The nematocysts of some Scleractinian coral species from the Sulawesi Sea, Indonesia

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Abstract. Nematocysts of corals are used either to defend against predators, to capture prey, to attach to suitable substrates, or to attack unrelated and neighboring cnidarians, intra and inter-specific aggression, and for the taxonomy. The objective of this study is to describe and illustrate the types, composition, and size dimesions of nematocysts of four Scleractinian coral species found in the reefs of the coast of Gorontalo and Manado in the Sulawesi Sea, Indonesia. Three coral species, *Montipora undata* (Bernard, 1897), *Hydnophora rigida* (Dana, 1946), and *Cycloseris vaughani* (Boschma, 1923) in the Manado Bay of North Sulawesi, and then *Acropora batunai* (Wallace, 1997) in the Tontayuo coast of Gorontalo were studied. Three colonies from each coral species were examined. Coral tissues derived from the different coral colonies were used to examine nematocysts types, composition, and dimensions under a microscope (Olympus CX41) at a magnification of 1000X, which connected to the computer (ST24 monitor) with the application of Optica view 7 (software). The length and width of the capsules, and the length of the shafts of undischarged nematocysts were measured for each coral colony. There was no significant difference in the nematocyst composition of the sMpM-II type between *M. undata* and *C. vaughani*, and then between *H. rigida* and *A. batunai*, but markedly different between *M. undata* and *H. rigida*, between *M. undata* and *A. batunai*, between *H. rigida* and *C. vaughani*, and then between *C. vaugani* and *A. batunai*. There was a marked difference in the nematocyst composition of MpM-I type between *C. vaughani* and *A. batunai*, but no significant difference in the nematocyst composition of *s*HI type among *M. undata*, *H. rigida* and *C. vaughani*. There were no significant differences between the *C. vaughani* and *A. batunai* capsules in terms of their length and width as well as the shaft length of MpM-I type. No differences in length and width of *s*HI type capsules were found among *M. undata*, *H. rigida* and *C. vaughani*. Furthermore, no differences in length and width as well as the shaft length of *s*MpM-II type capsules were found among *H. rigida*, *C. vaughani* and *A. batunai*, while the significant difference was found between *M. undata* and three other coral species (*H. rigida*, *C. vaughani* or *A. batunai*). The nematocysts of four coral species studied varied in types, composition and size dimensions of nematocysts. The present study showed that the four coral species had their specific nematocysts types, composition, and dimensions.

Key Words: *Acropora batunai*, *Cycloseris vaughani*, *Hydnophora rigida*, *Montipora undata*, nematocyst.

Introduction. Cnidarians (hydras, jellyfish, anemones, and corals) are a diverse group of animals of interest to ecologists, evolutionary biologists, and developmental biologists (Steele et al 2011; Americus et al 2020). The faunal group of Cnidarians is one of the most widespread taxa around the globe and specialized predators able to paralyze and sting their prey throughout a wide range of venom (Boero 2013; Condon et al 2013; Bonello et al 2017; Killi et al 2020). The phylum Cnidaria has a unique intracellular organelle called nematocyst located in ectoderm layer of the Scleractinian coral tissue, which discharged by evaginating their tubular contents following certain appropriate stimuli (Paruntu et al 2000; Technau & Steele 2011; Yue et al 2020). Every nematocyst consists of a capsule, a tubule or a shaft, or a combination of the two, and intracapsular fluid and is contained in a cell called nematocyte (Mariscal 1974; Zenkert et al 2011). Nematocysts are classified based on the morphological characteristics of the discharged tubule and/or shaft, and over thirty different types of nematocysts have been recognized (Mariscal 1974; Ӧstman 2000; Yoffe et al 2012).

Nematocysts are typical cnidarian organelles that can discharge and release venom under physicochemical stimuli for predation and defense (Ӧstman 2000;

Balasubramanian et al 2012; Beckmann & Özbek 2012; Yue et al 2020; Americus et al 2020). Nematocysts are used either to defend against predators, to capture prey, to attach to suitable substrates, or to attack unrelated and neighboring cnidarians, and intra and inter-specific aggression (Moran et al 2012; Yoffe et al 2012; Ashwood et al 2020).

