterranean Sea (Zenetos et al. 2004; Carmona et al. 2013, 2014; Bariche et al. 2020).

Morphological description: the specimens were up to 7 mm of length and showed a translucent body, bracket-shaped orange mark on the notum, and a distinct coloration of the cerata and rhinophores that clearly distinguished this species from other congeneric (Voucher RM3_2641, Figure 4A) (Carmona et al. 2014).

Remarks: found on rocky bottoms at different bathymetries, from the intertidal zone to the shallow subtidal zone, associated with coralline algae and in a sulphur-rich environment. Its taxonomic status and presence in the Mediterranean Sea were defined by Carmona et al. (2013, 2014) on a molecular and morphological basis.

***Phidiana anulifera* (Baba, 1949)**

Known distribution: Western Pacific (Gosliner 1979; Bhave and Apte 2011; Chow et al. 2022).

Morphological description: specimens measured approximately 5 mm in length (Voucher RM3_2638, Figure 4D). They were recognized by the opaque white body, along with rhinophores and oral tentacles displaying a distinctive orange coloration, which is also evident on the body's sides.

Remarks: found on rocky bottoms in the subtidal zone, associated with photophilic biocenosis and sessile cnidarian of the order Actiniaria. The present report is the first for the whole Mediterranean Sea.

***Goniobranchus obsoletus* (Rüppell & Leuckart, 1830)**

Known distribution: Northern Indian Ocean, Red Sea (Rudman 1973) and Qatar in the Persian Gulf (*personal observations*). This species is currently reported as NIS in Cyprus (Kleitou et al. 2019) and Greece (Bariche et al. 2020).

Morphological description: specimens had a length of 30–35 mm and featured a milky white body with an orange-brown reticulation on the mantle, a band of orange along the mantle's edge, and a submarginal dark blue band. Rhinophores and gills were translucent orange (Voucher RM3_2984, Figure 5B).

Remarks: this species is ubiquitous and was found in a wide range of habitats, from the intertidal zone to shallow subtidal areas on rocky reefs, associated with sponges and various type of algae.

***Verconia simplex* (Pease, 1871)**

Known distribution: originally described from Polynesia, this species is known from Indian Ocean (Mozambique and South Africa) (MolluscaBase 2024b) and in the Western Pacific Ocean (Yonow 2008).

Morphological description: specimens measured 20 mm. Body colour ranged from white to pink, with a distinct orange mantle margin (Voucher RM3_2711, Figure 5D). Rhinophores and gills had orange tips that distinguish it from the similar *Verconia sudanica* (Rudman, 1985), the latter frequent in the whole Red Sea (Yonow 2018).

Remarks: this species is widely distributed and was found in various habitats ranging from the intertidal zone to shallow subtidal areas on rocky reefs. It was occasionally spotted under rocks and was often associated with encrusting sponges. This is also the first record for the whole Mediterranean Sea.

***Dendrodoris fumata* (Rüppell & Leuckart, 1830)**

Known distribution: Indo-Pacific province (Nimbs and Smith 2016; Yonow 2008). Reported as NIS in the Mediterranean Sea (Cevik et al. 2012).

Morphological description: specimens measured about 30–35 mm (Voucher RM3_2563, Figure 5E). It was found in the smoky grey form with dark patches. Mantle was wide with irregular undulating borders.

Remarks: found in intertidal to shallow subtidal, on rocky reefs and sandy sediments, often associated with sponges. The Mediterranean records of this species are dubious and deserve a future in depth analysis based on morphological and molecular analysis.

Niparaya cf. *regiscorona* (Bertsch, 1972)

Known distribution: West Pacific Ocean (Zamora-Silva and Malaquias 2018).

Morphological description: specimen with voucher RM3_3989 (Figure 6A) measured 4 mm in length. Body colour was white, characterized by tiny brownish dots on the foot and mantle borders. Cephalic shield was posteriorly acuminated and raised, caudal lobes were symmetric and the parapodia reduced.

Remarks: found in shallow subtidal zone on photophilic algae colonizing hard substrates. Future morphological and molecular analyses will clarify the taxonomic position of this specimen, considering that *N. regiscorona* could be a complex of species with very similar external morphology as previously stated by Zamora-Silva and Malaquias (2018): "specimens … collected in Hawaii and western Pacific localities seem to belong to two distinct species".

Non determined Heterobranchia newly reported from Lebanon

Among the 24 species found, five taxa were not recognizable at the species or genus taxonomic levels (Table 2, Figure 3). Those taxa have been provisionally reported as *Doto* cf. *coronata*, *Doto* sp., *Noumaella* sp., Discodorididae sp. and *Pleurobranchaea* sp. until further morphological and molecular analyses will define their correct taxonomic status. The finding of five unassignable species raises the possibility of Mediterranean species potentially new for science and the need to increase the sampling effort in this eastern part of the Mediterranean basin.

