Monitoring marine Heterobranchia in Lembeh Strait, North Sulawesi (Indonesia), in a changing environment

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Abstract. Lembeh Strait is a famous locality known worldwide for the beautiful underwater world. Biodiversity studies in Lembeh Strait have become of high interest, since human activities (fishery and tourism) increased, and climate change is threatening the environment. Few preliminary biodiversity studies have been performed mainly in regions close by, such as Bunaken National Park (BNP). However, nearly nothing is known from Lembeh Strait. The present study is the first contribution to fill the gap in our knowledge on its biodiversity in this area. Target group is the marine Heterobranchia, as this was also the target group in the study of the BNP, to be able to compare these two regions. Sampling was done by diving in four sites of Lembeh Strait, (Nudi Fall, Makawidey, Nudi Retreat and Bajo) between 6 to 20 m depths. Pictures were taken from the substrate, before animals were sampled and subsequently preserved in 96% ethanol in the laboratory for further analysing. Seawater temperature and salinity were measured. Twenty-seven species from seven families and 11 genera of marine Heterobranchia were identified, most of them of the taxon Anthobranchia, two species of the Aeolidida and two species of the Sacoglossa. The localities differed with regard to the presence of species. Factors affecting biodiversity of marine heterobranchs are discussed.

Key Words: monitoring, biodiversity, Heterobranchia, Nudibranchia, Lembeh Strait.

Introduction. Lembeh Strait, a famous locality known worldwide for the species diversity underwater, is considered as one of the best places for muck diving. The strait, situated in the north-eastern tip of North Sulawesi is surrounded by the Celebes Sea in the north-west and Moluccas Sea in the east. Close to this area are further famous diving localities, e.g., the Bunaken National Park (BNP) or the Bangka Archipelago. These areas are part of the so-called Coral Triangle, a global hotspot of tropical marine biodiversity, which also embraces most Indonesian coastlines, the Philippines and other Indo-Australian countries. The Coral Triangle area, which covers only 1.6% (i.e., 5.7 million km^2) of our planet's oceanic area houses more than 600 different species of reef-building corals (Veron et al 2009), as well as more than 2000 coral reef fish species (Allen & Erdmann 2012). Numbers for other metazoan life forms are hardly known, although they probably comprise thousands of species, with a high number of undescribed taxa. This was recently shown in the Philippines for various groups of marine Heterobranchia, united in former times under the name "Opisthobrancha" (Gosliner et al 2015).

Major habitats in North Sulawesi, besides the mud and sandy areas in e.g., Lembeh Strait, are coral reefs (Ampou et al 2017; de Vantier & Turak 2004), sea grass beds (Wagey and Sake 2013), and mangroves (Calcinai et al 2017a). These marine ecosystems provide many ecosystem services, including coastal protection, livelihoods, food security, and nature based tourism industry, mainly sport diving and snorkeling (Moberg & Folke 1999). The high structural variety of these habitats and within the habitats ensures a high diversity of marine benthic organisms with varying life style, including e.g., economical important benthic filter feeders, such as the oyster *Pinctada maxima* (Jameson, 1901) in pearl industry (Ompi et al 2018), or the box mussel *Septiver bilocularis* (Linnaeus, 1758) (Ompi 1998). Some groups studied more in detail from North Sulawesi, e.g., Porifera (Calcinai et al 2017a; 2017b) and marine Heterobranchia (Kaligis et al 2018; Eisenbarth et al 2018), are important sources for bioactive compounds relevant in medical applications (Fisch et al 2017).

However, despite the fundamental importance of intact marine ecosystems for the economy and ecology, widespread anthropogenic impacts lead to their deterioration and destruction. Most of Indonesia's reefs have been destructed by human activities (Burke et al 2002). These human activities are intensified by a rapidly increasing population number, widespread and uncontrolled coastal development and increased exploitation of natural resources, including intensive fishing with frequently destructive methods (e.g. dynamite and cyanide fishing) or collection of key species (Suharsono & Kiswara 1984; Cowan et al 2017).

In recent years the unregulated increase in touristic activities also led to an increase in number of new resort complexes at Indonesian coastlines, where coral reefs and mangroves are destroyed during or for their construction (Kamagi et al 2016). The lack of proper wastewater management additionally increases the direct threat to the coastal ecosystems (Lasut et al 2005). All these direct human local activities go hand in hand with global threats such as global warming and strong El Niño events, which cause catastrophic coral bleaching events (Madin et al 2018; Wouthuyzen et al 2018). Heavy coral bleaching was recorded from almost all Indonesian waters in 2010 and 2016 (Wouthuyzen et al 2018), however has less effected North Sulawesi (Ampou et al 2017; Wouthuyzen et al 2018).