Various types of nematocysts generally have been considered as one of the useful characters in the taxonomy of different orders of Cnidarian (Mariscal 1974; Őstman 2000; Yoffe et al 2012). The nematocysts of Scyphozoa have been studied for a long time, and many descriptions and classifications have been suggested by the scientific community (Őstman 2000; Morabito et al 2014; Yue et al 2020). The cnidom in many species of Scyphozoa and cnida morphology have been used for their classification to distinguish them (Killi et al 2020; Yue et al 2020). The nematocysts of Hydrozoa have also been examined in detail for their utility in taxonomy (Őstman 2000; Balasubramanian et al 2012). The study of the nematocysts in Actiniaria is at a more advanced stage, as the procedure of giving data on the cnidae distribution and sizes of nematocysts has become conventional in actinian taxonomy (Mariscal 1974; Paruntu et al 2000). Morphology of eight nematocyst types and the effect of venom of the two cnidarian species were observed in *Velella velella* and *Aurelia aurita* from the Mediterranean Sea (Killi et al 2020). Lang (1984) reported that the nematocysts have rarely been used for the taxonomy of Scleractinians.

The Coral Triangle region is a equator marine area situated between the Pacific and the Indian Oceans, and a priority for marine biodiversity conservation because it has the highest species richness, and endemicity, of any marine region globally (Asaad et al 2020). It consists of six countries (Indonesia, Malaysia, Philippines, Papua New Guinea, Timor-Leste, and Solomon Islands), and has a gross domestic product worth $1.2 trillion per year in the socio-economic context of the marine ecosystems and > 120 million people benefit directly from its ecosystem goods and services (Foale et al 2013; Asian Development Bank 2014). The Coral Triangle is a global hotspot of marine biodiversity, has > 76% of the world's shallow-water reef-building coral species and 50% of razor clams, and it is six out of seven of the world's sea turtles and the largest mangrove forest in the world (Veron 2000; Zhang et al 2012; Walton et al 2014; Saeedi et al 2017; Paruntu et al 2018; Rumengan et al 2021). Indonesia's marine area is the center of the world's coral diversity, the distribution of which is more concentrated in eastern Indonesia, one of which is the Sulawesi Sea, the center of tropical biodiversity rich in coral species (Veron 2000; Woo et al 2013). However, study on nematocysts of coral species for taxonomic informations (e.g. nematocyst types, composition and dimensions) in coastal areas of Indonesia is still unique and limited to very few coral species, as reported by Paruntu et al (2013), Kodoati et al (2021), and Sagrang et al (2021). The purpose of this study is to describe and illustrate the types, composition and size dimesions of nematocysts of four Scleractinian coral species found in the reefs of the coast of Gorontalo and Manado in the Sulawesi Sea, Indonesia.

Material and Method

Coral colonies collection and preservation. Corals colonies of *Montipora undata*, *Hydnophora rigida*, and *Cycloseris vaughani* were collected from the reefs of Malalayang coast in Manado, North Sulawesi, while *Acropora batunai* was collected in the reefs of Tontayuo coast, Batudaa Pantai, Gorontalo, Sulawesi Sea, Indonesia (Figure 1). Coral colonies were fixed and preserved in 70% alcohol and decalcified in a 1:1 mixture of range of 10-40% formalin and 10-40% acetic acid in tap water. Decalcified tissues were kept in 70% alcohol and rinsed in water just before observation.