Doto cf. *coronata* (Gmelin, 1791)

Morphological description: specimen with vouchers RM3_2985 (Figure 3A) was observed at station 8 (Table 1) and measured 3 mm in length. The body was translucent-white, with a high density of small black dots running throughout the mantle. White spots were present on rhinophores, oral tentacles, and along the border of the foot. Even if species identification is not trivial in this genus, the specimen here observed could be identified as *Doto coronata* due to the lack of black pigmentation on the rhinophores, the presence of black dots throughout the body and the characteristic shape of the cerata (Martinsson et al. 2021). However, we prefer to conservatively classify

it as *Doto* cf. *coronata* until additional information, coupled with molecular analysis, will clarify the systematics of the Mediterranean *Doto* species.

Remarks: the specimen was found in circalittoral zone, in correspondence of a freshwater spring.

***Doto* sp.**

Morphological description: specimen with voucher RM3_2988 (Figure 3D) was observed at station 8 (Table 1) and measured 4 mm in length. The body was translucent-white, with a high density of small black dots running throughout the mantle. White spots were present on rhinophores, oral tentacles, and along the border of the foot. Two curved white lines were visible on the back, between the first and the second row of cerata, the latter pale white coloured and with a black dot on the top of each process.

Remarks: additional morphological and molecular information will be necessary to clarify the taxonomic position of this specimen that was found in circalittoral zone, in correspondence of a freshwater spring.

***Noumeaella* sp.**

Morphological description: the two individuals with vouchers RM3_2643 (Figure 4C) and RM3_2644 measured 7 mm in length and shared the same body colour pattern and general shape. In particular, the body was elongated and milky white coloured on the foot while the dorsum, the rhinophores and the head showed a black coloration with creamy irregular spots throughout the body. The rhinophores showed pronounced creamy papillae on the posterior side that are typical of the genus. The same creamy colour characterized the apical part of the rhinophores and the cephalic tentacles (Figure 4C).

Remarks: the two individuals were observed in the station 8 at 35 m of depth in circalittoral zone and in correspondence of a freshwater spring (Table 1). The prevalence of dark brown colour of this *Noumeaella* species does not match with any of the described species, although it looked quite similar to the species named *Noumeaella* sp.12, *sensu* Gosliner et al. (2015). Further in-depth analyses will be necessary to define its systematic status.

Discodoridae sp.

Morphological description: the specimen catalogued with voucher RM3_2652 (Figure 5G) was about 25 mm long. The body was translucent grey with white patches on the mantle, rhinophores were dark grey.

Remarks: this dorid was found in circalittoral zone, in station 2 at 38 m of depth (Table 1) and in correspondence of a hot water spring. Based on the general shape of the body it could be identified as belonging to the family Discodoridae even if the external morphology of this specimen does not allow a confident identification both at the species and genus

taxonomic levels. Some external diagnostic characters, like the presence and shape of the caryophyllids, can recall the genus *Jorunna* Bergh, 1876 (Camacho-García and Gosliner 2008), however, considering the weak morphological separation that exists between some Discodorididae genera (as happens for example between *Jorunna*, *Rostanga* Bergh, 1879, *Taringa* Er. Marcus, 1955, *Tayuva* Er. Marcus & Ev. Marcus, 1967, *Thordisa* Bergh, 1877), a certain placement in one genus rather than another is to date impossible. For all these reasons it is here conservatively indicated as Discodorididae sp. until a systematic review of the whole family using an integrative taxonomy approach will be carried out. However, even if a precise taxonomic assignment is not possible, we can say that in our knowledge it is not an already know Mediterranean species.

Pleurobranchaea sp.

Morphological description: the three specimens with vouchers RM3_2536 (Figure 6B), RM3_2541 and RM3_2547 (Table 1) measured 20–25 mm. The external morphology of the three individuals was congruent to each other and consisted of a translucent grey body and rhinophores, with homogenously distributed white dots all over the body, the cephalic portion, and the upper part of the foot (Figure 6B).

Remarks: the three specimens were collected from station 9 at 65 m of depth. They were collected "by catch" during fishing activities in the circalittoral zone, in correspondence of a muddy bottom characterized by sponges and corals. The external morphology recalled *Pleurobranchaea britannica* Turani, Carmona, Barry, Close, Bullimore & Cervera, 2024, recently described based on specimens from the North Atlantic and the Gulf of Cadiz: in particular, the general livery corresponds with the white stippling, the margin of the frontal velum slightly wavy and the absence of a caudal spur. However, further in-depth analyses will be necessary to verify this hypothesis.

