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Article

First Survey of Heterobranch Sea Slugs (Mollusca, Gastropoda) from the Island Sangihe, North Sulawesi, Indonesia

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Abstract: Indonesia is famous for its underwater biodiversity, which attracts many tourists, especially divers. This is also true for Sangihe Islands Regency, an area composed of several islands in the northern part of North Sulawesi. However, Sangihe Islands Regency is much less known than, e.g., Bunaken National Park (BNP, North Sulawesi). The main island, Sangihe, has recently experienced an increase in tourism and mining activities with potentially high impact on the environment. Recently, monitoring projects began around BNP using marine Heterobranchia as indicators for coral reef health. No information about this taxon exists from the remote islands in North Sulawesi. The present study represents the first monitoring study ever and focuses on marine Heterobranchia around Sangihe. In total, 250 specimens were collected, which could be assigned to Sacoglossa (3), Anthobranchia (19), and Cladobranchia (1). Despite the low number (23 versus 172 in BNP), at least eight species (35%) are not recorded from BNP, probably indicating differences in habitat, but also influence of a strong El Niño year in 2016. Here we also report for the first time a *Chromodoris annae* specimen mimicking *C. elisabethina*, and the discovery of a new *Phyllidia* species.

Keywords: Sangihe; North Sulawesi; Indonesia; Heterobranchia; sea slugs; biodiversity; monitoring; tourism

1. Introduction

Indonesia is an archipelagic country with a coastline of more than 100,000 km. Coral reefs, sea grasses, and mangrove forests cover approximately 50,875 km^2, although this number does not take into consideration the remote areas [1,2]. These tropical ecosystems with a high species and habitat diversity have a tremendous ecological and economic value to nature and humans. They contribute substantially to the community's income as well as to the national economy. However, many anthropogenic activities form a threat to these natural habitats. Suharsono [3] found that the condition of more than 20% of Indonesian coral reefs were in poor condition and only 6.5% were considered healthy. More recent studies in Indonesia suggest the additional decline of healthy reefs influenced by natural disturbances (e.g., Utama and Hadi, [4]) and up to 50% are severely damaged (e.g., Rudianto and Bintoro [5]).

North Sulawesi is known as a mega-diverse area, and therefore very popular for diving and snorkeling tourists. Thus, the pressure on the reefs has increased dramatically in the last few years. Based on Badan Pusat Statistik Provinsi Sulawesi Utara [6], the number of foreign visitors visiting North Sulawesi Province, via the International Airport of Sam Ratulangi, Manado, approached nearly 11,000 visitors alone in February 2018. This is an increase of 27% compared to January 2018. Comparing foreign visitors in February 2018 with February 2017, the number augmented by more than 100% [6]. Thus, the pressure on the reefs has increased dramatically in the last years. A few local studies conducted in Bunaken National Park (BNP), North Sulawesi, over 10 years clearly indicate a declining state of coral coverage and coral reef fish, and this is related to an increased number of local and foreign visitors, in addition to an increased number of permanent residents [7]. Another study identified diving and snorkeling activities as a major source of the decline in living coral coverage by comparing different sites around Bunaken Island [8]. Undisturbed areas had a live coral coverage of nearly 55% in 3 m depths, while areas with snorkelers and divers showed coverage of only 17% at this depth.

Sangihe Islands in North Sulawesi Province is less known to tourists. It is one of the most northern groups of islands in Indonesia, with Sangihe as the largest island covering an area of approximately 500 km^2. The area geographically connects North Sulawesi with Mindanao (Philippine Islands) and forms the eastern boundary of the Celebes Sea. However, biogeographically it is still part of the Wallacea, marked by the Wallace line, which runs between Sangihe Islands and the Philippines. Sangihe has come into focus recently by advertising adventurous diving tourism, including visits to the active underwater volcano Mahengetang in a depth of less than 10 m [9]. Being promoted recently as one of the tourist destinations in the Sangihe Islands Regency, the area is liable to experience a huge pressure on its environment in the near future by many more visitors, both national and international, and a higher demand for hotels, resorts, and diving centers. Higher levels of tourist activities are usually accompanied by threats to ecosystems, such as increased farming, aquaculture, and fisheries due to additional needs of temporary visitors and/or permanent residents. Additionally, Sangihe has come into the focus of mining companies. Since 2007, East Asia Minerals Corporation and local partners were granted exploration permits from the local government within an area of 42,000 ha in the south of Sangihe. The first gold and silver production phase within this Sangihe Gold Project was scheduled for the end of 2018, but did not start yet in 2019 [10]. In terms of minimizing the negative impacts on the environment in the future and helping to build up a sustainable use of the natural resources on and around Sangihe, investigation of the biodiversity in this still rather undisturbed region is paramount. In contrast to BNP, which is already highly affected by diving and snorkeling tourism, monitoring activities in Sangihe Islands Regency with only 12 resorts [11] could provide a good opportunity to study the impact of new infrastructure for tourists and their activities in the marine habitats, as well as other economically important activities on the environment.

Diversity and health of coral reefs is reflected by a diversity of marine organisms, including marine Heterobranchia. These sea slugs use a highly diverse food spectrum, with a high affinity to their specific diet. This spectrum covers nearly all sessile organisms (algae, poriferans, cnidarians, ascidians, bryozoans, tunicates). Thus, this group was already used for monitoring coral reef diversity in North Sulawesi [12–15]. Because marine Heterobranchs are also very attractive to tourists, additional data are and will be available through citizen science due to documentation in websites or personal information and provision of images on personal bases. This was shown lately by Nimbs et al. [16] and Nimbs and Smith [17] where long-term documentation of scientists and recreational divers led to the identification of new tropical species introduced in Port Stephens, on the central New South Wales coast of Australia, and Tasman Sea. In order to monitor potential damage to the environment around Sangihe, irrespective of its original cause, we have started with a first survey in 2016, focusing on marine Heterobranchia. Here we present the first results from this collecting period and compare our results with former studies in Bunaken National Park [13,14] and other areas in and around Indonesia.

2. Materials and Methods

Sampling was carried out during daytime from 3 to 7 August 2016 at seven sites around the island Sangihe (Figure 1, Table 1). Seven scientists and students (three with less and four with good collecting experience from former studies, including the BNP studies) collected in a depth range from the eulittoral to maximum of 28 m. On average, the bottom time for each collecting activity was 60 minutes. In total, underwater searching period correlated to approximately 50 working hours around the island. Additionally, about 10 working hours in total were spent collecting while snorkeling. Specimens were photo-documented in the field on the original substrate before being collected individually. Most specimens were identified before preservation using identification books and original literature [18–30], as well as websites (e.g., The Sea Slug Forum [31]). Regarding species validity, the World Register of Marine Species [32] was used. The sea slugs were usually preserved in 96% alcohol for further study (including barcoding). All animals were recorded with metadata that are available in the database Diversity Collection (Part of Diversity Workbench) using the data brokerage service of the German Federation for Biological Data (GFBio) [33]. Geographic names were not available for all collection sites. We then used the name of the village close to the respective study area. This is the case for the villages of Palahanaeng and Talengen. Available data on the distribution of respective sea slugs are downloaded from the Global Biodiversity Information Facility (GBIF). For visualization in maps, the geographic information system ArcGIS, release 10.0, was used. The material is registered in the Sam Ratulangi University (UNSRAT, Manado) reference collection under the number SRU2016/01.

Table 1. Details on collection sites (Figure 1). When sites do not have a geographic name, we used the name of the village nearby. Abbreviations of localities are used in Table 2.

Name	Abbreviation	Area and Geographic Data	Date of Collection
Ship Wreck	ShW	3°36′28.00″ N 125°29′38.00″ E	04.08.2016
Tahuna Bay South	TBS	3°35′59.40″ N 125°29′23.40″ E	04.08.2016
Mendaku	Men	3°22′01.94″ N 125°34′26.67″ E	03.08.2016
Palahanaeng (village)	Pal	3°35′18.92″ N 125°34′26.67″ E	07.08.2016
Talengen (village)	Tal	3°34′49.92″ N 125°34′34.93″ E	05.08.2016
Manalu	Man	3°32′08.87″ N 125°37′25.46″ E	06.08.2016
Sapaeng	Sap	3°34′55.81″ N 125°34′49.04″ E	06.08.2016

Figure 1. Details on North Sulawesi with collection sites in Sangihe (upper insert, and see also Table 1) and the collection area around Bunaken Island [13,14] (lower insert) for comparison.

Traditional barcoding genes (partial CO1 and partial 16 S) were analyzed for most specimens to verify identification. DNA-Isolation has been carried out by means of QIAgen®DNeasy Blood and Tissue-Kit (QIagen, Hilden, Germany), following the manufacturer's instructions. Partial sequences of mitochondrial CO1 (about 680 bp) and ribosomal 16 S (about 450 bp) were amplified by polymerase chain reaction using the primers LCO1490-JJ (5′–CHACWAAYCATAAAGATATYGG-3′) and HCO2198-JJ (5′-AWACTTCVGGRTGVCCAAARAATCA-3′) [34] for CO1 and 16 Sar-L (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sbr-H (5′-CCGGTCTGAACTCAGATCACGT-3′) [35] for 16 S. Amplification of CO1 was performed by an initial step (95 °C for 15 min) followed by 40 touch-down cycles of denaturation (94 °C for 35 s), annealing (55 °C for 90 s) and extension (72 °C for 90 s), with a final extension step 72 °C for 10 min. For 16 S rRNA, the PCR started with an initial step (95 °C for 15 min), denaturation (94 °C for 45 s), followed by 34 touch-down cycles, annealing (56 °C for 45 s), extension (72 °C for 90 s), and final extension step at 72 °C for 10 min. PCR products were sequenced by Macrogen Europe Laboratory (Amsterdam, Netherlands). The software GENEIOUS Pro 7.1.9 (Biomatters Ltd., Auckland, New Zealand) was used to extract the consensus sequence between the primer regions, to construct the final alignments, including sequences from the National Center for Biotechnology Information (NCBI, Bethesda, Maryland, USA), in order to analyze species assignment.