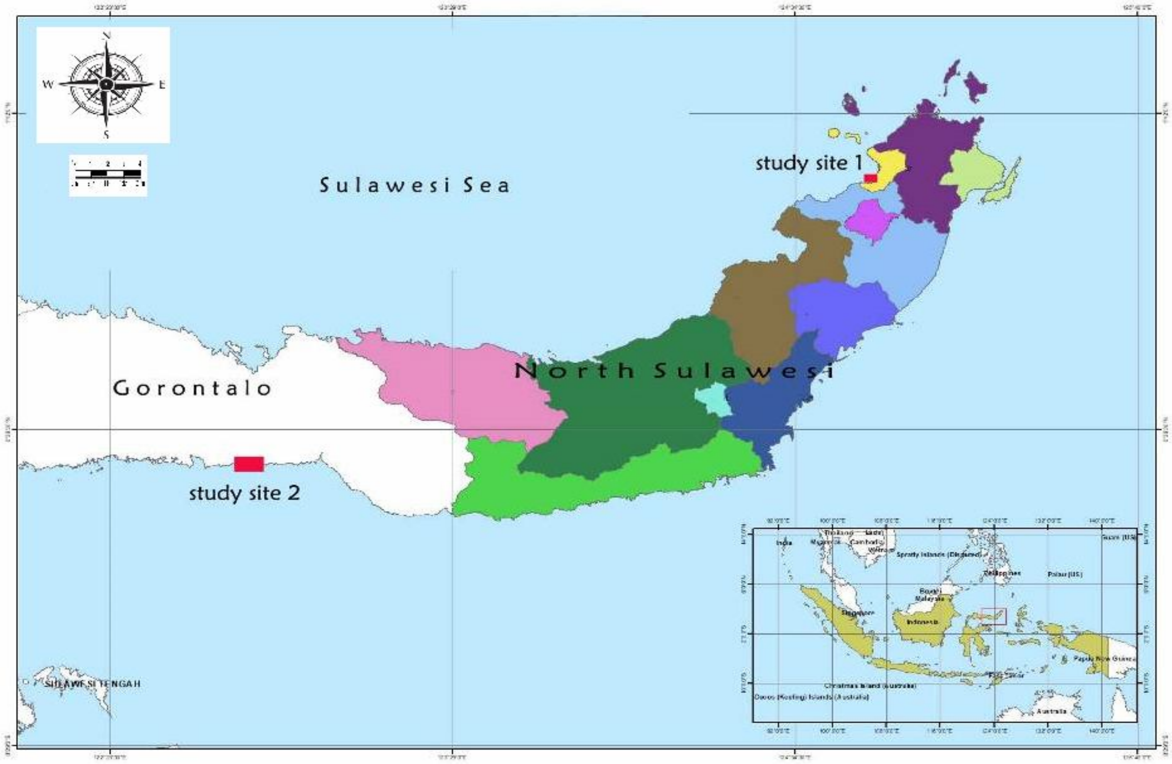

Figure 1. Location of *M. undata*, *H. rigida* and *C. vaughani* coral colonies sampling in the reefs of Malalayang coast, Manado in North Sulawesi (study site 1), and *A. batunai* coral colonies sampling in the reefs of Tontayou beach, Batudaa Pantai in Gorontalo (study site 2), Sulawesi Sea, Indonesia.

Observation of nematocyst and measurement of nematocyst dimensions. Tissues were peeled from the coral skeleton if necessary and squashed under a cover slip on a slide glass to observe nematocysts. Three colonies of each *M. undata*, *H. rigida*, *C. vaughani* and *A. batunai* were used, squashed tissues of each coral species were observed for each colony under a microscope (Olympus CX41) at a magnification of 1000X, which connected to the computer (ST24 monitor) with the application of Optica view 7 (software).

Capsules length and width and the shaft length of undischarged nematocysts were measured in the computer by using the application of Optica view 7, except large holotrichous izorhiza (*l*HI) of *M. undata* which was discharged nematocyst. The shaft length of undischarged microbasic p-mastigophore (MpM) was measured as the distance between the tip of the nematocyst and the tip of the V-shaped notch of the shaft (Figure 2). Range from 1 to 41 nematocysts of each type was measured for each coral species. Nematocysts of corals were identified according to Mariscal (1974).

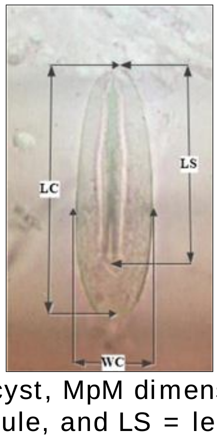

Figure 2. Measurement of nematocyst, MpM dimension. LC = length of capsule, WC = width of capsule, and LS = length of shaft.