Discussion

The three Blue Tyre expeditions carried out at 14 stations along the Tyre peninsula in Lebanon allowed to observe 65 specimens belonging to 24 sea slug species, increasing the total number of species known for Lebanon (of the relative taxonomical orders) from 35 to 55 (Table 2). The Nudibranchia order received the biggest increment, with its number almost doubled (16 added species out of the 19 already known for the area). This result is particularly important and suggests that a more extensive sampling effort, both in terms of number of investigated sites and samplings throughout the year, should be undertaken to properly investigate this promising area. Moreover, the low percentage of nudibranchs reported in the past, compared to a greater amount of "shelled" Heterobranchia, could be a consequence of

the sampling techniques used to study molluscan diversity. Heterobranchia are very sensitive to the procedures used to collect them since their soft and delicate bodies can be easily damaged by hard collecting methods. Moreover, species identification is almost impossible for nudibranchs without *in vivo* observations or analysis of diagnostic images of living organisms. This point is crucial since the methods commonly used to study molluscs, especially gastropods, rely on observing the shells of dead specimens or individuals preserved in ethanol. In this regard, the addition of only one species to each of the other investigated orders (*i.e.* Cephalaspidea, Pleurobranchida, Sacoglossa and Umbraculida) is in line with what discussed so far and confirms the greater effort in previous studies to unravel the diversity of "shelled" Heterobranchia. The Sacoglossa order, characterized by some shell-less genera, is an exception in this sense. The present study significantly contributes to the list of known Mediterranean Heterobranchia, which has considerably increased with the addition of four species: *Verconia simplex*, *Dendrodoris fumata, Phidiana anulifera*, and *Lomanotus vermiformis*, reported here for the first time in the Mediterranean basin (Table 2). Another interesting result is the high ratio between the number of taxa that could not be assigned to a species level (5) that could potentially be cryptic or new to science and the number of total species observed (24). This is noteworthy and highlights the extraordinary potential of the Mediterranean Sea as a hot spot of Heterobranchia diversity and its ability to host endemic or hidden diversity. Regarding non-indigenous (NI) Heterobranchia found in the Lebanese waters, this study reports the highest percentage reported so far for the Heterobranchia group with 42% of the collected specimens coming from extra Mediterranean localities. To better explore the diversity of Lebanese sea slugs, sampling was carried out across a wide range of habitats and employed a variety of techniques. In fact, focusing only on specific transects (with a statistically significant replicas) could be misleading in this group considering the direct association between some heterobranchs species and their food source that could determine cases of local abundance/ paucity according to the presence/absence of the prey. On the other hand, this lack of a standardize protocol does not allow to do statistically supported comparison between different stations and seasons. However, even though the absence of a species may not be very significant (because it could be the consequence of a less sampling effort), its presence is a particularly relevant aspect giving some useful information on the biological cycle or on the temperature preference of that species. Interestingly during the autumn expedition most of the species observed were NIS followed by some common Mediterranean species (Supplementary material Table S1) suggesting that increase of seawater temperature and possible summer heatwaves make the studied area unsuitable for native and more sensitive species, which are mostly of temperate to boreal affinity. Even if these latter speculations are based on preliminary explorations, similar observations were reported for

other molluscs from different areas of the Levantine basin (Albano et al. 2020, 2021) suggesting a possible trend of native species loss also in the Heterobranchia group due to global warming. Another interesting insight regarding the NIS observed in Lebanon is related to their geographical origin that, in most of the cases, is Indo/Pacific (Table S1) with species coming from very distant localities instead of the closer Red Sea, opening new questions on the possible vector of dispersal. In fact, as reported in other taxa (Albano et al. 2024), the naval traffic could represent the primary way of introduction for these species into the Mediterranean Sea even if more data, including a molecular characterization of the different populations from the whole distribution range, are required to exclude possible cases of cryptic diversity. However, this hypothesis opens interesting insights also for a conservation perspective.

Finally, the diversity of Heterobranchia provides a valuable framework for tracking the effects of global warming and can serve as an indicator of possible shifts in habitat biodiversity (Nimbs et al. 2016; Goddard et al. 2016, 2018; Azzola et al. 2022; Chow et al. 2022). Constant and effective monitoring of heterobranchs in this strategic Mediterranean area could yield important results for the entire community of sessile invertebrate organisms linked by prey-predator relationships to these specialized marine molluscs.

Conclusions

The study of Heterobranchia inhabiting Lebanon marine coastal areas significantly increased the number of species reported from the study area and the entire Mediterranean Sea. In fact, the total number of Lebanese species (belonging to the heterobranch orders investigated) rose from 35 to 55, with the highest increase in the Nudibranchia list, which almost doubled, and included four new records for the whole Mediterranean basin. Among the newly reported species, five out of 24 were not assigned to the species taxonomic level and require further analysis to determine if they belong to already known species or are new to science. Finally, the striking percentage of NIS (42%) among the total recorded taxa, especially considering that no other area of the Mediterranean Sea has such a high percentage, calls for a swift and effective action. Given this, Lebanese coastal waters have a central role in NIS detection into the Mediterranean basin, making the study of this strategic geographic area even more important.

Authors' contribution

GF, SP, LMF, AT: research conceptualization; GF, LMF, AT, YT: sample design and methodology; LMF, AT, YT: investigation and data collection; GF, ET: data analysis and interpretation; GF, MB, SP: ethics approval; MB, SP: funding provision; GF, LMF: roles/writing – original draft; GF, LMF, AT, YT, MB, ET, SP: writing – review and editing.

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Ethics and permits

This research complies with restrictions in terms of collected sample size, environmental survey of the collection sites, and the use of hand-net picking of specimens (harmless and not destructive), as well as 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).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Locus typicus, presence/absence and estimate of abundance of all the species observed.

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