Diversity and Distribution of Natural Populations of *Eucheuma* J. Agardh and *Kappaphycus* Doty In Nusa Tenggara Barat, Indonesia

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ABSTRACT The genera *Kappaphycus* Doty and *Eucheuma* J. Agardh are amongst the most economically-important seaweeds in Indonesia. Several species of *Kappaphycus* and *Eucheuma* are cultivated in many farms in Indonesia including Nusa Tenggara Barat (NTB). However the cultivated *Kappaphycus* is influenced by environmental changes. Variation in seaweed germplasm is necessary in order to produce elite lines with required quantity. More than 10 wild specimens of *Eucheum/Kappaphycus* species are obtained in the NTB marine waters, including *E. spinosum* (green and brown), *E. striatum* (green and brown), *Eucheuma/Kappaphycus* sp-*1* and *Eucheuma/Kappaphycus* sp-*2*. Distribution of the species in NTB has been mapped, showing that Lombok Island has more diverse species of *Eucheuma* compared to Sumbawa Island. In addition, morphological and physiological characterisation of the species have been undertaken, and it is observed that there are morphological differences in the same species obtained from different coastal zones, and thus indicating morphological plasticity of the species to different environmental conditions.

(**Keywords:** West Nusa Tenggara, *E. spinosum, E. striatum, E. edulis, E. serra,* natural diversity, seaweeds)

INTRODUCTION

The genera *Kappaphycus* Doty and *Eucheuma* J. Agardh are currently considered as the most economically important seaweeds in the world. The two genera serve as raw materials for carrageenan, a major component of dietary fibre [1] which is used as stabilizer, gelling agent, thickener, binder and additive in various food and pharmaceutical industries [2]. Today *Kappaphycus alvarezii* is a major cultivated carrageenan-producing species in Indonesia followed by *K. striatum* and *Eucheuma spinosum.* In addition, the production and processing of these species have become a highly valuable and profitable livelihood activity in many marginal seafarming communities in Indonesia including West Nusa Tenggara.

It is suggested that the two most commercially cultivated species of *K. alvarezii s*train Tambalang and *K. alvarezii s*train Moumere are introduced species from the Philippines. The seedlings of *Kappaphycus and Eucheuma* are obtained only by vegetative propagation, and therefore the same strains dominate seafarming in Indonesia. *K. alvarezi* has been introduced to more than twenty countries with two reported cases where *Kappaphycus* plants have spread from the introduction sites and adversely impacted the native habitats [3]. In addition, it is suggested that *K. alvarezii* is less adaptive to environmental changes and impacts of clmate change, as indicated by the occurrence of thallus bleaching and thus production reduction of *K. alvarezii* by more than 50% [4] when the seawater temperature increased by 2°C in Nusa Lembongan, Bali. Similarly, the National seaweed production in Indonesia also decresased in 2010. Therefore, the use of elite and more adaptive varieties developed from native species will be more favourable to environmental variation. Such strain is yet to be available.

This paper presents the diversity and distribution of *Eucheuma* and *Kappaphycus* collected from cultivation sites and from the wild populations of the NTB marine waters. Nusa Tenggara Barat is a province in central Indonesia with two main and many small islands, and located between the Wallace's and Weber's Lines. This information is important for both understanding the seaweed biodiversity in the NTB as well as contributing to the development of elite *Kappaphycus* and *Eucheuma* strains for cultivation.