3. Results

250 specimens were collected comprising 23 species (Table 2, Figures 2 and 3). These can be assigned to the *Sacoglossa* (3) and within the *Nudibranchia* to *Anthobranchia* (19) and *Cladobranchia* (1) (Figures 2 and 3). Out of the 250 specimens, identification was verified by barcoding for 236 specimens (partial CO1 and 16 S genes, see NCBI accession numbers in Table 3). Distribution of the species based on data in GBIF, including the new results from the island Sangihe, are depicted in Figures 4–6 with a restriction to the Indian and Western Pacific Ocean.

Figure 2. Sacoglossa and Anthobranchia: (**A**) *Elysia pusilla*, Elpu16Sa-3; (**B**) *Thuridilla gracilis*, Thgr 16Sa-2; (**C**) *Plakobranchus* cf. *papua*, Ploc16Sa-2; (**D**) *Notodoris serenae*, Aese16Sa-2; (**E**) *Chromodoris dianae*, Chdi16Sa-2; (**F**) *Chromodoris annae*, Chan16Sa-2; (**G**) *Chromodoris annae* mimicking *C. elisabethina*, Chel16Sa-1; (**H**) *Chromodoris strigata*, Chst16Sa-1; (**I**) *Glossodoris* cf. *cincta*, Glci16Sa-1; (**J**) *Goniobranchus geometricus*, Goge16Sa-2; (**K**) *Goniobranchus reticulatus*, Gore16Sa-1.

Table 2. Species records around Sangihe with details about specimens and locality, as well as first authorities. Species recorded in Eisenbarth et al. [14] around BNP are indicated in the last column.

Taxon	Species Name	Localities							Depths (m)	Number of Specimens	Size (mm)	Eisenbarth et al. [14]
		TBS	ShW	Man	Pal	Men	Sap	Tal				
Sacoglossa	*Elysia pusilla* (Bergh, 1871)	1	-	-	-	2	-	-	2	3	2–6	x
	Thuridilla gracilis (Risbec, 1928)	-	-	-	3	-	2	1	4–10	6	20–30	x
	Plakobranchus cf. *papua* (Meyers-Muñoz & van der Velde, 2016)	1	-	-	-	1	-	-	5–15	2	25,30	-
Anthobranchia	*Notodoris serenae* (Gosliner & Behrens, 1997)	-	-	2	-	-	-	-	24–27	2	60,90	x
	Chromodoris dianae (Gosliner & Behrens, 1998)	-	-	1	-	6	-	-	15–27	7	5–45	x
	Chromodoris annae (Bergh, 1877)	-	-	1	1	2	5	4	5–23	13	8–41	x
	Chromodoris strigata (Rudman, 1982)	-	-	-	-	1	-	-	15	1	10	x
	Glossodoris cf. *cincta* (Bergh, 1888)	-	-	-	1	-	-	1	8, 13	2	21, 48	x
	Goniobranchus geometricus (Risbec, 1928)	1	-	-	1	-	2	-	6–19	4	10–15	x
	Goniobranchus reticulatus (Quoy & Gaimard, 1832)	2	-	-	-	-	-	-	6, 9	2	25, 55	x
	Hypselodoris tryoni (Garret, 1873)	-	-	-	-	-	3	-	10, 16	3	25–60	x
	Phyllidia ocellata (Cuvier, 1804)	2	-	2	1	-	2	-	4–18	7	16–35	x
	Phyllidia picta (Pruvot-Fol, 1957)	6	-	3	2	2	6	2	1–15	21	13–30	-
	Phyllidia spec. (Phsp3_16Sa-1)	-	-	-	-	-	1	-	1	1	25	-
	Phyllidia madangensis (Brunckhorst, 1993)	-	-	-	-	-	-	1	8	1	28	-
	Phyllidia coelestis (Bergh, 1905)	3	-	1	1	-	1	3	3–12	9	7–32	x
	Phyllidia varicose (Lamarck, 1801)	19	-	2	10	2	15	10	3–15	58	7–87	x
	Phyllidiella lizae (Brunckhorst, 1993)	3	-	3	1	3	-	2	5–23	12	6–68	-
	Phyllidiella pustulosa (Cuvier, 1804)	19	4	11	15	2	15	11	1–23	77	12–47	x
	Phyllidiella nigra (van Hasselt, 1824)	-	-	-	-	-	1	-	8	1	29	x
	Phyllidiopsis krempfi (Pruvot-Fol, 1957)	1	-	2	6	-	6	-	6–28	15	14–50	-
	Phyllidiopsis shireenae (Brunckhorst, 1990)	-	-	1	-	-	1	-	8, 15	2	77, 81	-
Cladobranchia	Aeolidioidea (Flsp16Sa-1)	-	-	-	-	1	-	-	2	1	1	-

Table 3. Species used in this study, identification number, and Genbank accession numbers as also mentioned in Diversity Workbench.

Family	Species Name	ID	GenBank Accession Numbers	
			16 S	CO1
Chromodorididae (Bergh, 1891)	*Chromodoris dianae* (Gosliner & Behrens, 1998)	Chdi16Sa-1	MN104702	MN320502
		Chdi16Sa-2	MN104703	MN320503
		Chdi16Sa-3	MN104704	MN320504
		Chdi16Sa-4	MN104705	MN320505
		Chdi16Sa-5	MN104706	MN320506
		Chdi16Sa-6	MN104707	MN320507
		Chdi16Sa-7	MN104708	MN320508
	Chromodoris annae (Bergh, 1877)	Chan16Sa-1	MN104690	MN124751
		Chan16Sa-2	MN104691	MN124752
		Chan16Sa-3	MN104692	MN124753
		Chan16Sa-4	MN104693	MN124754
		Chan16Sa-5	MN104694	MN124755
		Chan16Sa-6	MN104695	MN124756
		Chan16Sa-7	MN104696	MN124757
		Chan16Sa-8	MN104698	MN124758
		Chan16Sa-9	MN104699	MN124759
		Chan16Sa-10	MN104700	MN124760
		Chan16Sa-11	MN104701	MN124761
		Chan16Sa-12	MN104702	MN124762
		Chel16Sa-1	MN104709	MN124763
	Chromodoris strigata (Rudman, 1982)	Chst16Sa-1	MN104710	MN365022
	Glossodoris cf. *cincta* (Bergh, 1888)	Glci16Sa-1	MN104711	MN339440
		Glci16Sa-2	MN104712	MN339441
	Goniobranchus geometricus (Risbec, 1928)	Goge16S-1	MN104715	MN339442
		Goge16S-2	MN104716	MN339443
		Goge16S-3	MN104717	MN339444
		Goge16S-4	-	MN339445
	Goniobranchus reticulatus (Quoy & Gaimard, 1832)	Gore16Sa-1	MN104719	MN339446
		Gore16Sa-2	MN104720	MN339447
	Hypselodoris tryoni (Garret, 1873)	Goca16S-1	MN104713	MN339448
		Goca16S-2	MN104714	MN339450
		Goku16Sa1	MN104718	MN339449

Table 3. *Cont.*

Family	Species Name	ID	GenBank Accession Numbers	
			16 S	**CO1**
Phyllidiidae (Rafinesque, 1814)	*Phyllidia picta* (Pruvot-Fol, 1957)	Phpic16Sa-1	MN217674	MN248545
		Phpic16Sa-5	MN217680	MN248543
		Phpic16Sa-6	MN217675	MN248546
		Phpic16Sa-8	MN217671	MN248540
		Phpic16Sa-9	MN217669	MN248539
		Phpic16Sa-10	MN217672	MN248542
		Phpic16Sa-11	MN217679	MN248549
		Phpic16Sa-12	MN217678	MN248547
		Phpic16Sa-13	MN217676	MN248548
		Phpic16Sa-14	MN217681	MN248544
	Phyllidia spec.	Phsp616Sa-3	MN217677	MN248550
		Phspec116Sa-2	MN217670	MN248541
		Phsp316Sa-1	MN217673	MN265389
	Phyllidia ocellata (Cuvier, 1804)	Phoc16S-1	MN173896	MN173896
		Phoc16S-2	MN173895	MN173895
		Phoc16S-4	MN173894	MN173894
		Phoc16S-5	MN173893	MN173893
		Phoc16S-6	-	MN173892
		Phoc16S-7	MN173891	MN173891
	Phyllidia coelestis (Bergh, 1905)	Phco16Sa-1	MN172238	MN234119
		Phco16Sa-2	MN172237	MN234113
		Phco16Sa-3	MN172236	MN234115
		Phco16Sa-4	MN172235	MN234118
		Phco16Sa-5	MN172234	MN234112
		Phco16Sa-7	MN172233	MN234116
		Phco16Sa-9	MN172232	MN234114
		Phco16Sa-10	MN172231	MN234112

Table 3. *Cont.*

Family	Species Name	ID	GenBank Accession Numbers	
			16 S	**CO1**
		Phva16Sa-2	MN243776	-
		Phva16Sa-3	MN243779	-
		Phva16Sa-4	MN243778	MN248554
		Phva16Sa-5	MN243774	-
		Phva16Sa-7	MN243747	-
		Phva16Sa-8	MN243735	-
		Phva16Sa-9	MN243783	MN248572
		Phva16Sa-10	MN243750	-
		Phva16Sa-11	MN243761	-
		Phva16Sa-12	MN243781	-
		Phva16Sa-13	MN243760	MN248571
		Phva16Sa-15	MN243782	MN248555
		Phva16Sa-16	MN243775	-
		Phva16Sa-17	MN243759	-
		Phva16Sa-18	MN243780	-
	Phyllidia varicosa (Lamarck, 1801)	Phva16Sa-20	MN243758	MN248556
		Phva16Sa-21	MN243734	MN248563
		Phva16Sa-22	MN243777	-
		Phva16Sa-23	MN243773	-
		Phva16Sa-24	MN243757	MN248568
		Phva16Sa-25	MN243746	-
		Phva16Sa-26	MN243733	-
		Phva16Sa-27	MN243771	MN248573
		Phva16Sa-28	MN243748	-
		Phva16Sa-29	MN243745	-
		Phva16Sa-30	MN243740	-
		Phva16Sa-31	MN243770	MN248567
		Phva16Sa-32	MN243768	-
		Phva16Sa-33	MN243767	MN248574
		Phva16Sa-34	MN243756	-
		Phva16Sa-36	MN243772	-
		Phva16Sa-37	MN243755	MN248569
		Phva16Sa-38	MN243763	-
		Phva16Sa-39	MN243744	-

