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Thallus variation of *Sargassum polycystum* from Central Java, Indonesia

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Abstract. *Widyartini DS, Widodo P, Susanto AB. 2017. Thallus variation of* Sargassum polycystum *from Central Java, Indonesia. Biodiversitas 18: 1004-1011. Sargassum polycystum* C. Agardh has characteristic of highly polymorphic thalli among individuals in its population. The shape and size of thalli are variable among the individuals. The study was aimed at knowing the variation of *S. polycystum* thalli grown in different beaches of Central Java. The data showed that the thalli of the *S. polycystum* grown in the Karimunjawa had more variation than those grown in the Menganti Beach, as obviously seen on its leaves and vesicles. There was a relationship between salinity and pH of the Menganti Beach of the length of vesicle *S. polycystum*, but nitrate and phosphate content weren't correlated of variation thalli. Temperature, salinity, and pH of Karimunjawa Beach were correlated with the vesicle's color, nitrate and phosphate correlated of the width as well as upper and lower surfaces of the leaves, phosphate also correlated with the leaf edge and vesicle diameter. Environmental factors in the Menganti Beach had not significantly affected the thalli variation, but salinity and pH had an effect on vesicle length. Temperature, salinity, and pH in the Karimunjawa Beach significantly affected the vesicle color, nitrate and phosphate affect the width of leaves as well as their upper and lower surface. Phosphate also affected the edge of the leaf and the vesicle diameter

Keywords: Environmental factors, Karimunjawa Beach, Menganti Beach, thalli variations

INTRODUCTION

The *Sargassum* seaweeds have characteristic of highly polymorphic thalli among individuals in its population. Thallus variation temporary different in individuals, between individuals, the environment, and between local variations. These variations exist due to genetic polymorphism and environment (Cheang et al. 2008). Polymorphism thallus forms occur in the population in the habitat and the same time. Morphological variation in *Sargassum* range is quite large so that limits species and varieties are still confusing and cause difficulties in classification. *Sargassum* is the richest species amongst other algae. *Sargassum* mostly grows on the beach which characterized with coral reefs as its substrate (Guiry and Guiry 2010) and morphologically as Phaeophyceae most complex genera (Noikasar and Ajisaka 2009).

Distribution of the species is very widespread, especially in the tropics and subtropics formed a dense forest on the ocean floor (Mattio and Payri 2010). Although the taxonomy of *Sargassum* has focused since 1985 (Yang 1995; Wong 2004), but still need the observation of the systematics (Mattio et al. 2010). *Sargassum* is typically built up of a holdfast, stipe cylindrical or flattened by primary up to tertiary branching. Thallus leaves elliptic to oblong, the leaf edge dentate, wavy, and curved or tapered edges. Vesicles are rounded or oval, to float (Lin 2011).

Sargassum polycystum C. Agardh is one species of the brown seaweeds (Phaeophyceae), an alginate producer, an important chemical compound since it has high economic value. The alginate content is strongly related to part of the thalli being extracted, a method of extraction, and the seaweeds cultivation area (Zailanie et al. 2003; 2016). Based on morphological characters thallus *S. polycystum* distinguished on *S. polycystum* var. *linearifolium* Y. Chiang, *S. polycystum* f. *crinitum* Reinbold, f. *festivum* Grünow, f. *intercedens* Grünow, f. *proliferum* Grünow. (Guiry and Guiry 2016). Characterized varieties of distinguishing thallus leaves. Forma is the lowest level of the species, characterized by a character that is not important, such as colors.

The *Sargassum* is widely spread starting from the beach up to coral reefs along the littoral and sublittoral areas. They are perennial growing throughout the year. This seaweeds could either reproduce generatively by producing spores or alternatively through a part of the thallus; their dispersal however strongly depends on either weather or season (Saraswathi et al. 2003; Rao et al. 2014). In Indonesia, *S. polycystum* is quite dominant in the beaches with dominant coral reef substrates and strong tides. Coral reefs are the best place for the seaweeds to grow since the thallus could stick strongly holdfast so the thallus does not release easily from its substrate (Kadi 2005). LSD test showed that almost all quantitative characters differed significantly among three species studied (data not shown). However, no significant difference was observed between *S. ilicifolium* and *S. tenerrimum* for maximum leaf length and also between *S. ilicifolium* and *S. glaucescens* maximum of leaf length to leaf petiole length ratio although these characters were significant when all 3 species were analyzed together (Noormohammadi et al. 2011).