MATERIALS AND METHODS

Eucheuma and *Kappaphycus* samples were collected from various locations around Nusa Tenggara Barat. The cultivated samples were obtained from various seaweed farms in Lombok and Sumbawa islands (Pengantap, West Lombok,; Gerupuk, Central Lombok; Serewe, East Lombok; Teluk Ekas, East Lombok; Kertasari, West Sumbawa; Labuhan Mapin, Sumbawa; Terano, Sumbawa; Hu'u Dompu, and Teluk Waworanda, Bima). Wild samples were collected from locations away from seaweed farms and they were collected from Gili Indah (Gili Air, Gili Meno and Gili Trawangan – North Lombok), Gili Genting and Bangko-Bangko (West Lombok), Tanjung Ann and Kute (Central Lombok), Teluk Ekas, Labuan Haji, Pulur (East Lombok). Teluk Kaung (Sumbawa) and Jelenge (West Sumbawa). The samples were observed for general morphology, then preserved as herbaria and for carrageenan content determination. Carrageenan (semi-refined carrageenan) was extracted according to method described by Misha et al. (2006) with some modification. To extract the semi-refined carrageenan, 3 g. of dried-seaweed was placed in a jar containing 150 ml of KOH solution (pH 9.0) (1:50, w/v), and heated in a water bath at *ca.* 90°C for 3 hours with frequent stirring. The mixture was filtered, and the supernatant was placed again in a water bath for another 2 to 3 hours until the volume was reduced by half. The supernatant was cooled at room temperature for 1 hour, and the gel was precipitated with 225 ml of ethanol (96%) (1: 3, v/v), and incubated overnight. Semi refined carrageenan was separated by filtration and dried at 60°C for 2 days.

All molecular work (DNA isolation, amplification *cox 2-3 spacer,* and sequencing) was undertaken at the Institute of Ocean and Earth Sciences (IOES, University of Malaya, Malaysia). Isolation of genomic DNA, amplification of mitochondrial *cox2-3 spacer* molecular marker and sequencing was undertaken as described by [5].

RESULTS AND DISCUSSION

Kappaphycus and *Eucheuma* from cultivation areas in NTB .

Survey of cultivated species in NTB resulted in 10 specimens which can be grouped into seven cultivated varieties, *i.e* six varieties *of Kappaphycus* and one variety of *Eucheuma*. They are *K. alvarezii* Tambalang brown, *K. alvarezii* Tambalang green, *K. alvarezii* Moumere brown, *K. alvarezii* Moumere green, *K. striatum* brown, *K. striatum* green, and *E. spinosum* (Table 1, Figure 1 to 5). Based on these collections, the distribution of cultivated and wild *Kappaphycus* and *Eucheuma* in Nusa Tenggara Barat are mapped for Lombok Island and is presented in Figure 8.

The morphology of cultivated *K. alvarezii* obtained from different locations is quite similar, with few morphological variations occurring in color and size depending on the location. For example, the thallus size and height of plants cultivated in Lombok Island were smaller than those in Sumbawa Island. In addition the variant obtained in Lombok Island has lighter color than the variant obtained from Sumbawa Island. Similarly, *K. alvarezii* strain Tambalang green obtained from Gerupuk (Central Lombok) has lighter color than that obtained from Pengantap (West Lombok) (Table 4, Appendix 1). More distinct morphological differences can be seen between *K. alvarezii* Tambalang and *K. alvarezii* Moumere. The Moumere has bigger, blunt-ended thallus with nodulation (tumor-like nodule) on the thallus surface while the Tambalang has smooth surface and thallus with pointed end.. It has also fewer branches than the *K. alvarezii* Tambalang. The *K. striatum* has also very similar characteristics but is different from the *K. alvarezii* Tambalang and Moumere. The *K. striatum* has lighter color, short thallus, more branches and dichotomus- or trichotomus-type of branches while *K. alvarezii* has verticullate-type of branches with long thallus. The green *K. striatum* was obtained in three cultivated areas, namely Pengantap (West Lombok), Serewe (East Lombok) and Kertasari (West Sumbawa) while the brown one was obtained only from Pengantap (West Lombok). With regards to *E. spinosum*, quite similar morphology was seen between the variant cultivated in Pengantap and Serewe, with both having spines and cartilaginous thallus.

Ecotype of *Kappaphycus* and *Eucheuma* from NTB .