Measurement of nematocyst composition. Measurement of nematocysts composition was carried out only on the main types of nematocysts, while the minor types of nematocysts were ignored because they were difficult to identify and count. The number of each type of cnidae was counted under a Olympus CX41 microscope at a magnification of 1000X. Range from 1 to 41 nematocysts of each type was counted in 15 different areas displayed on a computer monitor for each coral species. The relative abundance of nematocysts was compared between coral species, and the statistical test was performed.

Statistical analysis. Comparisons between the size dimensions of nematocysts (LC, the length of the capsule; WC, the width of the capsule; and LS, the length of the shaft) from the coral species were conducted using t-test. Variations between the dimensions of nematocysts (LC, WC and LS) among the coral species were compared using one-way ANOVA, followed by BNT-Test. Mean values and standard deviation were generated for each of the capsule length and width, and the shaft length of nematocysts. Significance differences was defined at $p < 0.01$. Statistical analyses were performed using Excel program.

Results and Discussion

Nematocysts of M. undata. Adult colonies of *M. undata* possessed at least microbasic *b*-mastigophore (MbM), type II small microbasic *p*-mastigophore (*s*MpM-II), type II large microbasic *p*-mastigophore (*l*MpM-II), small holotrichous isorhiza (*s*HI), and large holotrichous isorhiza (*l*HI) (Figure 3). MbM, *s*MpM-II, *l*MpM-II, and *s*HI were in undischarged nematocysts, while *l*HI was in discharged nematocyst. *s*MpM-II contained a shaft longer than two thirds of the capsule length. *l*HI and *l*MpM-II are specific to adult colonies of *M. undata* which were occasionally observed.

Figure 3. Nematocysts of *M. undata*. A. MbM; B. *s*MpM-II; C. *s*HI; D. *l*HI; and E. *l*MpM-II. Scale bar = 200 µm for all photos.

Nematocysts of H. rigida. Adult colonies of *H. rigida* possessed at least *s*MpM-II and *s*HI (Figure 4). *s*MpM-II and *s*HI were in undischarged nematocysts.

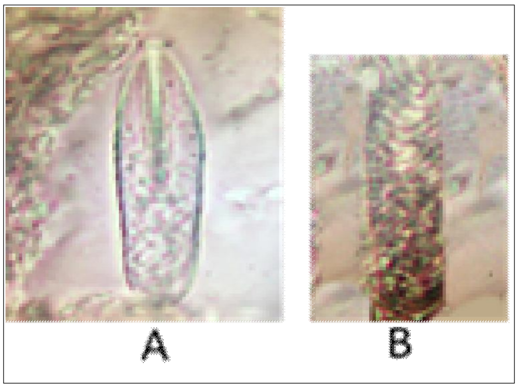

Figure 4. Nematocysts of *H. rigida*. A. *s*MpM-II and B. *s*Hl.

Nematocysts of C. vaughani. Adult colonies of *C. vaughani* had at least type I microbasic *p*-mastigophore (MpM-I), *s*MpM-II), type II large microbasic *p*-mastigophore (*l*MpM-II), *s*HI, and *l*HI (Figure 5). MpM-I contained a shaft shorter than a half of capsule length, while MpM-II had a shaft longer than two thirds of the capsule length. All nematocysts were in undischarged nematocysts. *l*HI and *l*MpM-II are also specific to adult colonies of *C. vaughani* which were rarely observed.

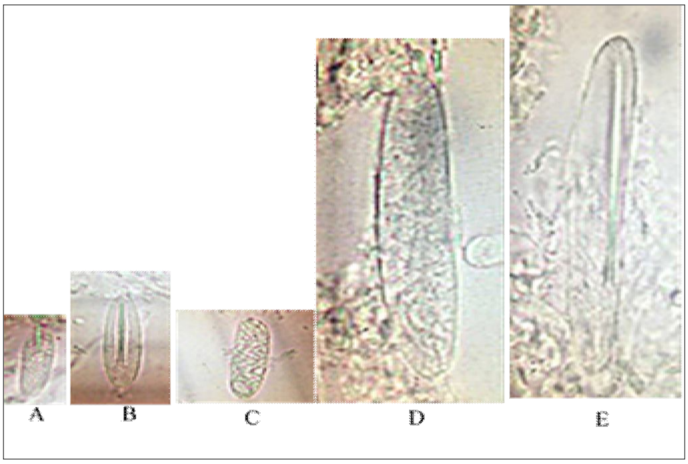

Figure 5. Nematocysts of *C. vaughani*. A. MpM-I; B. *s*MpM-II; C. *s*HI; D. *l*HI; and E. *l*MpM-II.