Table 3. *Cont.*

Family	Species Name	ID	GenBank Accession Numbers	
			16 S	**CO1**
		Phva16Sa-40	MN243754	MN248557
		Phva16Sa-41	MN243739	-
		Phva16Sa-42	MN243749	MN248562
		Phva16Sa-43	MN243764	MN248565
		Phva16Sa-44	MN243766	MN248561
		Phva16Sa-45	MN243741	MN248564
		Phva16Sa-46	MN243738	-
		Phva16Sa-47	MN243737	MN248558
		Phva16Sa-48	MN243753	-
		Phva16Sa-49	MN243743	-
		Phva16Sa-50	MN243742	MN248559
		Phva16Sa-52	MN243765	MN248560
		Phva16Sa-53	MN243762	MN248570
		Phva16Sa-54	MN243752	-
		Phva16Sa-55	MN243751	-
		Phva16Sa-56	MN243769	MN248566
		Phva16Sa-57	MN243736	-
		Phva16Sa-58	MN243732	-
	Phyllidiella lizae (Brunckhorst, 1993)	Phli16Sa-1	MN243971	MN248575
		Phli16Sa-2	MN243973	MN248577
		Phli16Sa-5	MN243972	MN248576
		Phli16Sa-6	MN243974	MN248578
	Phyllidiella pustulosa (Cuvier, 1804)	Phpu16Sa-1	MN243977	MN248624
		Phpu16Sa-2	MN244015	MN248636
		Phpu16Sa-3	MN243991	MN248601
		Phpu16Sa-4	MN243992	MN248606
		Phpu16Sa-5	MN243996	MN248608
		Phpu16Sa-6	MN244006	MN248602
		Phpu16Sa-7	MN244007	MN248594
		Phpu16Sa-8	MN243999	-
		Phpu16Sa-9	MN243980	MN248627
		Phpu16Sa-13	MN243969	MN248581

Table 3. *Cont.*

Family	Species Name	ID	GenBank Accession Numbers	
			16 S	CO1
		Phpu16Sa-14	-	MN248580
		Phpu16Sa-15	MN243960	MN248585
		Phpu16Sa-18	MN243983	MN248632
		Phpu16Sa-20	-	MN248590
		Phpu16Sa-23	MN243962	MN248586
		Phpu16Sa-24	MN243978	MN248625
		Phpu16Sa-25	MN244008	MN248595
		Phpu16Sa-26	MN243979	MN248626
		Phpu16Sa-27	MN244009	MN248596
		Phpu16Sa-28	MN243970	MN248591
		Phpu16Sa-29	MN243955	MN248639
		Phpu16Sa-30	MN244000	MN248620
		Phpu16Sa-31	MN243963	MN248587
		Phpu16Sa-33	MN243985	MN248614
		Phpu16Sa-34	MN244017	MN248637
		Phpu16Sa-35	MN243957	MN248640
		Phpu16Sa-36	MN244011	MN248597
		Phpu16Sa-38	MN243997	MN248609
		Phpu16Sa-39	MN244010	MN248598
		Phpu16Sa-40	MN243981	MN248628
		Phpu16Sa-46	MN244001	MN248620
		Phpu16Sa-48	MN243975	MN248590
		Phpu16Sa-50	MN243958	MN248641
		Phpu16Sa-52	MN244002	MN248621
		Phpu16Sa-53	MN243968	MN248584
		Phpu16Sa-55	MN244081	-
		Phpu16Sa-56	MN243994	MN248605
		Phpu16Sa-60	MN244014	MN248634
		Phpu16Sa-61	MN244006	MN248613
		Phpu16Sa-62	MN243995	MN248607
		Phpu16Sa-68	-	MN248610
		Phpu16Sa-69	MN244016	MN248635
		Phpu16Sa-70	MN244018	MN248638
		Phpu16Sa-71	MN243986	MN248616
		Phpu16Sa-73	MN243998	-

Table 3. *Cont.*

Family	Species Name	ID	GenBank Accession Numbers	
			16 S	CO1
		Phpu16Sa-74	MN243956	MN248642
		Phpu16Sa-75	MN243987	MN248617
		Phpu16Sa-76	MN243989	MN248615
		Phpu16Sa-77	MN243993	MN248604
		Phpu16Sa-79	MN243988	MN248619
		Phpu16Sa-80	MN244019	MN248600
		Phpu16Sa-84	MN244012	MN248599
		Phpu16Sa-85	MN243990	MN248618
		Phpu16Sa-86	MN244003	MN248611
		Phpu16Sa-87	MN243982	MN248629
		Phpu16Sa-90	-	MN248630
		Phpu16Sa-91	MN243984	MN248631
		Phpu16Sa-92	MN243967	MN248592
		Phpu16Sa-94	-	MN248603
		Phpu16Sa-95	MN244004	MN248612
		Phli16Sa-4	MN243976	MN248623
		Phli16Sa-7	MN244013	MN248633
	Phyllidiella nigra (van Hasselt, 1824)	Phpu16Sa-64	-	-
		Phfi16Sa-1	MN244067	MN248643
		Phfi16Sa-2	MN244068	MN248644
		Phpu16Sa-19	-	MN248652
		Phpu16Sa-47	MN244076	MN248654
		Phpu16Sa-54	MN244077	MN248653
		Phpu16Sa-57	MN244071	MN248651
		Phpu16Sa-58	MN244074	MN248650
	Phyllidiopsis krempfi (Pruvot-Fol, 1993)	Phpu16Sa-65	MN244072	MN248649
		Phpu16Sa-66	MN244073	MN248647
		Phpu16Sa-67	MN244069	MN248645
		Phpu16Sa-72	MN244070	MN248646
		Phpu16Sa-82	-	MN248658
		Phpu16Sa-83	MN244080	MN248657
		Phpu16Sa-88	MN244075	MN248646
		Phpu16Sa-93	MN244078	MN248655
	Phyllidiopsis shireenae (Brunckhorst, 1990)	Phsh16Sa-2	MN244082	MN248659

Figure 3. Anthobranchia and Cladobranchia/Aeolidioidea: (**A**) *Hypselodoris tryoni,* Goku16Sa-1; (**B**) *Phyllidia ocellata,* Phoc16Sa-3; (**C**) *Phyllidia picta,* Phpic_16Sa-13; (**D**) *Phyllidia* spec., Phsp3_16Sa-1; (**E**) *Phyllidia madangensis,* Phma16Sa-1; (**F**). *Phyllidia coelestis,* Phco16Sa-1; (**G**) *Phyllidia varicosa,* Phva16Sa-6; (**H**) *Phyllidiella lizae,* Phli16Sa-4; (**I**) *Phyllidiella pustulosa* complex, Phpu16Sa-29; (**J**) *Phyllidiella pustulosa* complex, Phpu16Sa-91; (**K**) *Phyllidiella pustulosa* complex, Phpu16Sa-95; (**L**) *Phyllidiella nigra,* Phpu 16Sa-64; (**M**) *Phyllidiopsis krempfi* Phpu16Sa-58; (**N**) *Phyllidiopsis shireenae,* Phsh16Sa-2; (**O**) Aeolidioidea Flsp16Sa-1.

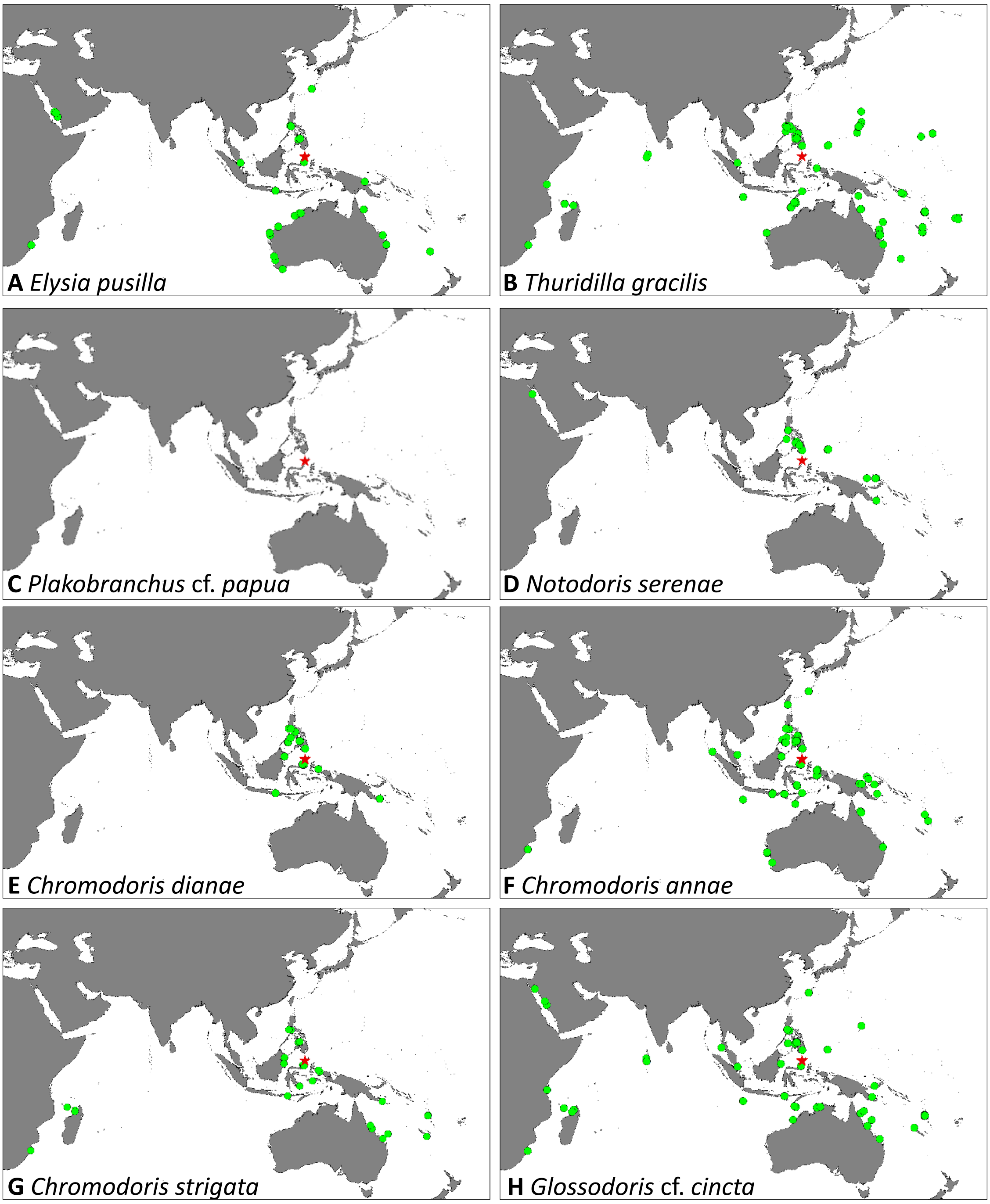

Figure 4. Distribution data of respective species in the Indo-Pacific Ocean. Data from this study (Sangihe) and downloaded from GBIF. (**A**) *Elysia pusilla* [36]; (**B**) *Thuridilla gracilis* [37]; (**C**) *Plakobranchus* cf. *papua* (no data in GBIF available yet); (**D**) *Notodoris serenae* [38]; (**E**) *Chromodoris dianae* [39]; (**F**) *Chromodoris annae* [40]; (**G**) *Chromodoris strigata* [41]; (**H**) *Glossodoris* cf. *cincta* [42].