In order to observe changes in species composition, irrespective of its cause, a base line of knowledge on species diversity is needed (Madduppa et al 2012; Nimbs et al 2016; Ponti et al 2016). A suitable taxon to survey changes in the environment was shown for marine Heterobranchia (Nimbs et al 2016; Kaligis et al 2018; Eisenbarth et al 2018, and literature herein). About 6000 species are recorded with around 3000 in the Indo-Pacific (Gosliner et al 2015). Scientists, amateur naturalists, and divers are attracted by their diversity in colour, shapes and lifestyles. Only recently, they were target of a larger survey in Bunaken National Park (Kaligis et al 2018; Eisenbarth et al 2018), resulting in 215 species, with 69 undescribed species, 34 of which have never been recorded before. Despite their famousness in Lembeh Strait, there is only one scientific survey performed in this region in 2016, where 11 species were reported (Pungus et al 2017). Since the southern part of Lembeh Strait is dominated by the international harbour of Bitung, with a growing fishery and fish processing industry (the second main economic pillar of this region), a monitoring of the area is necessary to guide sustainable use for both, fishery and tourism.

The present study aims to record diversity of marine heterobranch species and environmental conditions at selected famous diving spots in Lembeh Strait to provide the first data for a continuous monitoring program in this very special region.

Material and Method

Sampling sites. Sampling sites in Lembeh Strait are depicted in Figure 1. The strait is 16 km long, about 1-2 km wide and stretching between the Sulawesi main Island and Lembeh Island (Yusron & Susetiono 2005). Lembeh Strait's topography is dominated by adjacent mountain areas, which also form the coastlines. Shores are usually steep, going down to 10 m depth, followed by flat bottoms composed of soft sediments down to 20 m depth. Four sampling sites have been chosen, three along the mainland: Nudi Retreat (N 01°29'093", E

125°14'459"), Makawidey (N 01°28'510", E 125°14'190"), and Nudi Fall (N 01°27'674", E 125°13'602"), and one at Lembeh Island: Bajo (N 01°28'58", E 125°15'18").

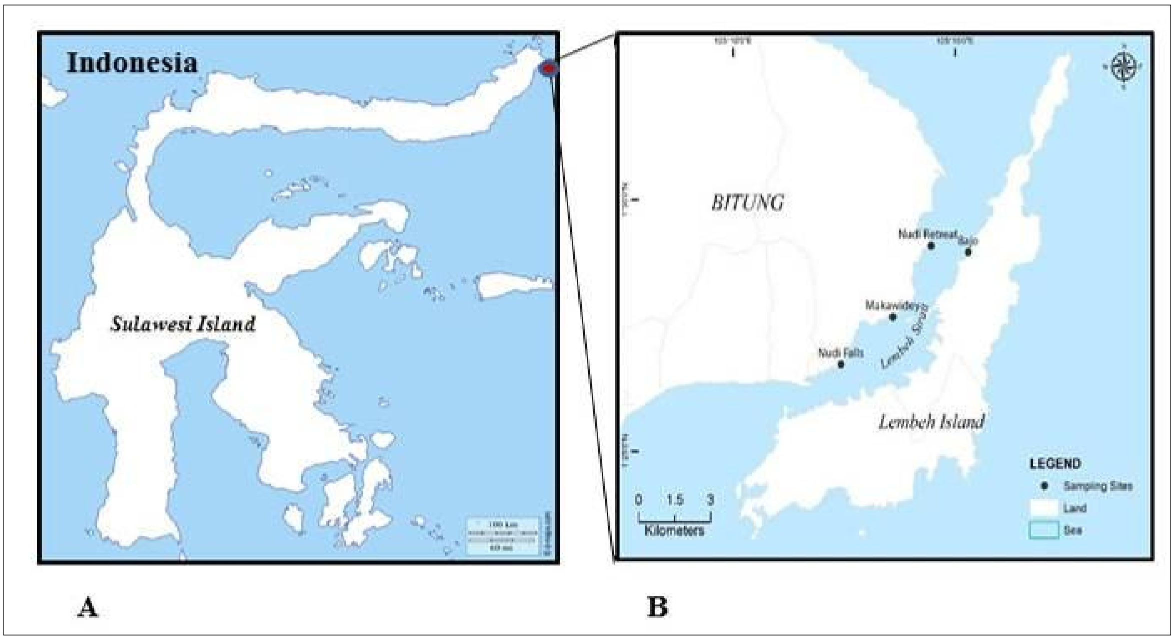

Figure 1. Overview of Sulawesi Island, Indonesia (A), and Detail four sampling sites in Lembeh Strait (B): Nudi Fall, Makawidey, Nudi Retreat, and Bajo.