Environmental conditions will show different characteristics of the various thallus forms, the content of alginate and resistance to environmental factors limiting or its ability to produce metabolites. The content of the alginate is affected by the passage of the thallus extracted, the extraction method used and the place to grow (Zailanie et al. 2003; Rasyid 2010). All parts of the thallus have the ability to perform photosynthesis process although different, so the result is not the same alginate. Prabha et al. (2012) stated that seaweed morphology is strongly influenced by the physiological tolerance of seaweed adapt to environmental factors such as salinity, temperature, depth, nitrate and phosphate, pH and the waves as limiting factors in marine waters.

The purpose of this study is to determine the morphological thallus variations of *S. polycystum* of different beaches. The study of morphological variations can indicate the relationship of morphology, expression of genes and environmental factors grew to compounds alginate. This makes the foundation doing research using parts of the thallus *S. polycystum* derived from Karimunjawa and Menganti Beaches in Central Java.

MATERIALS AND METHODS

Study area

Sargassum polycystum samples were taken from Menganti and Karimunjawa Beaches, Central Java, Indonesia. Menganti is located in the Karangduwur village, Ayah Subdistrict, Kebumen District, Central Java, Indonesia. Geographically, the Menganti Beach lies at 109° 24′ 44,3″ E and 7° 46′ 21,5″ S and 0 m altitude. While Karimunjawa Beach in the northern part of Central Java, directly facing the South China Sea. This Karimunjawa area is geographically located between 5°40’39’-5°55’00’ S and 110°05’57’-110°31’15’ E. Administratively, belongs to the subdistrict of Karimunjawa, Jepara District, Central Java, Indonesia. Karimunjawa is 45 miles from Jepara or about 60 miles from Semarang. The current comes from the South China sea forces to the eastern tip with the velocity of 8 to 25 cm.sec^{-1} (Figure 1).

Menganti Beach in Kebumen, the southern part of Central Java, dealing directly with the Indian Ocean. Sea water clarity of 4,75 m, shallowness on the beach of 150 cm. Current velocity 0,6 m.sec^{-1}. Slope 7° wave tides of 1,5-2 m. Rainy season exists between October and April, while dry season during July to September. Seasonal distribution of seaweeds at Island was shown among the different season, species diversity was rich in the post-monsoon season followed by monsoon season and less in pre-monsoon season (Prathep 2005; Josephine et al. 2013). Geologically, the beach is cast material leads to affect the white sands color on the beach including a good panoramic view (Sidicq 2015) and steep slope. The base substrate in the form of steep cliffs Menganti Coast, rubble and sand as waves of loud collision of the Indian Ocean. Coral substrate showing that the area is stable and constantly traversed by strong currents. Menganti coastal waters temperature ranges between 31-35°C, ranging between 29-30‰ salinity, water pH range of 7-8 (Widyartini et al. 2012).