Nematocyst of A. batunai. Adult colonies of *A. batunai* contained at least MpM-I and *s*MpM-II (Figure 6). MpM-I and *s*MpM-II were in undischarged nematocysts.

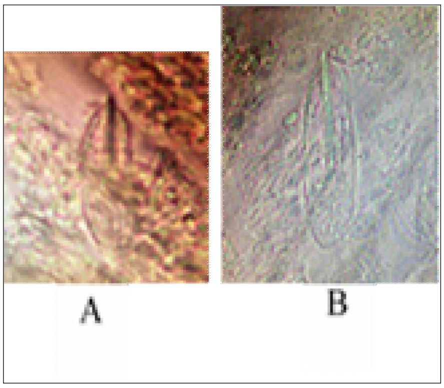

Figure 6. Nematocysts of *A. batunai*. A. MpM-I and B. *s*MpM-II.

Dimension of nematocyst. The average capsule length and width of MbM of *M. undata* were 190.39 µm and 42.62 µm respectively, while its average length of the shaft was 143.77 µm. The MpM-I of *C. vaughani* had average capsule length and width of 200.79

µm and 56.15 µm respectively, and its average shaft length of 86.41 µm, while *A. batunai* had an average capsule length and width of 208.12 µm and 60.00 µm respectively, and its average shaft length of 90.35 µm. The *s*MpM-II of *M. undata* possessed an average capsule length and width of 262.00 µm and 48.22 µm respectively, and its average shaft length of 152.77 µm, *H. rigida* had an average capsule length and width of 324.22 µm and 74.08 µm respectively, and its average shaft length of 205.89 µm, *C. vaughani* had average capsule length and width of 254.04 µm and 71.78 µm respectively, and its average shaft length of 150.05 µm, while *A. batunai* had an average capsule length and width of 304.46 µm and 71.71 µm respectively, and its average shaft length of 184.17 µm. The *s*HI of *M. undata* contained an average capsule length and width of 280.00 µm and 49.44 µm respectively, *H. rigida* had average capsule length and width of 320.71 µm and 72.41 µm, while *C. vaughani* had an average capsule length and width of 228.43 µm and 61.99 µm respectively. The *l*HI of *M. undata* had capsule length and width of 1205 µm and 288.6 µm respectively, while the length and width capsules of *C. vaughani* was 743.20 µm and 169.70 µm, respectively. Furthermore, the length and width of the capsules and shaft length of *l*MpM-II of *M. undata* were 1269 µm and 243.3 µm and 963.1 µm respectively, while *l*MpM-II of *C. vaughani* had capsules length and width, and shaft length of 834.9 µm, 163.60 µm, and 628.2 µm, respectively. The dimension of nematocysts of the above coral species is shown in Table 1.

Table 1
Dimensions of nematocysts at some species of Scleractinian corals, i.e., *M. undata*, *H. rigida*, *C. vaughani* and *A. batunai*