Sampling and identification. Specimens were collected directly from substrate in the field by Scuba diving at each site from 28-29 March, and 29-30 April 2018. Totally 32 dives with 8 dives in every sampling site were involved. Each collecting dive usually lasted 45 min. A line transect was laid with 25 m length and 2 m widths at the ground starting from 6 to 20 m depth. Two divers followed along this line transect collecting all sea slugs observed in this area. After diving along the transect, specimens were also collected outside of the line transect. Three line transects were performed on each site. Pictures of the slugs were taken in situ still sitting on the original substrate. Collected specimens were preliminarily identified by using identification books (Debelius & Kuiter 2007; Gosliner et al 2008; Gosliner et al 2015), and scientific publications (Martynov & Korshunova 2012; Stoffels et al 2016; Kaligis et al 2018). All individuals were identified to species level. Validity of species names was checked with the help of the World Register of Marine Species (WoRMS). Animals were fixed in 96% EtOH and a small piece of members of the Chromodorididae and Phyllidiidae was used for barcoding DNA. Remaining specimens are deposited and registered at the reference collection of Faculty of Fisheries and Marine Sciences, University of Sam Ratulang Manado.

DNA-isolation has been carried out by means of QIAgen® DNeasy Blood and Tissue-Kit, following manufacturer's instructions. Partial sequences of mitochondrial CO1 (ca. 680bp) and ribosomal 16S (ca. 650bp) were amplified by polymerase chain reaction (PCR) using the primers LCO1490-JJ (5'-CHACWAAYCATAAAGATATYGG-3') and HCO2198-JJ (5'-AWACTTCVGGRTGVCCAAARAATCA-3') (Astrin & Stüben 2008) for CO1; 16Sar-L (5′-CGCCTGTTTATCAAAAACAT-3') and 16Sbr-H (5′-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al 1991). 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 sec), annealing (55°C for 90 sec) and extension (72°C for 90 sec), with a final extension step 72°C for 10 min. For 16S rRNA, the PCR started with an initial step (95°C for 15 min), denaturation (94°C for 45 sec), followed by 34 touch-down cycles, annealing (56°C for 45 sec), extension (72°C for 90 sec) and final extension step at 72°C for 10 min. PCR products were sequenced by Macrogen Europe

Laboratory (Amsterdam, Netherlands). GENEIOUS Pro 7.1.9 was used to extract the consensus sequence between the primer regions, construct the alignment for each gene using the default parameters and for creating the final alignments.

Salinity and water temperature were measured at all localities. Seawater samples in 5 L vials were taken from the seawater surface, and in 6-15 m above the sea bottom and measured with the help of a refractometer. Seawater temperature was also measured by using liquid thermometer (Thomas scientific) at the same depths.

Results. Water salinity measured at all four sampling sites showed continuously 32‰. Seawater temperature close to surface level was 29°C, and 28°C below 10 m. Most species were found in coral rubble. However, some species, mainly from the family Chormodorididae, were associated to sponges within the coral rubble (Figures 2B-H), or to tunicates (Nembrotha) (Figure 3H).

In total 27 species, belonging to 11 genera and 7 families, were found at the four localities (Table 1 and Figures 2-4). Most of the species (14) can be assigned to the family Chromodorididae. Phyllidiidae was represented with 5, Discodorididae, Plakobranchidae and Flabellinidae with two species each. Aegiridae and Polyceridae had one species representative each. Thus, Anthobranchia was represented in highest numbers. No other members of marine Heterobranchia were collected.

With regard to localities, Nudi Fall showed the highest number of species (17), followed by Nudi Retreat (12), Makawidey (11), and finally Bajo with just one species (Figure 5). Taking only those localities with higher diversity in consideration, the overlap of species (3) in these three localities is not very high (Figure 6).