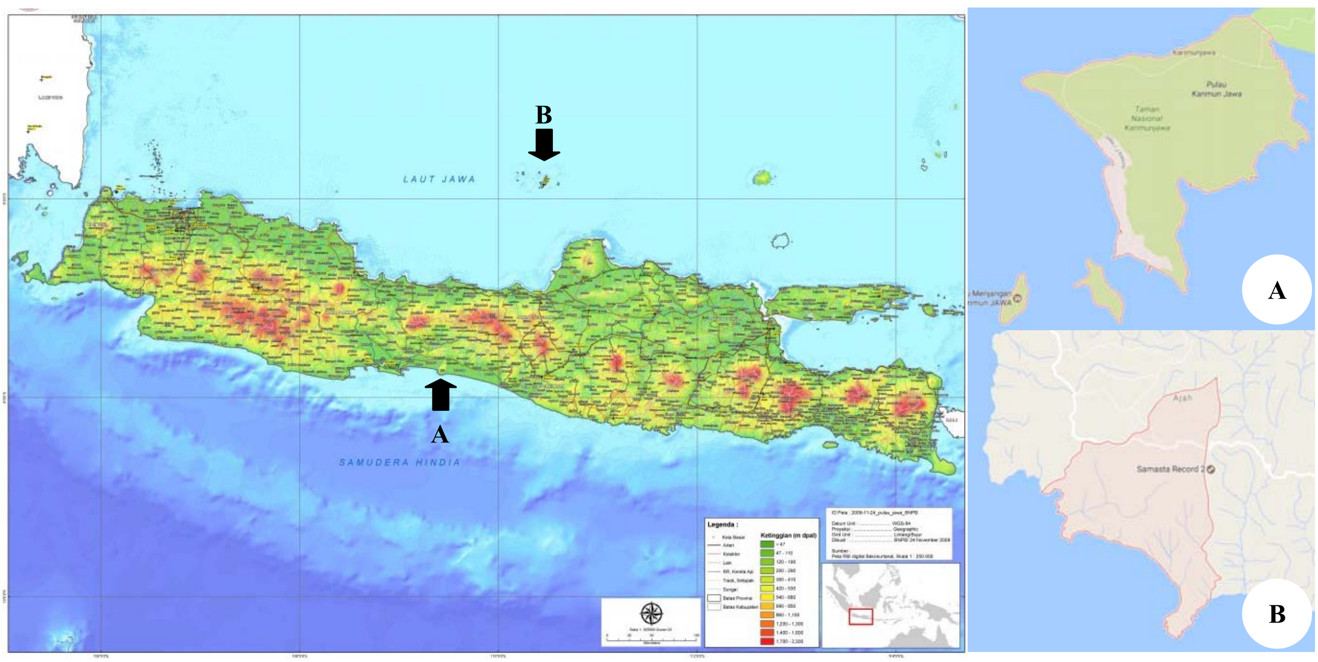


Figure 1. Location of Menganti Beach (A) and Karimunjawa Beach (B) in Central Java, Indonesia

The ocean currents are strong currents, consisting of tidal currents and nontidal currents. Flows on the Karimunjawa Beach derived from the Java Sea that brings water masses towards the Java Sea to the east. The velocities surface water flow in marine waters average ranges between 8-25 $cm.sec^{-1}$. Temperatures at the surface ranged from 28-29°C; salinity ranges between 28-29 ‰. coastal waters pH range 7-9 (BTNKJ 2004; Yuwono 2015; Dinda et al. 2012).

A survey method was used in this study with sampling was done in a selected randomized design applying a quadratic method. Transects were set along the sea shore with an interval of 10 m. each transect has three plots at the $1m^2$ size. The *S. polycystum* seaweeds obtained within the transects was then described fully including its thalli. Samples were taken at three stations in Menganti Beach (Figure 2) and in Karimunjawa Beach (Figure 3).

High waves in coastal waters of Menganti is greater than those on Karimunjawa Beach. Differences in wave movement affect the habitat and how to grow seaweed. *S. polycystum* seaweed thrives on the substrate stable rock or coral. *Sargassum* growing in waters Menganti attached to the surface of the rock, visible when waters recede. *Sargassum* in the waters Karimunjawa stick to the basics and always stagnant waters. Waters with a smaller stream with small tidal influence. The current study noted if the growth of the *S. polycystum* in these two areas were different, the thallus of the Menganti Beach were stuck strongly to the reefs (holdfast), to make a good panoramic view like a brownish rug during the low tide. In contrast, *S. polycystum* seaweeds grow on the north (Karimunjawa), which characterized with the small tide, the thallus are stick on the reefs on the seabed as its substrate to make long thallus which appears on the sea's surface (Figure 4).

The coral reefs are the best place to strong-stick the thallus (*holdfast*) to allow the thallus keep on its substrate when there is high tide. Along the Indonesian beaches, the *S. polycystum* seaweeds almost dominated beaches areas characterized with stable reefs substrate and high tides (Kadi 2005; Montesinos et al. 2008)). The *Sargassum* seaweeds disperse in almost all tropical and subtropical beaches, most of them grow in the shallowing reefs or even become the benthos or pelagic to the reefs (Boaden 1995). The brown alga, *S. polycystum*, was widely distributed and found at all sites, while *Padina* and *Acanthophora* were the most abundance species during the dry season (Josephine et al. 2013).

Procedures

Sampling of S. polycystum

In order to minimize some temporary and seasonal effects and to keep the natural morphology, all specimens were collected at the same growing period, to before the seaweeds loss their leaves and develop its reproduction structures. Samples were placed spatially (10m interval) by means to minimize individual grouping from the same plant like germlings *Sargassum* which has a short dispersal range. For the morphological analysis purpose, of each population, 30 full individuals were collected. Specimen from both places (Menganti-Kebumen) and (Karimunjawa-Jepara) were taken during the low tide to allow easy dive.