Nematocysts types	*Coral species*	*LC (µm)*	*WC (µm)*	*LS (µm)*
MbM	*M. undata*	190.39±19.74 (27)	42.62±20.15 (27)	143.77±32.09 (27)
	H. rigida	-	-	-
	C. vaughani	-	-	-
	A. batunai	-	-	-
MpM-I	*M. undata*	-	-	-
	H. rigida	-	-	-
	C. vaughani	200.79±44.63 (41) a	56.15±14.33 (41) a	86.41±28.22 (41) a
	A. batunai	208.12±40.43 (5) a	60.00±23.17 (5) a	90.35±18.01 (5) a
*s*MpM-II**	*M. undata*	262.00±17.12 (10) a	48.22±6.14 (10) b	152.77±27.75 (10) a
	H. rigida	324.22±43.88 (35) a	74.08±8.51 (35) a	205.89±26.94 (35) a
	C. vaughani	254.04±65.47 (29) a	71.78±9.50 (29) a	150.05±44.97 (29) a
	A. batunai	304.46±36.26 (28) a	71.71±4.67 (28) a	184.17±13.51 (28) a
*s*HI	*M. undata*	280.0±69.15 (3) a	49.44±12.98 (3) a	-
	H. rigida	320.71±143.9 (10) a	72.41±25.90 (10) a	-
	C. vaughani	228.43±33.57 (18) a	61.99±2.96 (18) a	-
	A. batunai	-	-	-
*l*HI	*M. undata*	1205±24.04 (2)	288.6±8.06 (2)	-
	H. rigida	-	-	-
	C. vaughani	743.20±0(1)	169.70±0 (1)	-
	A. batunai	-	-	-
*l*MpM-II	*M. undata*	1269±0 (1)	243.3±0 (1)	963.1±0 (1)
	H. rigida	-	-	-
	C. vaughani	834.90±0 (1)	163.60±0 (1)	628.20±0 (1)
	A. batunai	-	-	-

Note: The number in the parentheses represents the number of nematocysts measured. Three colonies from each coral species were examined. Coral tissues derived from different colonies were used. Range from 1 to 41 nematocysts were measured to calculate average values for each coral species. Line (-) indicates that no data was found. LC, the length of the capsule; WC, the width of the capsule; LS, the length of the shaft. Means±SD. The different characters indicate that the values are significantly different from those at other coral species (t-Test; single-factor ANOVA & BNT-Test, **p < 0.01). Values are not significantly different at the 0.05 level share the same characters for each test. The dimension of MbM, *l*MpM-II, and *l*HI was not done the statistical test, because of single or fewer data.

Composition of nematocyst. Only *M. undata* possessed MbM which was absent in the other three species, *H. rigida*, *C. vaughani* and *A. batunai*. Two corals, *C. vaughani* and *A. batunai* had MpM-I which were absent in *M. undata* and *H. rigida*. Two corals, *M. undata* and *C. vaughani* possessed *l*HI and *l*MpM-II which were absent in *H. rigida*, and *A.*

batunai. Furthermore, *s*HI was found in *M. undata*, *H. rigida*, and *C. vaughani* which was absent in *A. batunai*. The nematocysts composition of *M. undata* possessed 60% MbM, 26.67% *s*MpM-II, 2.2% *l*MpM-II, 6.67% *s*HI, and 4.44% *l*HI. *H. rigida* contained 77.78% *s*MpM-II and 26.67% *s*HI. *C. vaughani* contained 45.56% MpM-I, 32.22% *s*MpM-II, 1.11% *l*MpM-II, 20.00% *s*HI, and 1.11% *l*HI. Furthermore, *A. batunai* had 15.9% MpM-I and 84.1% *s*MpM-II. Nematocyst composition values of *M. undata* and *C. vaughani* were early reported by Kodoati et al (2021) and Sagrang et al (2021) (Figure 7).

There was no significant difference in the nematocyst composition of *s*MpM-II between *M. undata* and *C. vaughani*, and then between *H. rigida* and *A. batunai*, while the differences were found between *M. undata* and *H. rigida*, between *M. undata* and *A. batunai*, between *H. rigida* and *C. vaughani*, and then between *C. vaughani* and *A. batunai* (single-factor ANOVA and BNT-Test, $p < 0.01$). The nematocyst composition of MpM-I was marked difference between *C. vaughani* and *A. batunai* (t-Test, $p < 0.05$), while no apparent difference was found in the nematocyst composition of *s*HI among three coral species, *M. undata*, *H. rigida* and *C. vaughani* (single-factor ANOVA-Test, > 0.05).