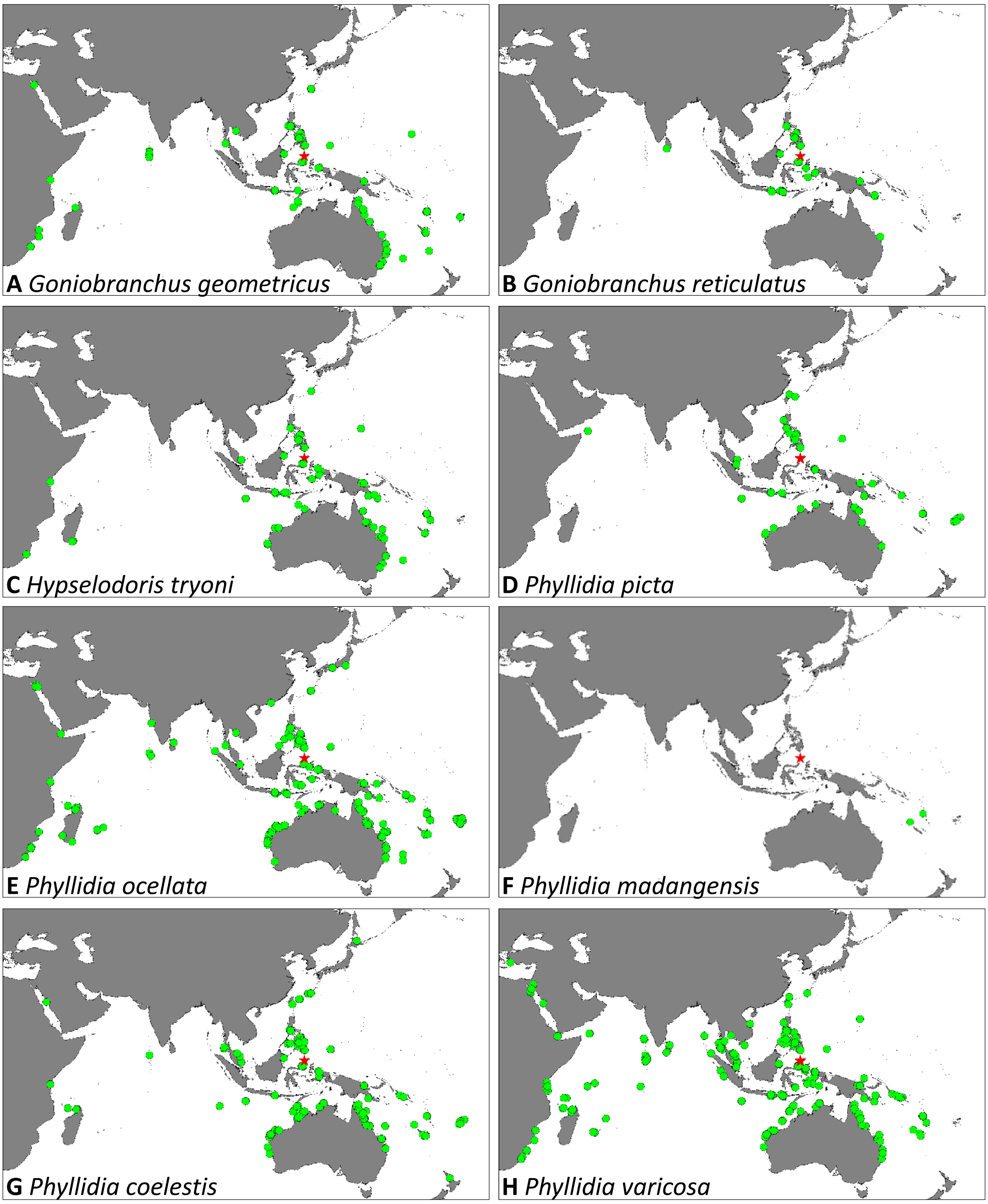

Figure 5. Distribution data of respective species in the Indo-Pacific Ocean. Data from this study (Sangihe) and downloaded from GBIF. (**A**) *Goniobranchus geometricus* [43]; *(***B**) *Goniobranchus reticulatus* [44]; (**C**) *Hypselodoris tryoni* [45]; (**D**) *Phyllidia ocellata* [46]; (**E**) *Phyllidia picta* [47]; (**F**) *Phyllidia madangensis* [48]; (**G**) *Phyllidia coelestis* [49]; (**H**) *Phyllidia varicosa* [50].

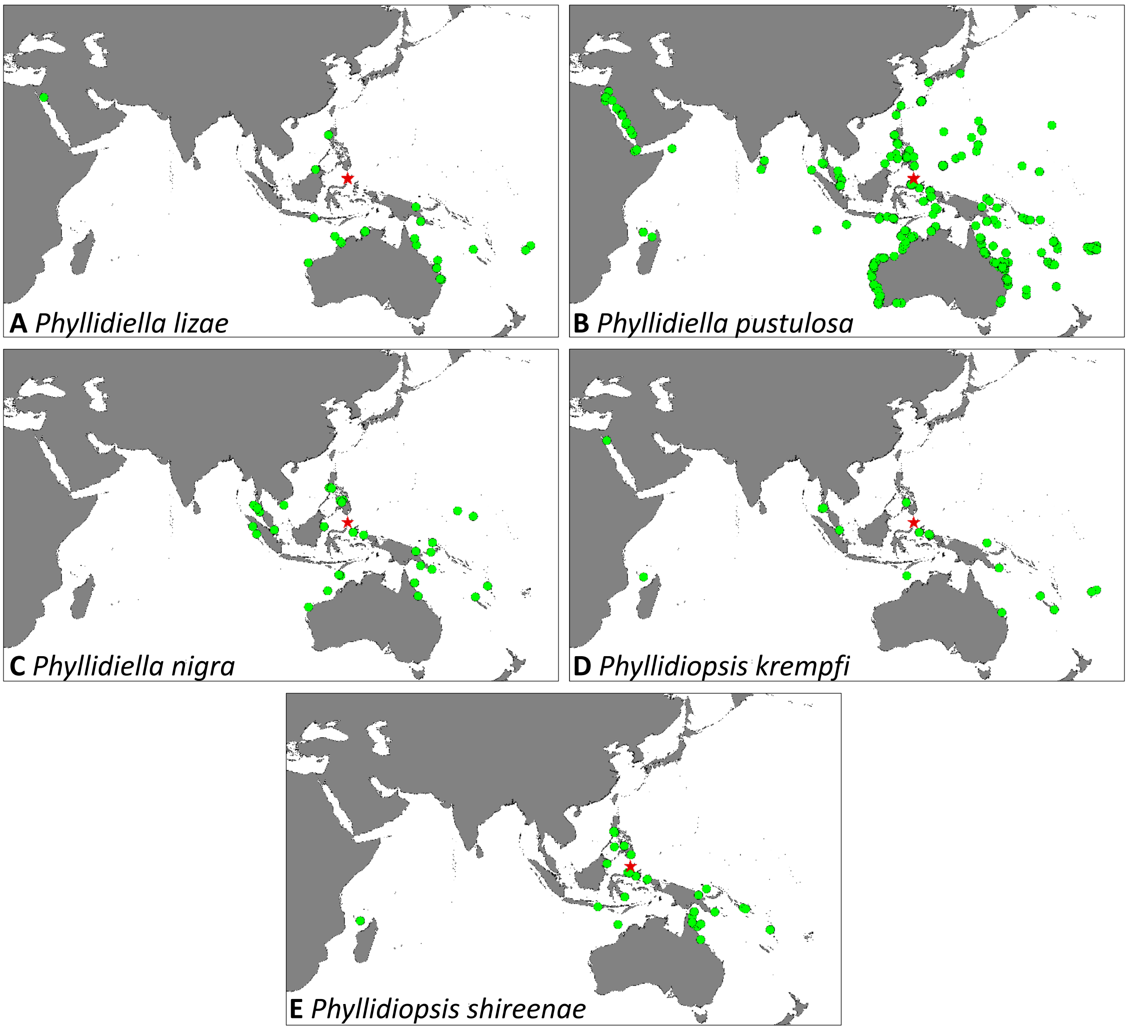

Figure 6. Distribution data of respective species in the Indo-Pacific Ocean. Data from this study (Sangihe) and downloaded from GBIF. (**A**) *Phyllidiella lizae* [51]; (**B**) *Phyllidiella pustulosa* [52]; (**C**) *Phyllidiella nigra* [53]; (**D**) *Phyllidiopsis krempfi* [54]; (**N**) *Phyllidiopsis shireenae* [55].

Systematics
HETEROBRANCHIA
SACOGLOSSA
PLAKOBRANCHOIDEA
Family: Plakobranchidae Gray, 1840
Elysia, Risso, 1818
Elysia pusilla, Bergh, 1871 (Figures 2A and 4A, Table 2)

Description

Three specimens of *Elysia pusilla* with lengths of 2–6 mm were collected from Mendaku and Tahuna Bay South (Figure 2A). All three specimens had the typical green coloration with whitish rhinophores.

Remarks

One specimen from Mendaku was crawling out of a patch of the chlorophyte *Caulerpa racemosa*; another one at the same locality was sitting on a *Halimeda* species with small thalli. The third specimen from Tahuna Bay South was associated with *Halimeda* cf. *macroloba*. The species is widely distributed in the Indo-Pacific Ocean (Figure 4A).