Observation of thallus morphology

All individual specimens were observed for their morphology based on 14 morphological characters like branch-thallus, leaves, and vesicles. Length, width, and diameter of each parameter were observed by applying such equipment with 0.01 mm accuracy. Such characters like leaf's edge, a form of the particular individual are grouped as morphological category parameters and scored. Leaves and vesicles were taken randomly.

Observations of environmental parameters

Parameters of temperature (°C), salinity ($^o/_{oo}$), pH were observed on the growing areas directly. While Nitrate and phosphate content were analyzed in the environmental laboratory of Unsoed. Chlorophyll, Fe, and Mg content were analyzed in the Laboratory of Plant Physiology, Universitas Jenderal Soedirman, Purwokerto, Indonesia.

Data analysis

Data of morphological variations were then performed in a table of characters, the matrix with their multivariate ones. Data of environmental factors and thallus morphology were analyzed with the statistical program of SPSS 16.0 which was used to know the correlation between them and the GLM in order to differentiate environmental factors of the Menganti-Kebumen and Karimunjawa-Jepara beaches toward the thalli morphological variations.

RESULTS AND DISCUSSION

Morphological analysis

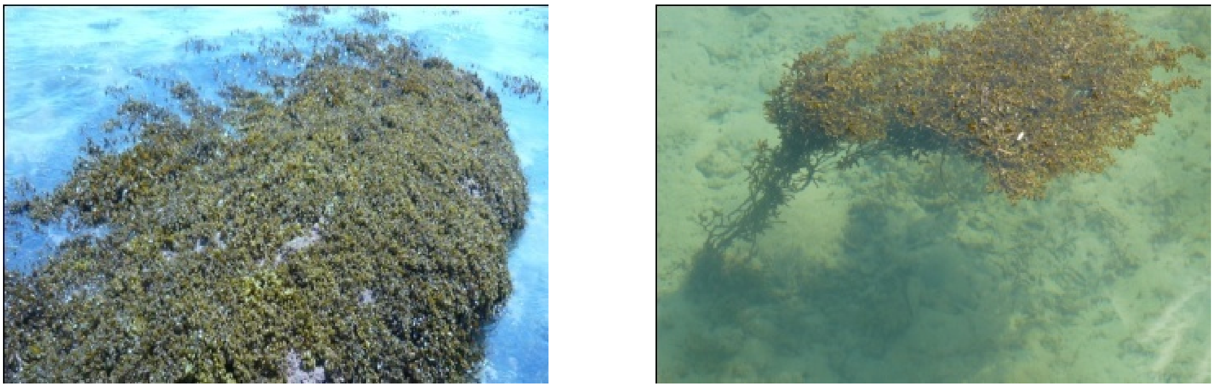
The *Sargassum* seaweeds are characterized with very polymorphic thalli. The high polymorphisms exist in inter-individuals intra-population. There are some variations in the form and size of thallus branch, leaves, and vesicles within its population. The *Sargassum* thallus has such a leaf-like formation called as phylloid, air bladder or vesicle, fake branch (stalk) or stipe and *holdfast* in discoid. Individual, intra-individual, environment and local variations differences on size as well as other variations exist only temporarily. Morphological analysis of the thalli of *S. polycystum* seaweeds from Menganti-Kebumen and Karimunjawa-Jepara Beaches showed if there were variations in form and size of the stipe-thalli, leaves, and vesicles (Figure 5). Visually, it could be distinguished if the *S. polycystum* seaweeds from the Menganti Beach has brown-greenish to dark brown thalli in compared with that of from the Karimunjawa Beach which has light brown. The last group of seaweeds thalli has more fucoxanthin pigments on its thalli than the first group. The brown seaweeds contain chlorophyll a, b, and fucoxanthin, which consists of violaxanthin, flavoxanthin-a, and neo fucoxanthin-b, xanthophyl to make the brown effect on the thalli (Megayana et al. 2011; Limantara and Heriyanto 2010).

A B C

Figure 2. The station sampling in Menganti Beach, Kebumen, Central Java, Indonesia. A. Station I (eastern tip), B. Station II (next to the TPI), C. Station III (western tip).

A B C

Figure 3. The station sampling in Karimunjawa Beach, Jepara, Central Java, Indonesia. A. Station I (left of the pier), B. Station II (right of the pier), C. Station III (far from the pier).