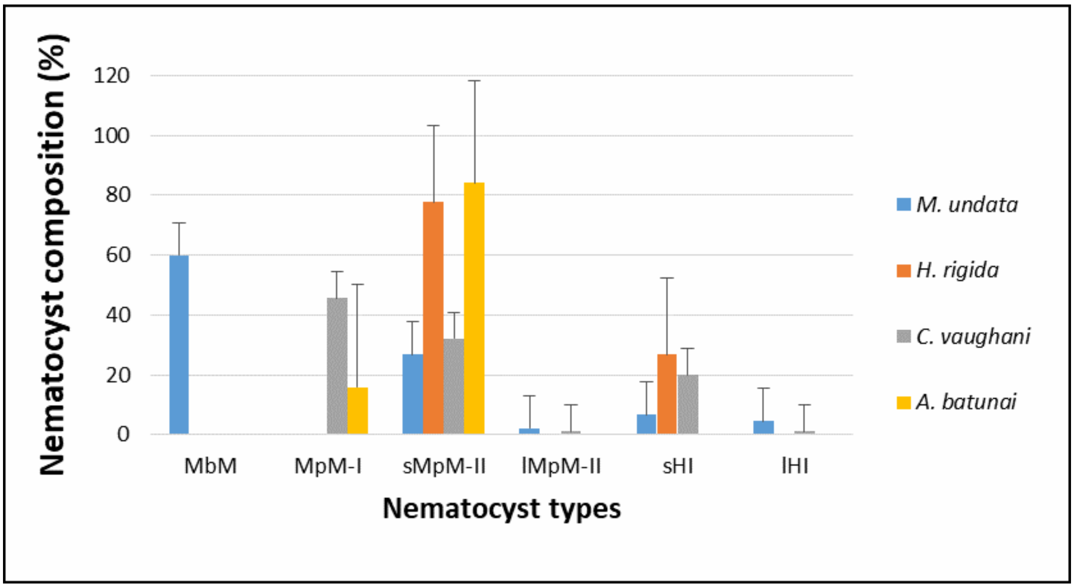

Figure 7. Nematocyts composition at some species of Scleractinian corals.

Discussion. Undischarged MbM of *M. undata* had a capsule, tubule, spines, lacks V-shaped notch at the base of the shaft, and narrow shaft (Figure 3A), while undischarged MpM possessed a capsule, tubule, spines, and V-shaped notch at the base of shaft (Figures 3B, 3E, 4A, 5A, 5B, 5E, 6A, and 6B). Noted that MbM had a capsule, lacks V-shaped notch at the base of the unfired, narrow shaft, the rod-shaped shafts, and the first coil upwards of tubule at end of the shaft, the different spines-patterns on the shaft, and the distal tubule armed with tiny spines, while MpM had a capsule, narrow tubule, spines of the broad shaft and narrow tubule, the V-shaped notch (V) at end of unfired, board shaft (Őstman 2000; Yue et al 2020). Paruntu et al (2000) stated that MpM had two types of MpM, called MpM-I and MpM-II. Undischarged MpM-I had a shaft shorter than a half of the length of the shaft, while MpM-II had a shaft longer than two-thirds of the length of the capsule. MpM-I were observed in *C. vaughani* and *A. batunai* (Figures 5A and 6A), while MpM-II were observed in *M. undata*, *H.rigida*, *C. vaughani* and *A. batunai* (Figures 3B, 3E, 4A, 5B, 5E, and 6B). The two types of MpM-II are tentatively called *s*MpM-II) and *l*MpM-II. sMpM-II was observed in *M. undata*, *H.rigida*, *C. vaughani* and *A. batunai* (Figures 3B, 4A, 5B, and 6B), while *l*MpM-II was found in *M. undata* and *C. vaughani* (Figures 3E and 5E). Undischarged *s*HI of *M. undata*, *H. rigida* and *C. vaughani* had capsules, tubules and spines of tubules (Figures 3C, 4B, 5C, and 5D), while discharged capsule of *l*HI with tubule and spines was shown in Figure 3D. Őstman (2000) noted that HI had a capsule, tubule spined throughout, isodiametric tubule, tubule of uniform or nearly uniform thickness proximal to mid-point of the tubule. The two types of HI are also tentatively called *s*HI and *l*HI. *s*HI was observed in *M. undata*, *H. rigida* and *C. vaughani* (Figures 3C, 4B, and 5C), while *l*HI was found in *M. undata* and *C. vaughani* (Figures 3D and 5D).