Thuridilla, Bergh, 1872
Thuridilla gracilis, Risbec, 1928 (Figures 2B and 4B, Table 2)

Description

Six specimens with lengths of 20–30 mm were collected in front of Palahanaeng village (3 specimens), Talengen village (1 specimen), and in Sapaeng (2 specimens). All specimens show the typical black background and whitish to light green colored fine longitudinal lines. The anterior part of the foot, the tips of the rhinophores, and the tip of the tail show the typical orange color, but the orange rim of the parapodia is only very narrow. No distinct blue spots, which are described from some specimens of the form *bayeri*, are visible.

Remarks

One specimen from Sapaeng was observed on an algae looking very similar to *Dictyota*, the other from the same locality and one animal from Palahanaeng village were sitting close to the same algal species. The specimen from Talengen village was crawling on the base of small *Halimeda* thalli. The remaining two specimens were crawling on unspecified sediment. The species is widely distributed in the Indian and Western Pacific Ocean (Figure 4B).

Plakobranchus, van Hasselt, 1824
Plakobranchus cf. *papua*, Meyers-Muñoz and van der Velde, 2016 (Figures 2C and 4C, Table 2)

Description

Two specimens of *Plakobranchus* cf. *papua* with lengths of 25 and 30 mm were collected in Tahuna Bay South and Mendaku at depths of 5 and 15 m. Our animals show yellowish to white spots of various sizes arranged in a distinct pattern on a darker olive to green background. Our animals differ from the animals described and depicted by Meyers-Muñoz et al. [56] in so far as that they exhibit more spots and thus appeared lighter in color than the animals described from West Papua, Indonesia. However, our animals match with regard to the rhinophores, which are nearly completely violet in color.

Remarks

Recently, Yonow and Jensen [57] reviewed and discussed the complicated situation within the genus *Plakobranchus* with at least 14 species described from the Pacific Ocean. Many species have never been found again; descriptions were poor, rendering assignment of new material very difficult. The authors depict two specimens, one from Ambon, one from Malaysia, assigned tentatively to *P.* cf. *papua*. They look very similar to our specimens, especially in the number of spots and the arrangement of these. Eisenbarth et al. [14] assigned their specimens from Bunaken Island to *P. ocellatus*. In contrast to our specimens which were collected in depths of 5 and 15 m, the animals collected in BNP lived in the eulittoral. Both animals from our collection were crawling on sediment surrounded by various species of algae. No further distribution records are listed in GBIF (Figure 4C).

NUDIBRANCHIA
DORIDINA
Family: Aegiridae P. Fischer, 1883
Notodoris Bergh, 1875
Notodoris serenae Gosliner and Behrens, 1997 (Figures 2D and 4D, Table 2)

Description

Two specimens of *Notodoris serenae* with lengths of 60 and 90 mm were collected in Manalu at depths of 24 and 27 m. They show the same typical coloration as depicted in Kaligis et al. [13].

Remarks

Only *Notodoris serenae* from the family Aegiridae, which usually feeds on hexactinellid sponges, was collected during the present survey. Both animals were crawling on sediment. The species is mainly known from the Coral Triangle (Figure 4D).

Family: Chromodorididae Bergh, 1891
Chromodoris Alder and Hancock, 1855
Chromodoris dianae Gosliner and Behrens, 1998 (Figures 2E and 4E, Table 2)

Description

Seven specimens of *Chromodoris dianae* with lengths of 5–45 mm were collected in Manalu (1 specimen) and Mendaku (6 specimens) at depths of 15–27 m. The body is elongate and the color of this species is white with a tinge of blue and a pattern of distinct interrupted black lines and spots. The rhinophores are yellowish to orange, whereas the gills are white with yellow tips.

Remarks

Gosliner and Behrens [58] mentioned in their first description the similarity in color with *Chromodoris quadricolor* (Rüppell and Leuckart, 1830), another pale blue chromodorid. However, *C. quadricolor* has an orange marginal band. Our *Chromodoris dianae* specimens are very similar to those depicted in Yonow [27] and Kaligis et al. [13]. Our specimens were also mainly collected from sponges. The mantle glands of *C. dianae,* which can be seen clearly in the live animal, are well separated from each other and are highly ramified with digitate branches. Species records are mainly confined to the Coral Triangle (Figure 4E).

Chromodoris annae Bergh 1877 (Figure 2F,G and Figure 4F, Table 2)

Description

Thirteen specimens of *Chromodoris annae* with lengths of 8–41 mm were collected in Manalu, Palahanaeng village, Mendaku, Sapaeng, and Talengen village at depths of 5–23 m (Table 2). Our specimens show the typical blue color with darker miniature spots. They are lacking a mid-dorsal longitudinal line and any small black dots within the blue areas. The rhinophores exhibit the typical yellow color. However, one specimen shows differences in coloration by exhibiting a lighter blue and an interrupted black line in the middle.

Remarks

Some *Chromodoris* species are difficult to distinguish by color only [13]. *Chromodoris elisabethina* Bergh, 1877 looks similar to *Chromodoris annae*, but *C. annae* usually does not have a median black line and the blue areas of the mantle are not uniform blue as is the case of *C. elisabethina* (Rudman, 1982). However, we collected one animal in front of Talengen village (Figure 2G) which is quite similar to *C. elisabethina*: the specimen shows the usual elongate bluish body with the mantle margin encircled by a black, a white, and finally a yellow band. However, the animal mimicking *C. elisabethina* had additionally a medially lying black line, which was interrupted several times. Barcoding and comparison with our unpublished sequences, and the few available from NCBI, clearly indicate its correct assignment to *C. annae*, and therefore provides here the first example of mimicry involving *C. annae* and *C. elisabethina*. Mimicry forms between members of the Phyllidiidae and Chromodorididae are depicted in several identification books [19,20], and described in Cheney et al. [59] and Padula et al. [60]. However, mimicry between closely related *Chromodoris* species was described in a broader context for the first time only recently [61]. This is the first example of *Chromodoris annae* mimicking *C. elisabethina*. The species is widely distributed in the Indo-Pacific Ocean, including subtropical areas (Figure 4F).

Chromodoris strigata Rudman, 1982 (Figures 2H and 4G, Table 2)

Description

Only one specimen of *Chromodoris strigata* with a length of 10 mm was collected in Mendaku at a depth of 15 m. The mantle of this specimen shows a white background with bluish tinges. The gills and rhinophores are the same yellow to orange as the mantle border. The yellow band along the mantle rim is interrupted.

Remarks

Although having similarities to many bluish to white *Chromodoris* species, *C. strigata* is easily recognised in this color group by the fading blue on white background as well as the areas of light yellow to white in the yellow mantle rim. This renders the species paler than other species [21]. Its distribution is recorded from the Indo-Pacific Ocean (Figure 4G).

Glossodoris Ehrenberg, 1831
Glossodoris cf. *cincta* (Bergh, 1888) (Figures 2I and 4H, Table 2)

Description

Two specimens of *Glossodoris* cf. *cincta* with lengths of 21 and 48 mm were collected in Manalu and Mendaku at depths of 8 and 13 m. The animals show an elongate to oval shape with mottled reddish brown and white on the notum. The gills and rhinophores are brown.

Remarks

Nudibranchs of the genus *Glossodoris* are moderately large and easily spotted. They are widely distributed in tropical and temperate reef environments around the world [23,62]. Most recently several new species with similar color patterns to *G. cincta* were described [63,64]. *Doriprismatica kyanomarginata* Yonow, 2018 differs from our specimen by having a diffuse inner yellow ribbon at the mantle margin, which is characteristic for this new species. Our animal is very close in coloration to *Glossodoris acosti* Matsuda and Gosliner, 2018. Especially the coloration of the mantle margin with a light blue outermost ring, followed by a dark green and then a lighter yellow-green ring is very similar in both species. However, the rings are wider in *G. acosti* and furthermore, the gills are mentioned to be larger, forming an arch opening to the posterior and with two distinct spirals. Our animal had all gill branches on one level and the arrangement was forming a complete circle. It thus resembles the animal depicted as *Glossodoris* cf. *cincta* in Matsuda and Gosliner [63]. Bergh [65] in his original description of *G. cincta* mentioned dark brown rhinophores with white dots and the gills with six larger branches in the anterior part, followed by eight smaller ones on each side in the posterior part of the circle. Thus, our specimen also differs from the original description. We therefore only tentatively assign our animal to *Glossodoris cincta*. The specimen was collected from a brownish sponge. According to GBIF data, *Glossodoris cincta* shows a broad distribution from the Red Sea until Fiji Islands (Figure 4H). However, difficulties in correct identification probably blur the correct distribution area.

Goniobranchus Pease, 1866
Goniobranchus geometricus (Risbec, 1928) (Figures 2J and 5A, Table 2)

Description

Four specimens of *Goniobranchus geometricus* with lengths of 10–15 mm were collected in Tahuna Bay South (1 specimen), Palahanaeng village (1 specimen), and Sapaeng (2 specimens) at depths of 6–19 m. Our specimens are rose colored with opaque white tubercles and a network of thick black lines in between the tubercles. The mantle rim is whitish. The translucent white gills and rhinophores have bright green to yellow tips.

Remarks

The color pattern of *Goniobranchus geometricus* from Sangihe is very similar to that depicted in various identification books, and is also shown by Yonow [27], Kaligis et al. [13], and Eisenbarth et al. [14].

The slug usually can be found under stones or coral rubble [20], where we also found our animals. The species is widely distributed in the Indo-Pacific Ocean (Figure 5A).

Goniobranchus reticulatus (Quoy and Gaymard, 1832) (Figures 2K and 5B, Table 2)

Description

Two specimens of *Goniobranchus reticulatus* with lengths of 25 and 55 mm were collected in Sapaeng at depths of 6 and 9 m. The specimens show an elongate body with a reticulated network of red lines over the surface mantle. The mantle rim exhibits a narrow white area. The rhinophores are white with red tips. The gills are reddish with the inner rachis opaque white.