A B

Figure 4. Thalli on its habitat in Menganti and Karimunjawa Beaches, Central Java, Indonesia. A. Holdfast on the reefs at Menganti Beach, B. Stipe on the seabed's reefs at Karimunjawa Beach

Different environmental factors of these two beaches may cause differently to the organisms live there due to nutrition content in these areas. Toward the life of seaweeds, distribution of nutrition leads to variation in thalli and so its alginate content. The seaweeds dispersal is related with its genetic character and environment (Cheang et al. 2008). The *Sargassum* seaweeds are abundantly grown along the beach with coral reefs substrate, with enough sunshine, shallowness of 4 m with the strong current, the salinity of 28-35 ‰, pH 6-8, and temperature of 25-32 ^{0}C (Ibrahim et al. 2014; Raymundo et al. 2007). This species of seaweeds are dispersed widely, including beaches and reefs in the littoral and sublittoral areas. They grow along the year, perennial but its dispersal is affected by season (Kadi 2005) and (Saraswathi et al. 2003). The reef is a strong sticking point holdfast so thallus not easily crashing waves. Coral as a stable habitat that brown seaweed holdfast able to stick with better than green and red seaweeds, *Padina*, *Sargassum*, and *Turbinaria* grew dominant (Ammar 2011; Domettila et al. 2015).

Leafy-thalli of *S. polycystum* from the Menganti Beach has an oval to long form (Figure 6) with the length varies from 1.6-3.8 cm, width 0.8-1.5 cm, dark brown with black spots. Meanwhile, the leafy thalli of the same seaweeds from the Karimunjawa Beach are oval to long with the length varies between 1.5-4.5 cm, width of 0.5-1.3 cm with light brown and black spots. The leaf's tip obtuse to truncate. The leaf's edge obtuse to truncate. The leaf's base truncates to obtuse. Leaf's upper and lower surface are brownish spots (Figure 7).

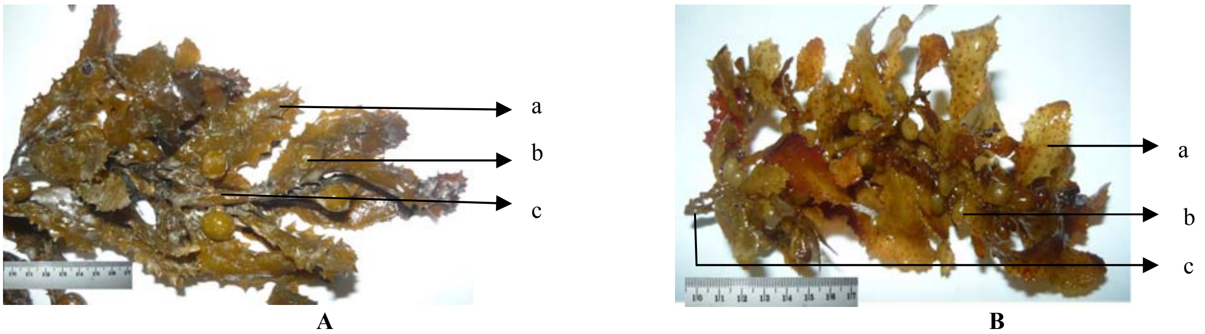


Figure 5. The *S. polycystum* thalli of Central Java, Indonesia. A. Menganti BeachB. Karimunjawa. a. Leaf, b. Vesicle, c. Stipe

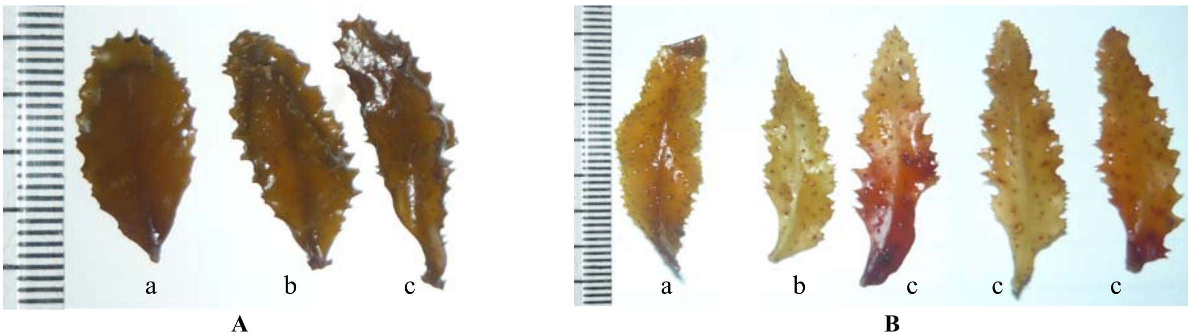


Figure 6. Leavy thalli of the *S. polycystum* of Central Java, Indonesia. A. Menganti, B. Karimunjawa. a. Ovale, b. Elliptic, c. Oblongate