In total, 135 specimens were collected in various depths from 6 to 20 m depth (Table 1). Following Fisch et al (2017), we defined four categories for species abundance, such as abundant (A), when more than 40 specimens were found, common (C) with 20 to 39, uncommon (UC) with 4 to 19 and rare (R) with 1 to 3 specimens (Table 1). None of the species in our samples was abundant. Only *Phyllidia ocellata* Cuvier, 1804 was found to be common in Lembeh Strait, while *Aegires villosus* Farran, 1905, *Chromodoris* cf. *elisabethina* (Bergh, 1877), *C. magnifica* (Quoy and Gaimard, 1832), *Goniobranchus fidelis* (Kelaart, 1858), *Nembrotha kubaryana* Bergh, 1877, *Phyllidia coelestis* Bergh, 1905, *Phyllidia elegans* Bergh, 1869, *Phyllidia varicosa* Lamarck, 1801, *Thuridilla gracilis* Risbec, 1928, and *Thuridilla lineolata* Bergh, 1905 were found rarely in this region. All other species were uncommon, but were represented at least with more than four specimens.

Table 1

Marine Heterobranchia in Lembeh Strait, with localities, depth, substrate and abundance, and NCBI accession numbers. Abundance is given as: common (C): 20-39 specimens; uncommon (UC): 4-19; and rare (R): 1-3 specimens, following the classification of Fish et al (2017)

Higher taxon affiliation	*Species name*	*Nudi Fall*	*Makawidey*	*Nudi Retreat*	*Bajo*	*Depth (m)*	*Substrate*	*Abun-dance*	*Voucher*	*GenBank accession numbers*	
										16S	*CO1*
Sacoglossa											
Plakobranchidae Gray, 1840	*Thuridilla gracilis* (Risbec, 1928)	-	-	X	-	> 9	Coral rubbles	R	Thra18LS1/ MO34	MK903016	MK903015
	Thuridilla ineolata (Bergh, 1905)	-	X	-	-	> 9	Dead corals	R	-	-	-
Nudibranchia, Doridina											
Polyceridae Alder and Hancock, 1845	*Nembrotha kubaryana* Bergh, 1877	X	-	-	-	> 9	Tunicate & dead corals	R	Nesp18LS1/ MO13	-	MK348902
Aegiridae P. Fischer, 1883	*Aegires villosus* Farran, 1905	-	X	-	-	> 9	Coral rubble & sponges	R	Aevi18LS1/ MO32	MK322444	-
Discodorididae Bergh, 1891	*Halgerda batangas* Carlson & Hoff, 2000	X	-	-	-	> 6	Coral rubble	UC	Haba18LS1/ MO02	MK322452	MK348905
									Haba18LS2/ MO04	MK322453	MK348903
									Haba18LS3/ MO03	-	MK348904
									Haba18LS4/ MO05	MK322454	-
	Jorunna funebris (Kelaart, 1859)	-	X	-	-	> 9	Coral rubble & sponges	UC	Jofu18LS1	-	-
Chromodorididae Bergh, 1891	*Chromodoris annae* Bergh, 1877	X	-	X	-	> 6	Coral rubble& dead corals	UC	Chan18LS2/ MO08	MK322445	-
									Chan18LS3/ MO09	MK322446	-
	Chromodoris colemani Rudman, 1982	-	X	X	-	> 9	Coral rubble	UC	-	-	-
	Chromodoris cf *elisabethina* (Bergh, 1877)	X	-	-	-	> 9	Coral rubble & sponges	R	-	-	-
	Chromodoris hamiltoni Rudman, 1977	X	-	X	-	> 6	Coral rubble & sponges	UC	-	-	-
	Chromodoris lochi Rudman, 1982	X	X	X	-	> 6	Coral rubble & sponges	UC	-	-	-
	Chromodoris magnifica (Quoy & Gaimard, 1832)	X	-	-	-	> 9	Ascidians and corals	R	-	-	-