Remarks

Our specimen is very similar to the animals depicted by Kaligis et al. [13] and Eisenbarth et al. [14], which were also identified as *G. reticulatus*. Barcoding and comparison with our unpublished sequences, and the few available from NCBI, indicate its correct assignment to *G. reticulatus*. However, Yonow [27] discussed *Chromodoris inopinata* Bergh, 1905 as a very common form in the Indo-Pacific and probably often misidentified as *G. reticulatus*. *C. inopinata* shows very similar color patterns as *G. reticulatus*. No CO1 sequences assigned to *C. inopinata* are available at NCBI GenBank yet. The records in GBIF show a more limited distribution than is known from *G. geometricus*, with findings mainly from the Coral Triangle (Figure 5B).

Hypselodoris Stimpson, 1855
Hypselodoris tryoni (Garrett, 1873) (Figures 3A and 5C, Table 2)

Description

Three specimens of *Hypselodoris tryoni* with lengths of 25–60 mm were collected in Sapaeng at depths of 10–16 m. The specimens show a cream to dirty brown mantle with bluish to dark violet spots. These spots are surrounded by a ring of white pigment and then a paler area. The rim of the mantle is purple. The gill and rhinophores are translucent white with the rachis of the gills brownish and the rachis of the rhinophores purple.

Remarks

Hypselodoris tryoni, *Goniobranchus leopardus* (Rudman, 1987), and *Goniobranchus cavae* (Eliot, 1904) are very similar in external appearance, exhibiting a dark cream background color with dark violet round patches surrounded by a light colored area. Additionally, *G. cavae* can be highly variable in color [30,66]. However, in *G. cavae* the gills and rhinophores are white with usually purple tips, whereas in *H. tryoni*, the rachis of the gills and rhinophores shows a purple coloration throughout the full length and the tips of the rhinophores are not distinctively purple. The species has a wide distribution in the Indo-Pacific Ocean with many records also from the subtropics (Figure 5C).

Family: Phyllidiidae Rafinesque, 1814
Phyllidia, Cuvier, 1797
Phyllidia ocellata Cuvier, 1804 (Figures 3B and 5D, Table 2)

Description

Seven specimens of *Phyllidia ocellata* with lengths of 18–35 mm were collected in Tahuna Bay South (2 specimens), Manalu (2 specimens), Palahanaeng village (1 specimen), and Sapaeng (2 specimens) at depths of 4–18 m. All our animals exhibit the typical yellow coloration with white tubercles, some of which are surrounded by black circles, followed by a thin white line. All other white tubercles are sticking out of the orange background color.

Remarks

Phyllidia ocellata with the yellow to orange background and the tubercles surrounded by black rings is unique in its coloration and therefore cannot be confused with any other *Phyllidia* species. Gosliner et al. [20] depicted color morphs that lack white tubercles, which were not found during the present study. The species is very common in the Indo-Pacific with a range into subtropics of Australia (Figure 5E).

Phyllidia picta Pruvot-Fol, 1957 (Figures 3C and 5E, Table 2)

Description

Twenty-one specimens of *Phyllidia picta* with lengths between 13 and 30 mm were collected in Tahuna Bay South (6 specimens), Manalu (3 specimens), Palahanaeng village (2 specimens), Mendaku (2 specimens), Sapaeng (6 specimens), and Talengen village (2 specimens) at depths of 1–15 m. All of our animals show an oval shape, with black reticulate pattern and single yellow tubercles on a blue background. The rhinophores are yellow and the foot sole has no black stripe.

Remarks

Brunckhorst [67] considered *Phyllidia picta* to be a junior synonym of *P. coelestis*, but Yonow [26] and Stoffels et al. [68] confirmed its validity. The species is not recorded from Bunaken National Park [13,14] but was reported from Ambon [29] and is also recorded in GBIF from few other places in Indonesia down to Australia (Figure 5D).

Phyllidia spec. (Figure 3D, Table 2)

Description

Figure 3D exhibits an unidentified *Phyllidia* specimen with a length of 28 mm. It was found only once in Talengen village. This specimen has an elongate to oval shape with greenish to greyish background and black lines between tubercles arranged in ridges. Tubercles are single rather than compound. The rhinophores are yellow. The foot sole shows a black line as is typical for *Phyllidia elegans* Bergh, 1869, to which it is very similar.

Remarks

The specimen cannot be assigned to any described species. Genetic information indicates no relationship to *P. elegans*, but to *Phyllidia picta*; however, therefore its assignment to the genus *Phyllidia* is confirmed. The specimen of the undescribed *Phyllidia* species depicted by Eisenbarth et al. [14] looks very different from ours.

Phyllidia madangensis Brunckhorst, 1993 (Figures 3E and 5F, Table 2)

Description

Phyllidia madangensis was collected in front of Talengen village with one specimen with a length of 28 mm. Our animal shows the typical features, the lack of the dark stripe on the foot sole and its overall blackish color. Few white tubercles capped in bright yellow are scattered over the notum. The rhinophores are dark yellow.

Remarks

Phyllidia madangensis is very similar to *P. carlsonhoffi* Brunckhorst, 1993; however, our animal has smaller tubercles and is more blackish than *P. carlsonhoffi*, as is depicted by e.g., Gosliner et al. [20]. Rudman [69] illustrated a specimen of *P. madangensis* with whitish tubercles, similar to our specimen, whereas the tubercles of some *P. carlsonhoffi* can be more bluish. *Phyllidia carlsonhoffi* also has tubercles more evenly distributed over the notum, whereas *P. madangensis* has sparsely scattered tubercles. Brunckhorst [67] described rhinophoral tubercles to occur in all *Phyllidia* species, but the presence of a small tubercle directly in front of each rhinophoral pocket appears to be unique to *P. madangensis*.

Our specimen did not really show this tubercle. However, the overall appearance and the coloration allow the assignment to *P. madangensis*, which is a rather rare species (Figure 5F).

Phyllidia coelestis Bergh, 1905 (Figures 3F and 5G, Table 2)

Description

Nine specimens of *Phyllidia coelestis* with lengths of 7–32 mm were collected in Tahuna Bay South (3 specimens), Manalu (1 specimen), Palahanaeng village (1 specimen), Sapaeng (1 specimen), and Talengen village (3 specimens). The specimens display the typical background blue color with three black lines. The line in the middle is interrupted by few single yellow tubercles, whereas the outer two lines run lateral to the smaller yellow tubercles. The rhinophores are yellow.

Remarks

Phyllidia coelestis is a smaller and widely distributed species (Figure 5G), which has neither a foot stripe nor a median tuberculate ridge. The species can be distinguished from other similar looking phyllidiids, such as *P. varicosa,* by the central black stripe on the notum, interrupted by large yellow tubercles. Additionally, it has a characteristic black Y-shaped pattern between and in front of the rhinophores. Brunckhorst [70] and Yonow [29] mentioned a dark form that has a central oval region where the ground color is black and only a marginal band around it depicts the bluish-white color.

Phyllidia varicosa, Lamarck, 1801 (Figures 3G and 5H, Table 2)

Description

Fifty-eight specimens with lengths of 7–87 mm were collected at all sampling sites, except ship wreck, at depths of 3–15 m. All specimens show a light blue background with yellow tubercles in rows and blackish lines between these tubercle ridges. The rhinophores are yellowish.

Remarks

Phyllidia varicosa is a large species that can be distinguished by its black stripe at the foot sole, which is absent in most *Phyllidia* species. It has three to six longitudinal, tuberculate notal ridges [67]. Our animals are quite similar to this description, with an elongate to oval shape, the yellow rhinophores, and the black stripe along the foot sole. The species is very common in the Indo-Pacific Ocean and also occurs in the subtropics of Australia (Figure 5H).

Phyllidiella, Bergh 1869
Phyllidiella lizae Brunckhorst, 1993 (Figures 3H and 6A, Table 2)

Description

Twelve specimens with lengths of 6–68 mm were assigned preliminarily to *Phyllidiella lizae*. They were collected in Tahuna Bay South (3 specimens), Manalu (3 specimens), Palahanaeng village (1 specimen), Mendaku (3 specimens), and Talengen village (2 specimens). All specimens show the pale pink background, pale pink tubercles and irregular, narrow black lines on the dorsum like a pale 'x'. The rhinophores are black with pink at the base.

Remarks

Brunckhorst [67] stated that *Phyllidiella lizae* is recognizable by its pale pink notum with simple, rounded, pale pink tubercles and narrow black lines crossing the dorsum. The rhinophores are black at the tip, pink in the central area, and white at the base. Other distinguishing characters are the pale, pinkish white oral tentacles and foot sole. Our animals match this description, except that the rhinophores are more pinkish at the base, instead of white. However, molecular data indicate cryptic speciation (A.P. unpublished data). Records in GBIF are confined to the coral triangle and the northern parts of Australia (Figure 6A).

Phyllidiella pustulosa (Cuvier, 1804) (Figure 3I–K and Figure 6B, Table 2)

Description

In this study, 77 specimens of *Phyllidiella pustulosa* with lengths of 12–68 mm were found at all sampling sites in Sangihe in depths of 1–23 m. Our animals have elongate bodies, and diverse color variations; from reddish to pink or even green tubercles surrounded by black lines.

Remarks

Stoffels et al. [68] described *Phyllidiella pustulosa* with a high intraspecific variation and cryptic speciation, based on molecular analyses. Already Brunckhorst [67] stated that ontogenetic variation also might have contributed to the confusion in the literature. Burghardt et al. [12] assigned one specimen to *Phyllidiella nigra,* which actually looks very similar to *P. pustulosa.* In our collection, *P. pustulosa* is the species with the highest number of color-morphs and our own unpublished molecular data confirm cryptic speciation. Thus, the broad distribution data in GBIF in the tropic and subtropic Indo-Pacific Ocean probably reflect the distribution of several cryptic species (Figure 6B).

Phyllidiella nigra (van Hasselt, 1824) (Figures 3L and 6C, Table 2)

Description

One specimen of *Phyllidiella nigra* with a length of 29 mm was collected in Sapaeng. This specimen has an elongate body, and its overall color appears blackish with pinkish to brownish tubercles. The tubercles are evenly scattered and not arranged in rows, however they cluster together, as typical for *Phyllidiella pustulosa*. The rhinophores are black.