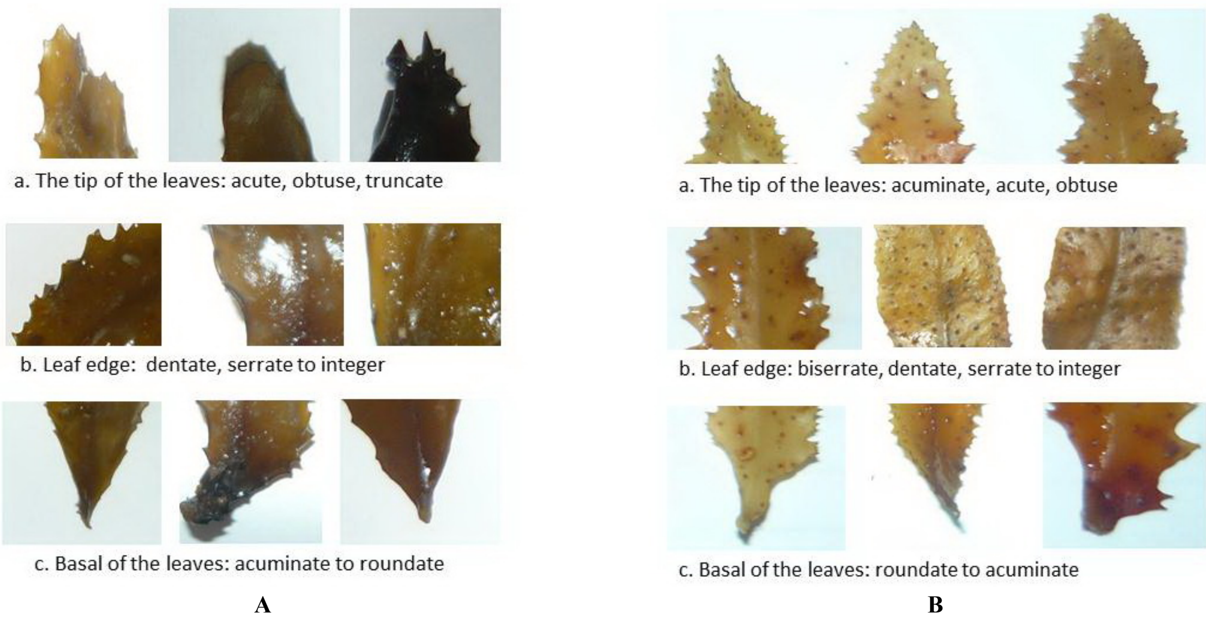


Figure 7. The shape of thalli leaves of the *S. polycystum* of Central Java, Indonesia. A. Menganti, B. Karimunjawa

Stipe of the *S. polycystum* taken from the Menganti Beach is cylindrical, rough, with the length of 2-34 cm, and dark brown. Meanwhile, the stipe-thalli of *S. polycystum* from the Karimunjawa Beach are cylindrical with the length of 6.8-47 cm and light brown color. The stipe-thalli of Karimunjawa *Sargassum* is also branch where the branch from the first branch then second, etc. The main stipe-stick on the deep seabed with stagnant water. The thallus of the main branch has a bigger diameter and darker color than the following branches. The thalli of Menganti *Sargassum* mostly are having the sole branch, primary branch, only one individual out of 30 individuals has a secondary branch (Cheang et al. 2008).

Vesicles of the Menganti *S. polycystum* are round with 0.1-0.9 cm diameter, dark brown but the one from Karimunjawa Beach has round to oval and 0.1-1.3 cm diameter and light brown in color (Figure 8). Vesicles are specific organ to help the seaweed's thallus buoyancy so this organ is completed with pneumatocyst or bladder, air bubbles contain CO_2 or O_2.