Survey of wild species in NTB resulted in nine specimens which can be grouped into five ecotypes of *E. spinosum* and four uncharacterized *Eucheuma/ Kappaphycus* variants (Table 3, Figure 6 and 7). The *E. spinosum* can be recognized by spines observed in the branch, and they are quite similar in morphology with cultivated species with some variation in color, size and branch type. The other uncharacterized groups are similar to *Eucheuma* and *Kappaphycus,* and they are

Table 1. Cultivated species of *Kappaphycus* and *Eucheuma* in Nusa Tenggara Barat

No	Location	Species
1	Pengantap, West Lombok	1. *E. spinosum* 2. *K. striatum* (brown) 3. *K, striatum* (green) 4. *K. alvarezzii* Tambalang (green)
2.	Teluk Gerupuk, Central Lombok	1.*K. alvarezii* Moumere 2. *K. alvarezii* Tambalang brown 3. *K. alvarezii* Tambalang greem
3.	Serewe, East Lombok	1. *K. alvarezii* Tambalang brown 2. *E. spinosum* 3. *K. striatum* green
4.	Teluk Ekas, East Lombok	1. *K. alvaresii* Tambalang brown 2. *K. alvarezii* Moumere
5.	Kertasari, West Sumbawa	1. *K. striatum* green
6.	Labuan Mapin, Sumbawa	1. *K. alvarezii* Tambalang brown
7.	Terano, Sumbawa	1. *K. alvarezii* Tambalang brown
8.	Hu'u Lakey Dompu	1. *K. alvarezii* Tambalang brown
9.	Teluk Waworoda, Bima	1. *K. alvarezii* Tambalang brown

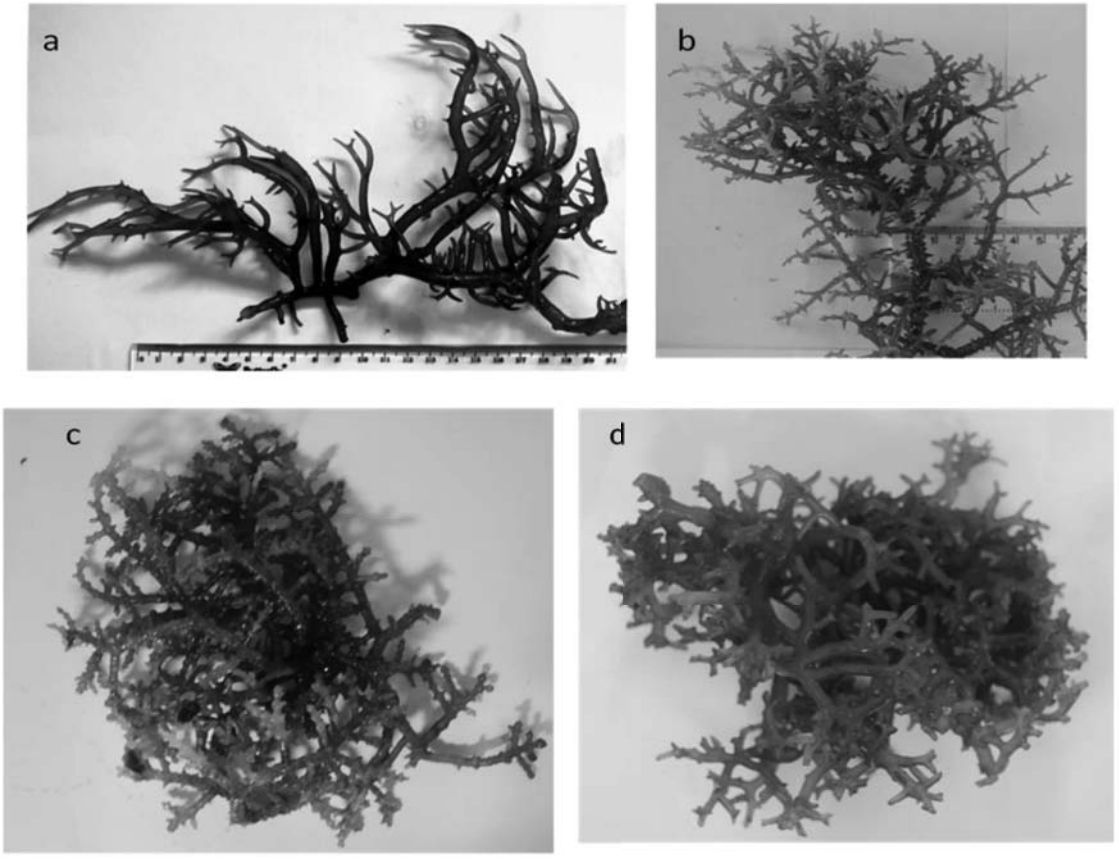

Figure 1. Four varieties of *Eucheuma and Kappaphycus* collected from cultivation areas in Pengantap, West Lombok are *Kappaphycus alvarezii* Tambalang green (a), *Eucheuma spinosum* (b), *Kappaphycus striatum* brown (c) and *Kappaphycus striatum* green (d).