	Chromodoris willani Rudman, 1982	-	-	X	-	> 6	Coral rubble & sponges	UC	-	-	-
	Glossodoris cincta (Bergh, 1888)	-	-	X	-	> 9	Coral rubble	UC	GLci18LS1/ MO30	MK322447	-
	Goniobranchus fidelis (Kelaart, 1858)	X	-		-	> 6	Coral rubble	R	Gofi18LS1/ MO07	MK322448	-
	Goniobranchus geometricus (Risbec, 1928)	X	-	X	X	> 6	Coral rubble	UC	Goge18LS3/M O06	MK322449	MK348906
									Goge18LS1/M O33	MK322451	-
	Goniobranchus kuniei (Pruvot-Fol, 1930)	X	-	-	-	> 9	Coral rubble & ascidian		-	-	-
	Goniobranchus reticulatus (Quoy & Gaimard,1832)	-	-	X	-	> 9	Coral rubble	UC	Gore18LS1/ MO24	MK322450	-
	Hypselodoris tryoni (Garett, 1873)	X	-	-	-	> 6	Coral rubble	UC	Hytr18LS2/ MO11	MK322455	-
	Hypselodoris maculosa (Pease, 1871)	X	X	-	-	> 6	Coral rubble & sponges	UC	-	-	-
Phyllidiidae Rafinesque, 1814	*Phyllidia coelestis* Bergh, 1905	-	X	-	-	> 9	Rubble corals	R	Phco18LS1/ MO21	MK852557	MK911039
	Phyllidia elegans Bergh, 1869	-	X	-	-	> 9	Coral rubble	R	-	-	-
	Phyllidia ocellata Cuvier, 1804	X	X	X	-	> 6	Coral rubble	C	Phoc18LS1/ MO28	MK911024	MK911038
									Phoc18LS2/ MO29	MK911025	MK911037
									Phoc18LS3/ MO22	MK911026	MK911033
									Phoc18LS4/ MO23	MK911027	MK911034
									Phoc18LS5/ MO15	MK911028	MK911035
	Phyllidia varicosa Lamarck, 1801	X	-	X	-	> 6	Dead corals	R	Phva18LS1/ MO26	MK911030	-
									Phva18LS3/ MO37	MK911031	MK852558
	Phyllidiella pustulosa (Cuvier, 1804)	X	X	X	-	> 6	Coral rubble	UC	Phpu18LS1/ MO16	MK911029	MK911036
									Phli18LS1/ MO18	MK911022	-
									Phli18LS2/ MO19	MK911023	MK911032

Nudibranchia, Cladobranchia											
Flabellinidae Bergh, 1889	*Coryphellina exoptata* (Gosliner & Willan, 1991)	X	X	-	-	> 6	Coral rubble	UC	-	-	-
	Coryphellina rubrolineata O'Donoghue, 1929	X	-	-	-	> 6	Coral rubble & sponges	UC	Flsp18LS1/ MO36	MK903017	-
Number of species		17	11	12	1						