Remarks

Brunckhorst [67] distinguished *Phyllidiella nigra* from conspecifics by its tall, rounded, dark pink to red tubercles, which are evenly distributed (not clustered) over the dorsum (e.g., specimens from Ambon in Yonow [29]). Stoffels et al. [68] already depicted several specimens with tubercles clustering and surrounded by black patterns. In our study, *P. nigra* appears blackish with darker pinkish tubercles, but the overall appearance is quite similar to the *P. nigra* specimens depicted in Stoffels et al. [68]. Our genetic analyses group this specimen with published sequences also assigned to *P. nigra*; however, the quality of our sequence is poor and needs repetition. The species is mainly recorded from the Coral Triangle and Northern Australia (Figure 6C).

Phyllidiopsis Bergh, 1876

Phyllidiopsis krempfi Pruvot-Fol, 1957 (Figures 3M and 6D, Table 2)

Description

Fifteen specimens of *Phyllidiopsis krempfi* with lengths of 14–50 mm were collected in just one locality, Palahanaeng village, at depths of 13–16 m. The oral tentacles are fused, as is typical for the genus. The animals are elongate to oval and have two black lines on the dorsum extending around the rhinophores, meeting in front of the rhinophores. Additionally, black lines run from these longitudinal lines perpendicularly toward the notum margin, similar to the patterns depicted by Stoffels et al. [68]. The color of our animals varies from reddish (Figure 3L) to pale pink. The rhinophores are black.

Remarks

Phyllidiopsis krempfi is characterised by a predominantly pink coloration and wide shape [67]. *Phyllidiopsis gemmata* (Pruvot-Fol, 1957) is very similar to *P. krempfi*, but Tibiriçá et al. [66] described characteristic differences. *Phyllidiopsis krempfi* has pink rhinophores with only the apical part in black, while *P. gemmata* has mainly black rhinophores with only the base pinkish [67]. Our animals therefore more resemble *P. gemmata*. However, the tubercles are simple in *P. gemmata*, while they are compound in *P. krempfi* [67], as this is the case in our animals. *P. gemmata* is also mentioned to be more elongate than

P. krempfi. This character is difficult to distinguish, when no other material is available for comparison. Molecular data confirm the assignment to *P. krempfi* and indicate a higher color variation as previously described. Records in GBIF are rare (Figure 6D) but reach from the Red Sea to Fiji Islands.

Phyllidiopsis shireenae Brunckhorst, 1990 (Figures 3N and 6E, Table 2)

Description

Two specimens of *Phyllidiopsis shireenae* with a length of 77 and 81 mm were collected in Manalu and Sapaeng at depth of 8 and 15 m. The body is elongate to oval, with a typical longitudinal mid-dorsal ridge, which is covered with large whitish tubercles. The body color is white with opaque white spots and a typical black lining. The foot is also white. The rhinophores are salmon pink.

Remarks

The specimens are very similar to the one depicted by Stoffels et al. [68] from the northern Moluccas and from Ambon [29]. Ours have the two black transversal lines connecting the longitudinal stripes in common with them. One of our specimens shows a black dot in the middle of the white ridge, similar to the animal depicted by Gosliner et al. [20]. Brunckhorst [67] considered the mid-dorsal crest as the characteristic feature of *Phyllidiopsis shireenae*, which is lacking in most other *Phyllidiopsis* species. Another characteristic is the salmon pink rhinophores. *Phyllidiopsis pipeki* Brunckhorst, 1993, *Phyllidiopsis burni* Brunckhorst, 1993, and *Phyllidiopsis fissuratus* Brunckhorst, 1993 differ from *P. shireenae* in having large compound tubercles, black or pale pink rhinophores, and pink to grey ventral coloration (white in *P. shireenae*) [67]. The species is mainly distributed from the Coral Triangle to Northern Australia (Figure 6E).

Aeolidioidea spec. (Figure 3O, Table 2)

Description

A tiny aeolidid species, probably a juvenile, with a length of 1 mm was collected in Mendaku at 1 m depth. The animal (Figure 3O) is whitish with orange rhinophores and with orange to opaque white cerata. The rhinophores showed irregular swellings or rings. Oral tentacles are short.

Remarks

There are many members of the Aeolidioidea with similar rhinophores, and similar cerata shape and arrangement, but overall habitus resembles probably most a *Flabellina* species. Proper identification will need barcoding methods, resulting in the complete loss of this specimen for further investigation.

4. Discussion

This is the first study describing the diversity of marine Heterobranchia around the island of Sangihe, Sangihe Islands Regency, North Sulawesi Province. Collecting at different locations (Tahuna Bay South, Ship Wreck, Mendaku, and Manalu, on the eastern coastline; in front of the villages of Palahanaeng and Talengen, and Sapaeng, on the western coastline) ensured the cover of differing habitats and degrees of exposure. Strong currents did not allow extensive sampling in many exposed areas, especially at the outer reef areas and drop offs. This first record is based on a high number of specimens (250), which can be assigned to 23 species (Table 2). The species number cannot compare with the higher numbers of other recent studies at North Sulawesi (Figure 7) [13,14], which might be due to several factors. Collecting time was lower than in BNP, but the differences in habitats were more pronounced. We observed a high sedimentation rate in the water column, resulting in many organisms (sponges, corals, and algae) being covered by a thin layer of silt or mud. This is probably caused by unusually heavy rainfall in this particular season and/or the higher impact of many small river systems close to the collection areas.

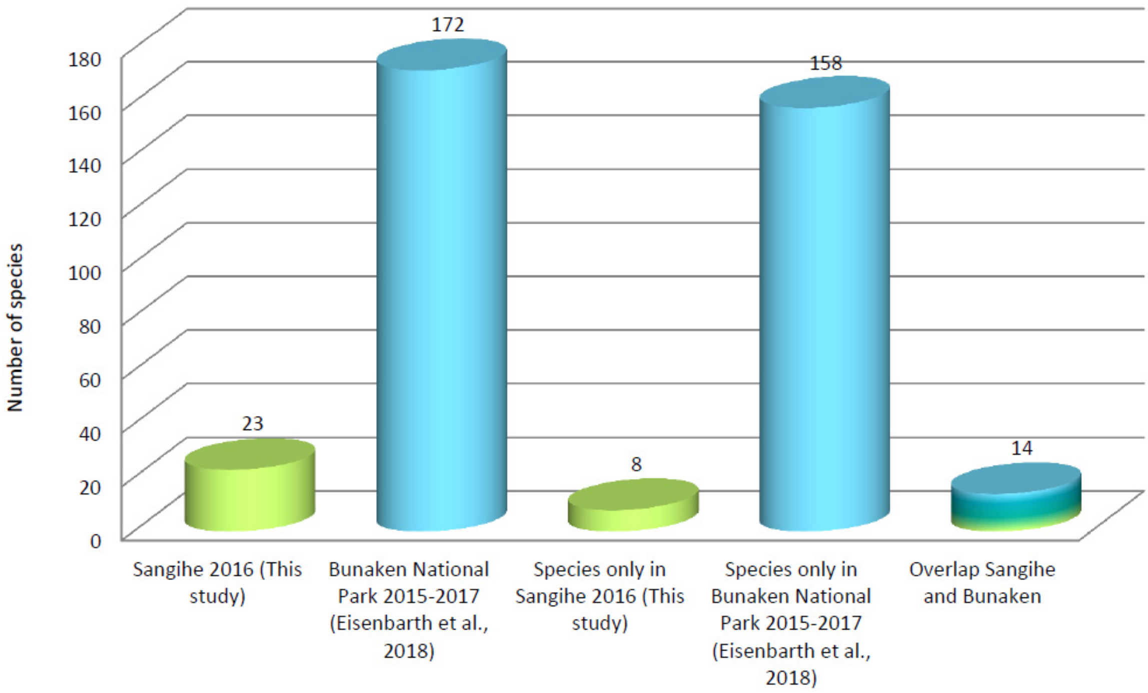

Figure 7. Comparison of species diversity in this study (Sangihe) with (Bunaken National Park) [14]. Note that one-third of the species collected in Sangihe were not found during surveys in Bunaken National Park.

Figure 8 and Table 2 provide detailed information about numbers of species/specimens found at the various collection localities around Sangihe. Collection time and effort were similar for all localities. The highest number of sea slug species was found at Sapaeng (13 species, 60 specimens), followed by Tahuna Bay South (11 species, 58 specimens), Palahanaeng village (11 species, 42 specimens), Manalu (11 species, 29 specimens), Mendaku (10 species, 22 specimens), and Talengen village (9 species, 35 specimens). The lowest overall species number was recorded on the Ship Wreck (1 species, 4 specimens), a locality highly influenced by Tahuna harbor and the city of Tahuna. Members of the Anthobranchia (with 238 specimens assigned to 19 species) were present in all seven localities, followed by sacoglossans (11 specimens assigned to 3 species), present in five localities. The Cladobranchia was represented by only one specimen, an unidentified member of the Aeolidioidea.

Recent studies have shown that several species represent cryptic species complexes, while species treated earlier as different taxa are simply color-variants of the same species [60,61,66,71–75]. With regard to our listed taxa, cryptic speciation has been recorded for the genus *Plakobranchus* [57]. We did not barcode our specimens, but the color patterns allow the tentative assignment to *P. papua*. We can confirm that *Phyllidiella pustulosa* is a species complex with similarly colored species or subspecies [68]. Therefore, our animals are tentatively assigned to this species, although they group within different clades (unpublished data; see also Stoffels et al. [68]). Color variation and mimicry appear quite common in Chromodorididae (e.g., Cheney et al. [59], Padula et al. [60], Layton et al. [61], Johnson and Gosliner [62], Epstein et al. [76]). Thus, identification only by color might lead to errors, and therefore we barcoded these taxa to verify identification by including sequences from our specimens into a preliminary phylogenetic analysis of this family (unpublished data). We could therefore identify the first mimicry forms within the species *Chromodoris annae* exhibiting the color of *C. elisabethina*.