A significant difference in nematocyst composition was found among the four coral species studied, *M. undata*, *H. rigida*, *C. vaughani* and *A. batunai*. Two large nematocysts, *l*HI and *l*MpM-II are specific to *M. undata* and *C. vaughani*, while MbM is only specific to *M. undata*. *l*HI and *l*MpM-II were absent in *H. rigida* and *A. batunai*, while MbM was absent in *H. rigida*, *C. vaughani* and *A. batunai*. MbM was most abundant type in *M. undata*, *s*MpM-II was most abundant type in *H. rigida* and *A. batunai*, while MpM-I was the most abundant type in *C. vaughani*.

The *l*HI, which was present in *M. undata* and *C. vaughani*, and might be used for defense against predators and capturing food. Paruntu et al (2000) reported that the *l*HI is a specific nematocyst to planulae or young polyps and never found in adult colonies of *Pocillopora damicornis*, and suggested that it could be used for capturing food and defense against predators at the development early stages. Discharged *l*HI of *Pachyseris rugosa* mesenterial filaments were found on the surface of the target tissue of the coral, *Acropora nobilis*, and suggested that it might be used for both digestions of prey and interspecific aggression (Paruntu et al 2000; Paruntu & Darwisito 2016). The MpM-I, which was most abundant in *C. vaughani* might be related to the appearance tentacles of that coral. The fungia coral, *C. vaughani* had very clearly visible tentacles around its mouth. Paruntu et al (2000) stated that the MpM-I are distributed in tentacles of adult coral colonies. The MpM is in general considered as a defensive nematocyst and also used for capturing prey by penetration (Mariscal 1974). The *l*MpM-II was only present in *M. undata* and *C. vaughani*, and it could be used for both digestion of prey and interspecific aggression. Paruntu & Darwisito (2016) stated that the discharged *l*MpM-II of *P. rugosa* mesenterial filaments were found on the target tissue of the *A. nobilis*, and he suggested that it might be used for both digestion of prey and interspecific aggression. The present study showed that the MbM was only present and most abundant in adult colonies of *M. undata*, and it might be used for aggression against predators. Earlier researchers have attributed a defensive/aggressive role to the MbM type (Paruntu et al 2000). The *s*MpM-II were present in all coral species studied, and suggested that this type of nematocyst might be used for interspecific aggression and digestion of prey as reported by Paruntu et al (2000).

The sMpM-II and sHI have almost observed in all adult colonies of coral species studied except for the sHI was absent in *A. batunai*. The MpM-I was only presence in *C. vaughani* and *A. batunai*. The dimensions of the nematocysts were similar to each other except for the width of the sMpM-II capsules in *M. undata* was different from the other width of sMpM-II (Table 1). These indicated the possibility that the nematocysts sizes of the same type are not dependent on the different coral species in the present study, but those might be influenced by the stages of the development of the coral. Paruntu et al (2000) suggested that the nematocyst sizes of the same type of *P. damicornis* tend to be influenced by the developmental stages of the coral. Hereafter there was a big variation in the nematocyst sizes of MpM-II and HI found in adult colonies of *M. undata* and *C. vaughani*. There was a possibility remains that there are two classes of the sizes of MpM-II (*s*MpM-II and *l*MpM-II) and HI (*s*HI and *l*HI) in adult colonies of *M. undata* and *C. vaughani*.

Conclusions. This present study showed that the compositions of nematocysts are different among different coral species. All coral species studied had specific nematocyst to each other. A possibility remains that the sizes of the same type of nematocysts are not influenced by the different coral species. Future researches will be recommended to study more numbers of coral species about the biology of nematocysts which were not yet studied in the coral reefs of Sulawesi Sea, Indonesia, especially the coastal areas of the North Sulawesi and Gorontalo Provinces as parts of the center of coral reef biodiversity in the world.

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