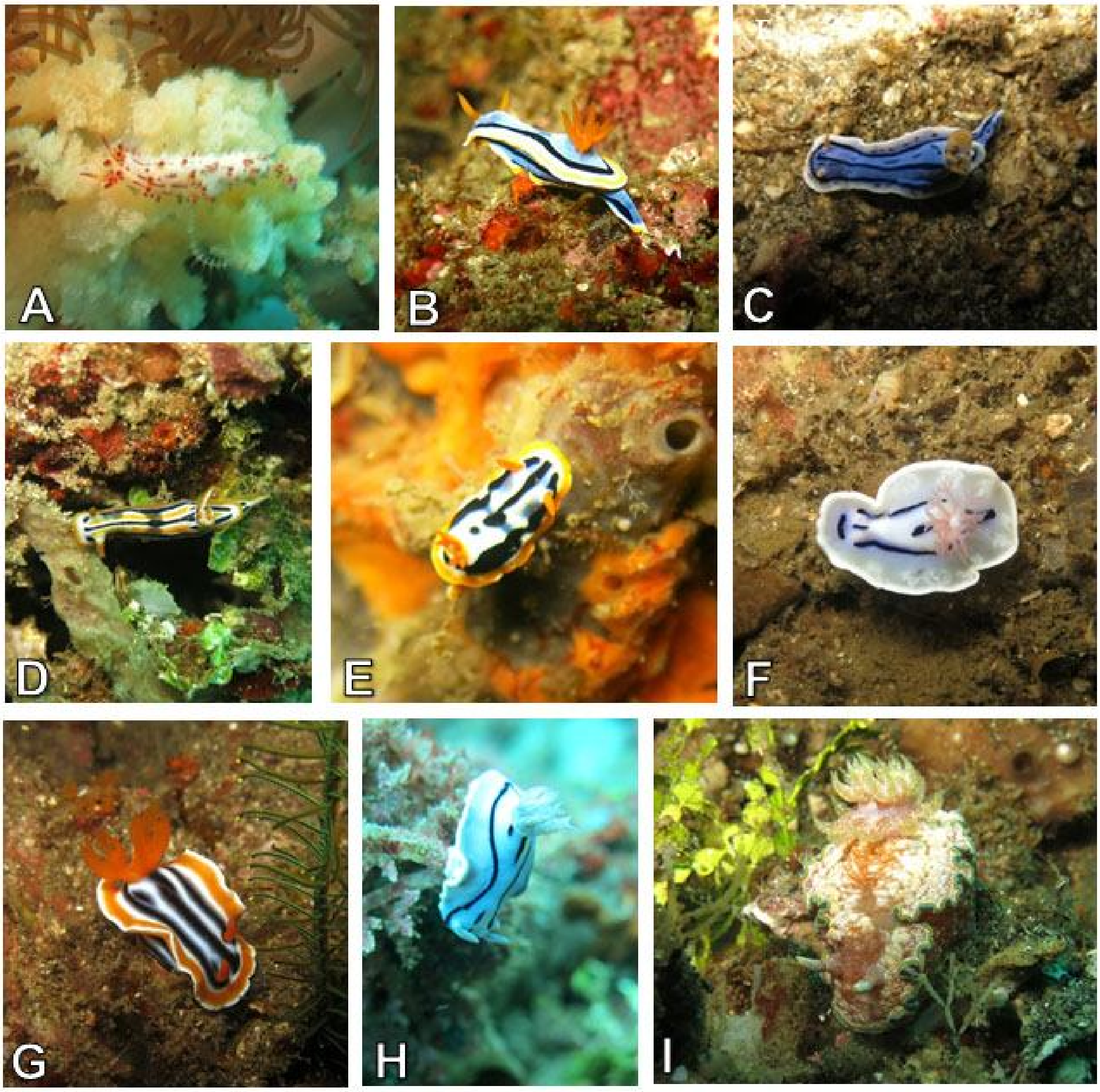

Figure 2. Anthobranchia: A. *Aegires villosus*, B. *Chromodoris annae*, C. *Chromodoris colemani*, D. *Chromodoris hamiltoni*, E. *Chromodoris* cf *elisabethina*, F. *Chromodoris lochi*, G. *Chromodoris magnifica*, H. *Chromodoris willani*, I. *Glossodoris cincta*.