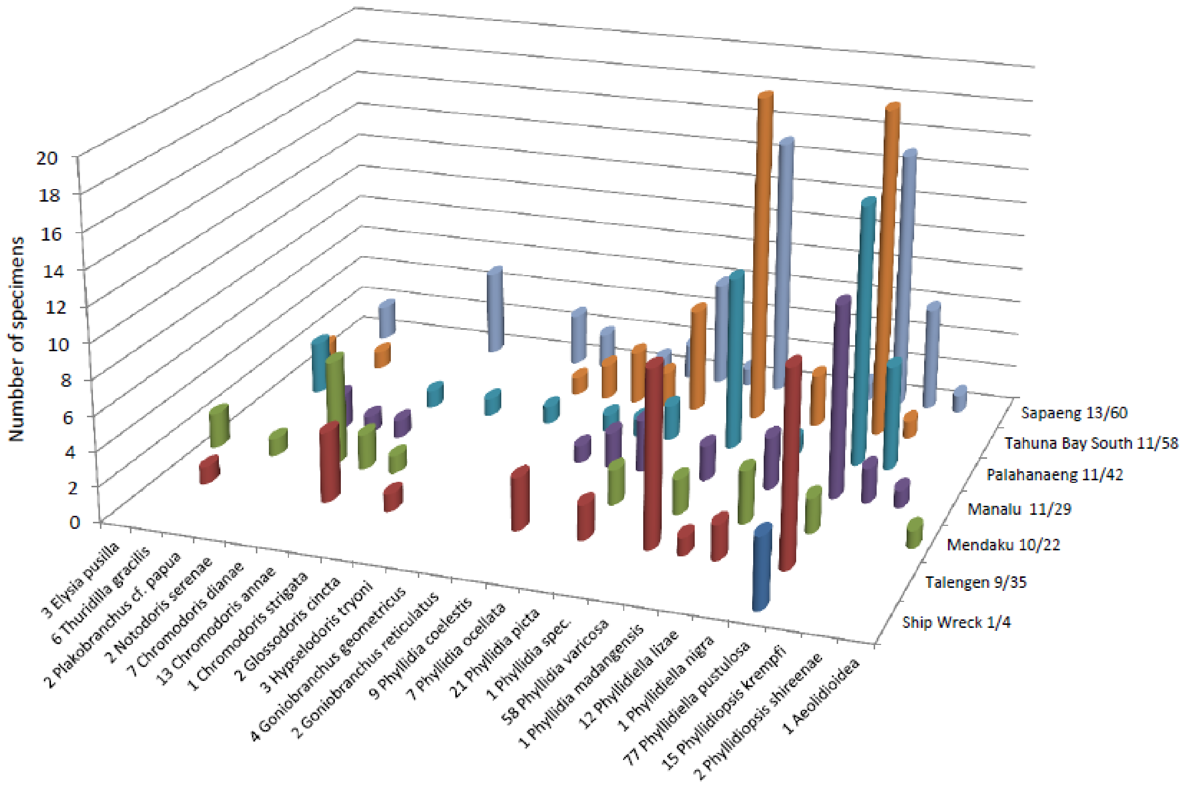

Figure 8. Comparison of marine Heterobranch species collected around the island Sangihe. The numbers in front of the species names indicate the number of collected specimens. The numbers after the locality names indicate the number of species collected, followed by the number of specimens.

Phyllidiidae show the highest dominance (three genera represented by 11 species, with 205 specimens) in our study. Of the five valid phyllidiid genera, *Reticulidia* and *Ceratophyllidia* were not present in our study. These genera are also not recorded from BNP, but *Reticulidia halgerda* Brunckhorst and Burn in Brunckhorst [50] was recorded from Ambon [29]. The second most commonly recorded group is the family Chromodorididae. Seventeen chromodoridid genera are recorded by WoRMS. In our study three genera are represented by nine species with 35 specimens; therefore, this family is not well represented in our collection. Only one further anthobranch family besides Chromodorididae was found, the hexactinellid sponge-feeding Aegiridae with *Notodoris serenae*. Thus, in total 19 anthobranch species are now recorded from Sangihe, in contrast to the 69 anthobranch species mentioned by Eisenbarth et al. [14] from BNP.

Interestingly, the number of cladobranchs with only one very tiny unidentified aeolidid species was extremely low, compared to other study areas close by, e.g., Ambon, Bali, Vietnam, Papua New Guinea, Taiwan, and Hong Kong (Table 4). According to these studies, usually one-quarter to one-third of collected nudibranchs comprise members of Cladobranchia (Table 4). A similar proportion of Anthobranchia to Cladobranchia as seen around Sangihe (20:1) was only recorded from Mauritius [77]. Eisenbarth et al. [14], covering the Bunaken National Park, mentioned 28 species of Aeolidioidea and in total 47 cladobranch species, compared to 69 anthobranch species (Table 4). The low cladobranch number around Sangihe might be explained by the more sheltered sampling localities with a dominance of algae and sponges, and no hydro-dynamically exposed areas so typical of outer reefs and necessary for their hydrozoan prey. The number of sacoglossan species (3) with 11 collected specimens is also rather low. However, our overall numbers are in line with other studies from Indonesia, which show the general dominance of Nudibranchia and particularly the Anthobranchia, versus all other marine heterobranch groups (Table 4). This is also consistent with the overall diversity in these different groups [78,79].

Table 4. Marine Heterobranch species records of several studies from the Indo-Pacific split into main taxa

	Acteonoidea	Cephalaspidea + Runcinacea	Anaspidea	Sacoglossa	Umbraculida	Pleurobranchomorpha	Anthobranchia	Cladobranchia	Total Species Number	References
Sangihe 2016	0	0	0	3	0	0	19	1	23	This Study
BNP 2015–2017	0	24	4	26	0	2	69	47	172	[14]
Ambon	0	11	6	12	0	4	90	15	138	[27,29,80]
Bali and Indonesia	3	12	7	11	0	9	128	35	205	[81]
Vietnam	0	11	7	6	1	6	95	25	151	[82]
Papua New Guinea	0	71	9	61	0	8	257	132	538	[83]
Taiwan	0	2	0	4	0	1	53	10	70	[84]
Hong Kong	0	0	0	0	0	0	40	14	54	[85]
Chagos Archipelago	0	2	1	2	0	0	30	6	41	[86]
Maldives	0	4	2	2	0	2	21	4	35	[25]
Marshall Islands	5	13	5	10	0	1	53	14	101	[87]
Lizard Island	4	28	6	21	0	4	66	29	158	[88]
Mauritius	0	5	5	0	0	2	22	1	35	[77]
Western Australia	7	22	12	21	2	6	115	31	215	[89]
Fiji Islands	10	30	6	26	1	6	127	45	251	[90]
New Caledonia	16	82	10	17	1	4	98	30	258	[91]
Heron Island	0	20	5	31	0	7	151	47	261	[92]
Red Sea	7	41	17	16	0	8	140	65	294	[28]
Great Barrier Reef	0	64	12	42	0	9	210	77	414	[92]
Lakshadweep Islands	1	6	5	9	0	4	27	8	60	[93]
New Caledonia	4	19	12	25	0	11	237	65	373	[94]
New South Wales	0	35	17	27	2	12	209	80	378	[95]
Tropical East Pacific	0	89	13	30	0	11	131	125	399	[96]

Comparing results from the collecting sites, a few species clearly dominate the various habitats: *Phyllidiella pustulosa* species complex (77 recorded specimens) was collected from all localities. The species has a high number of records (Figure 6B) which also indicates a very common distribution with high specimens' numbers; however, it has to be emphasized here that the map depicts actually a species complex with several cryptic species looking all very similar to *P. pustulosa*. The second most common species around Sangihe was *Phyllidia varicosa* (58), which is also very common in the Indo-Pacific (Figure 5H). *Phyllidia picta* (21) was also collected from all sites around Sangihe except Ship Wreck. *Phyllidiopsis krempfi* was found only at four sampling sites. With 15 specimens, it was quite common around Sangihe, but this species probably is not so commonly distributed in the Indo-Pacific (Figure 6C). It is also not recorded from BNP. *Chromodoris annae*, *Phyllidia coelestis*, and *Phyllidiella lizae* (13, 9, and 8 specimens respectively) were also found at only four sampling sites. *Phyllidia madangensis* seems to be very rare and our specimen probably represents the only record from Indonesia at the moment (Figure 5F).

In comparison to the study by Eisenbarth et al. [14] (2018) covering the Bunaken National Park (BNP) and including several collection periods between 2015 and 2017, the number of species is much lower (23 versus 172 species) (Figure 7). When including a former collection period in 2003 [12], the total species number increases to 215 in BNP. Interestingly, we collected seven species that are not yet recorded from BNP (Figure 7), despite the extensive studies around this area. Three of them were very common around Sangihe: *Phyllidia picta* (21 specimens), *Phyllidiella lizae* (12 specimens), and *Phyllidiopsis krempfi* (15 specimens). The other four were less common: *Phyllidia madangensis* (1 specimen), *Phyllidiella nigra* (1 specimen), *Phyllidiopsis shireenae* (2 specimens), and *Plakobranchus* cf. *papua* (2 specimens). An undescribed *Phyllidia* species was also collected, which is not recorded from BNP or any other locality. Since nothing can be said about the affiliation of the small aeolidid, the number might even be nine. Overlap of species when comparing these two areas in North Sulawesi was therefore less than 70%, despite the rather short distance of approximately 200 km.

By comparing our preliminary results on the largest island of the Sangihe Islands Regency, not only with the studies from North Sulawesi, but also with other studies from Indonesia and nearby countries, the overlap of species lies mainly in the most common phyllidiid species, including the *Phyllidiella pustulosa* complex and *Phyllidia varicosa*, as well as the chromodorid *Chromodoris annae*. Sangihe is still heavily under-sampled and more collecting events are necessary to better understand the marine Heterobranch fauna from this highly remote area. However, differences outlined here between species composition clearly show the distinctiveness of this region from other areas close by. With this first sampling period, we have created the first baseline for future biodiversity studies and monitoring projects, especially with regard to human activities.

Author Contributions: All authors except N.U. and A.P. were involved in collecting the animals. N.U., A.P., and H.W. analyzed the material; N.U. and H.W. wrote the manuscript and designed the figures; all other authors contributed with comments and corrections to the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare that they have no conflict of interests.

Availability of Data and Materials: The material was made available by the late Fontje Kaligis. Some material is used for further studies within the project funded by the Federal Ministry of Education and Research, Germany. Metadata of each individual is documented in the database Diversity Collection (Part of Diversity Workbench) using the data brokerage service of the German Federation for Biological Data (GFBio) [33]. Data are publicly available at www.gfbio.org for browsing and the archived data can be downloaded at https://doi.org/10.20363/heterobranchia-sangihe-prj-1.1. Photographs are available from Heike Wägele with copyright from Zoological Research Museum Alexander Koenig, Bonn. Material not used for further studies will be stored in the Sam Ratulangi University collection under the number SRU2016/01. Sequences are uploaded to NCBI.

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