Correlation analysis between Menganti environmental parameters and thalli variations showed a correlation between salinity and pH toward the variations in thalli ($p<0.05$) with the coefficient correlation of (r) = 0.416. There is also a correlation between salinity and pH to the vesicle's length. Meanwhile, analysis correlations between environmental factors of the Karimunjawa to the thalli variation showed there is a significant relationship between water temperature, salinity, pH and colour of the vesicles ($r=0.390$ and $p=0.033$); nitrate and phosphate content to the leaf width, upper and lower surfaces of the leaf thallus; and the phosphate content also corresponds to the leaf's edge and vesicle diameter ($p<0.05$) (Mayakun and Prathep 2005).

Data analysis of effect of environment to environment parameters using a GLM (*General Linear Model*) program showed a nonsignificant effect between water temperature, salinity, pH, nitrate and phosphate contents toward variations of *S. polycystum* thalli ($F_{calc}<F_{table}$ and $p>0.05$), but salinity and pH had an effect on vesicle length. The environment parameters of the Karimunjawa, however, showed a significant effect of the variations of the thalli ($F_{calc}<F_{table}$ $p<0.05$). There is a significant effect of water temperature, salinity, and pH to the vesicle's color, the content of nitrate and phosphate affects the length, width, edges, as well as the upper and lower surface of the seaweed S. polycystum seaweed ($F_{calc}>F_{table}$ and $p<0.05$).

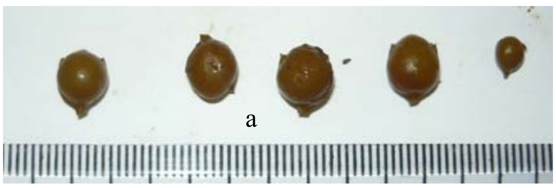
Generally speaking, leafy and stalky thalli, as well as the vesicles of *S. polycystum* from the Menganti Beach show a shorter, thicker and darker color than that of grown in the Karimunjawa Beach. This phenom might probably come from the quality of the environmental factors with higher water temperature and so high tides (Table 1).

The differences in their environment factors like salinity, shallowness, nitrate and phosphate content, pH, tides, lead to cause different growth rate as well as the sea weed's quality since they belong limiting factors of the sea weed's to grow optimally. The extreme environmental factors, however, lead to disturbing the seaweed to grow optimally, since they disturb reproduction phases, slowing down the growth rate and even causing death.

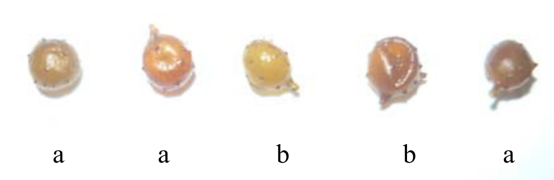
Morphological characters strongly depend on the physiological tolerance of the seaweed to adapt with its environmental factors like water temperature, salinity, and pH. Sea tides are also important factors in seaweed's growth since they affect the seaweed's existence in a particular area (Harley et al. 2012). The water temperature, however, is the main factor affecting the seaweed's growth. Increase water temperature may reduce the amount of dissolved oxygen content leads to affect photosynthesis processes of the seaweeds (Haas et al. 2014).

Table 1. Observation of physical chemistry parameters of the Menganti and Karimunjawa Beaches of Central Java, Indonesia

Environment parameters	Menganti Beach	Karimunjawa Beach
Water temperature (ºC)	31-35	28-29
Salinity (‰)	29-30	28-29
pH	6-7	7-8
Nitrate (ppm)	0.485-0.876	0.089-0.670
Phosphate (ppm)	0.438-0.499	0.334-1.162
Chlorophyll content ($mg.L^{-1}$)	23.11-26.32	16.07-18.03
Fe ($mg.L^{-1}$)	not detection	not detection
Mg ($mg.L^{-1}$)	1.779-2.096	0.779-2.165
Water clarity (m)	4.75	6-7
Shallowness (m)	1.50	2.31
Current velocity ($m.sec.^{-1)}$)	0.6	8-25
The tides (m)	1.5-2.0	0.2-0.45



A



B

Figure 8. Vesicles of *S. polycystum* of Central Java, Indonesia. A. From Menganti Beach, B. From Karimunjawa Beach. A. rounded, B. Ovale

Variations in water temperature also caused surface uses sea weed's dispersal in a particular area (Lin 2011), they can grow on the range of temperature between25-35ºC, and optimally at 25ºC. Optimum phosphate content in the water is 0.1-0.2 $mg.L^{-1}$, while nitrate content lies between 0.1-0.7 $mg.L^{-1}$ (Gutov et al. 2014). The too low water temperature, however, may affect the sea weed's physiological activities like stopping biochemical processes in the thallus, on the contrary, too high water temperature disrupt its enzyme and disturb biochemical processes in the thalli. Meanwhile, the too high or too low salinity level of a particular area will disrupt the osmosis processes of the seaweeds. Adaptation capacity of the seaweeds in facing the fluctuation of water salinity, by adjusting ions concentrations in the thalli to keep the concentration outside and inside of the thallus still balance (Lin 2011).