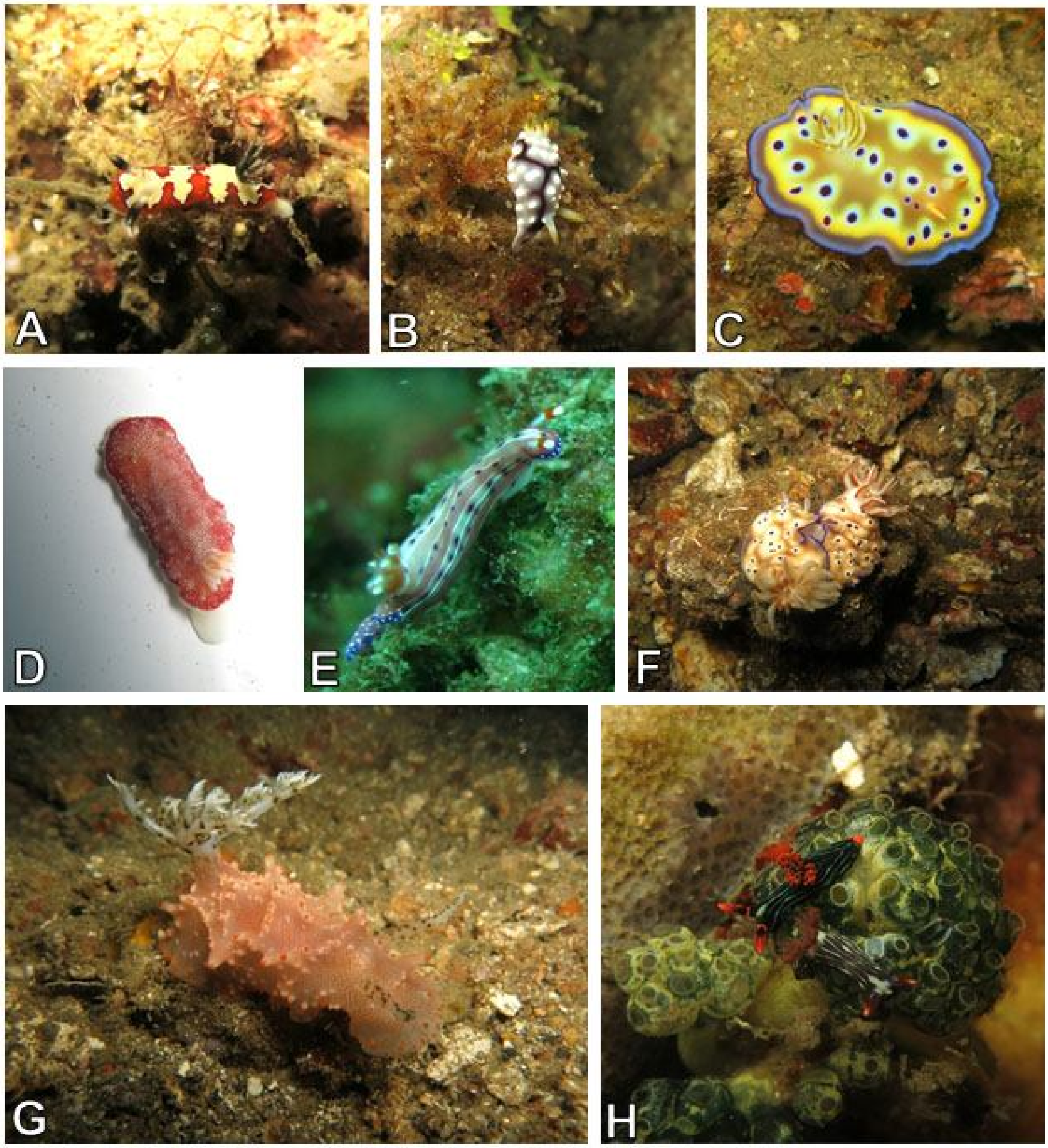

Figure 3. Anthobranchia: A. *Goniobranchus fidelis*, B. *Goniobranchus geometricus*, C. *Goniobranchus kuniei*, D. *Goniobranchus reticulatus*, E. *Hypselodoris maculosa*, F. *Hypselodoris tryoni*, G. *Halgerda batangas*, H. *Nembrotha kubaryana*.

Figure 4. Anthobranchia A-F, Cladobranchia G-H, Sacoglossa I-K: A. *Jorunna funebris*, B. *Phyllidia coelestis*, C. *Phyllidia elegans*, D. *Phyllidia ocellata*, E. *Phyllidia varicosa*, F. *Phyllidiella pustulosa*, G. *Coryphellina exoptata*, H. *Coryphellina rubrolineata,* I. *Thuridilla gracilis,* K. *Thuridilla lineolata*.

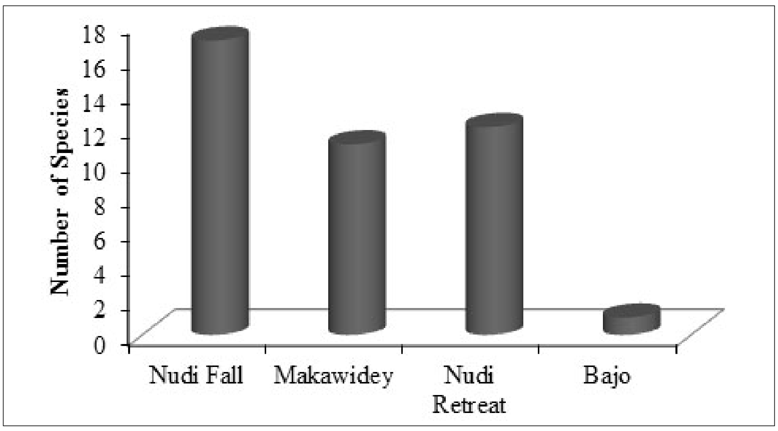

Figure 5. Number of species found at the four localities in Lembeh Strait.