Differences in water salinity lead to variations in colonization and morphological characters of the seaweeds (Choi et al. 2010). The range for optimum salinity varies between 33-40 ppm. Water pH is also one of the most important growing factors of the seaweeds. The pH level will affect total carbon molecules can be used by the sea weed's photosynthesis, optimum pH for optimum growth is 7, if the water pH is < 6, so the carbon molecules will be in dissolved CO_2 or carbonic acids and when the pH varies between 6-10, the carbon molecules will dissolve in bicarbonate ions HCO^{3-}, and carbon will be in the form of CO_3^{2-}if the water pH > 10 (Lin 2011; Litaay 2014).

Wave tides were also noted as one of several environment factors affecting the seaweeds habitat. *Sargassum* grows in intertidal zone with high and low tides and sublittoral zone by sticking its thallus to the strong substrate through its holdfast or buoyancy on the sea surface. Most of the *Sargassum* grow in coral reefs especially in the sand flat which dried, sandy, and having life and death corals during the low tide. In the low water temperature, Sargassum will grow with a larger size but different form (Boaden 1995; Choi et al. 2010). The red seaweeds pf *G. corticata* species taken from the low intertidal area has low antioxidant activities characters due to environmental stresses characters like improper water temperature, or low level of sunshine. On the other hand, brown seaweed of *S.tenerrum* taken from middle intertidal showed a higher antioxidant activity due to the low level of environmental stress (Yuwono 2015). Coral reef communities are in a state of change throughout their geographical range, factors contributing to this change include bleaching or the loss of algal symbionts, physical damage, and disease and increasing abundance of macroalgae (Ostrander et al. 2000; Raymundo et al. 2007; Andrew et al. 2008). Overfishing and nutrient loading have altered interactions among macroalgae and their herbivores, leading to significant increases in macroalgal cover (Yñiguez et al. 2008; Bahartan et al. 2010; Lapointe and Bedford 2010). The increased abundance of macroalgae negatively affects coral growth and recruitment, and this has long-term consequences on the physical structure of the reef (Costa et al. 2008; Andrew et al. 2008: Hafting et al. 2012).

In conclusion, the thallus of leaves and vesicles *S. polycystum* from Karimunjawa were more varied than those from the Menganti Beach. There was a correlation between water salinity and pH in Menganti Beach of the length of the vesicle *S. polycystum* ($p<0.05$) where the coefficient of correlation is (r)=0.416, but nitrate and phosphate content weren't correlated to the vesicle length. Whereas water temperature, salinity, and pH in Karimunjawa Beach were correlated with the vesicle's color (r=0.390 and p=0.033), nitrate and phosphate affect width as well as upper and lower surfaces of leaves, and phosphate also affects so the thallus edge and vesicle diameter ($p<0.05$). Water temperature, as well as the nitrate and phosphate contents in Menganti Beach, had no significant effect on thallus variation of *S. polycystum*, but salinity and pH had an effect on vesicle length. Whereas water temperature, salinity, and pH in Karimunjawa Beach have a significant effect on vesicle color, the content of nitrate and phosphate affects the width as well as the upper and lower surface of leaves. The phosphate also affected the edge of the leaf and the vesicle diameter of the *S. polycystum* ($F_{calc}<F_{table}$ $p<0.05$).

The general conclusion, thalli variation are influenced by growing environment, so polymorphism always occurs both in individuals and among individuals in *Sargassum*. Therefore, to convince the boundaries of a species need other supporting data, such as anatomy and genetic variation. The polymorphism species is influenced by genetic and environmental traits. According to Tentahen et al. (2011) analysis of the brown seaweed genome, *Ectocarpus siliculosus* use of genes for real-time PCR shows that both groups of these genes are the original operon and regulate alginate as a single non-glucose carbon source. These observations strongly support that genes participate for the detection and synthesis of alginates.

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