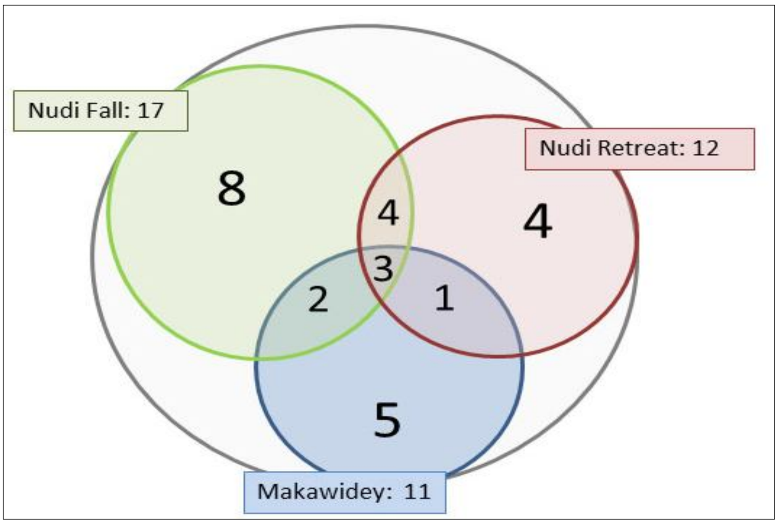

Figure 6. Venn diagram explaining overlap of species in the three most diverse localities. Digits indicate species numbers.

Disscusion. Marine Heterobranchia are a highly diverse group with representatives feeding on algae, sponges, cnidarians or tunicates. Some are even specialized feeders on eggs. The species represented in our study are mainly sponge feeders. Only *N. kubaryana* feeds on tunicates and the two *Coryphellina* species on hydrozoans. Thus, the high number of anthobranchs reflects the occurrence of a high variety of sponges in Lembeh Strait. The dominance of Anthobranchia is also shown for other areas in Indonesia, e.g., Papua New Guinea, Ambon or Bunaken National Park (summarized in Kaligis et al 2018). However, the low overlap of species in the three localities where most of the specimens were collected indicates already habitat differences. The overall number of species we found in Lembeh is considerably lower (only about 10%), than the number shown for Bunaken National Park (Eisenbarth et al 2018). However, scattered information from few publications (e.g., Tonozuka 2003; Cox 2017) and websites indicate a much higher diversity in Lembeh Strait.

The overall specimen number in our study (135) is high. Interesting is the difference in specimen numbers per species with regard to other areas in North Sulawesi. Whereas Chromodorididae are dominant in Lembeh Strait, Sangihe Island is dominated by Phyllidiidae (unpublished data of NU and HW). According to Kaligis et al (2018) and Eisenbarth et al (2018), BNP shows a high number of aeolid species and specimens. This might be due to the presence of many hydrozoan species in BNP, which were less observed in Lembeh Strait. The difference in food composition is certainly dependent on substrate and hydrodynamics of the region, and this certainly is one major factor in the different nudibranch species and specimen composition when we compare Lembeh Strait with BNP (Ampou et al 2017; Eisenbarth et al 2018).

Differences in species number among sites are probably more related to local conditions and the high structural variety of the habitats, as well as sampling problems. Bajo is an open area with extremely strong currents, which certainly impeded the same sampling effort compared to the other three sites. However, strong currents might also hinder larval settlement, which might be facilitated in the other sampling sites with less currents and zones of calm waters. Although data on larval development of tropic nudibranchs is rare, we assume most of the species do have a planktonic phase as is shown for many invertebrates (Ompi 2010; Ompi & Svane 2018). Variation in number of species among sites might also be controlled by other factors. The dominance of Chromodorididae and Phyllidiidae in comparison to other dorid species is also seen in other areas close by (Eisenbarth et al 2018). Chromodorididae and Phyllidiidae are protected by natural compounds, acting as defence system against predators. They displace warning colours, which allow them to sit on substrate obvious to fish or other predators with good visual systems. However, the compounds also protect against predators like sea anemones, mushroom corals, and pycnogonids (Stoffels et al 2016).

According to our results, industries' activities in the south of Lembeh Strait do not seem to affect the biodiversity of nudibranchs. Especially Nudi Fall, which is close to this area, showed a high diversity. However, our study is too small yet, to speculate about factors which regulate distribution of nudibranchs in Lembeh Strait.

We did not measure a critical temperature during our first survey in Lembeh Strait, which would threaten corals and induce coral bleaching. However, we did not assess health of corals with regard to diseases or other bleaching factors. Although only few marine heterobranchs feed on zooxanthellate hexacorals directly, these slugs in general need healthy coral communities, with a high diversity of especially sessile metazoan life forms. In the future surveys, we will additionally address the health situation of these communities and study other habitats, like mangroves and the few sea grass and algal areas in Lembeh Strait. This will help to get a better overview of the diversity of marine Heterobranchia and to create a base line for monitoring an area of high economic value for North Sulawesi, Indonesia.

Conclusions. Monitoring of marine Heterobranchia in Lembeh Strait has just started and the overall diversity is not known yet. Species diversity varied and probably depends on substrate availability and hydrodynamics of the region.

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