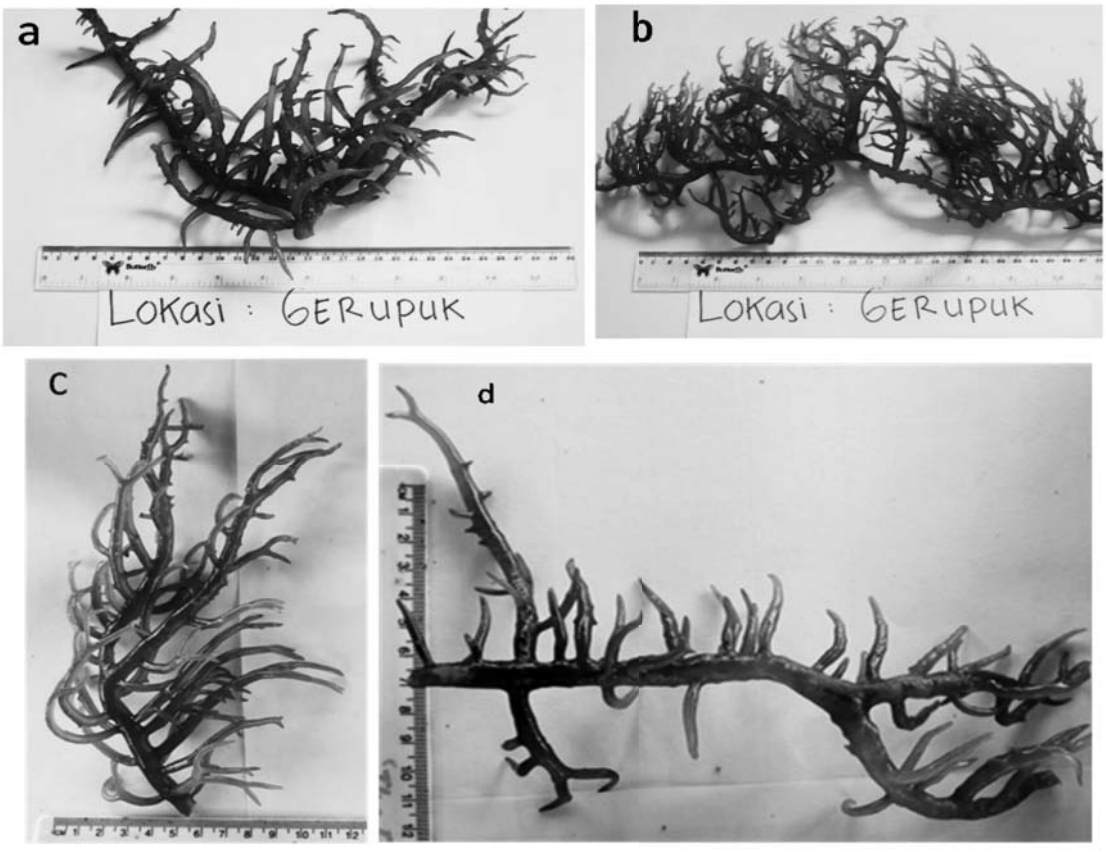

Figure 2. Three varieties of *Kappaphycus* collected from cultivation areas in Gerupuk, West Lombok are *Kappaphycus alvarezii* Moumere brown (a), *Kappaphycus alvarezii* Tambalang brown (b) and *Kappaphycus alvarezii* Tambalang green (c). Sometimes the *Kappaphycus alvarezi* Tambalang have a combined colour of brown and green (d).

Figure 3. Two varieties of *Kappaphycus* collected from cultivation areas in Teluk Ekas, East Lombok are *Kappaphycus alvarezii* Tambalang brown (a) and *Kappaphycus alvarezii* Moumere brown (b).

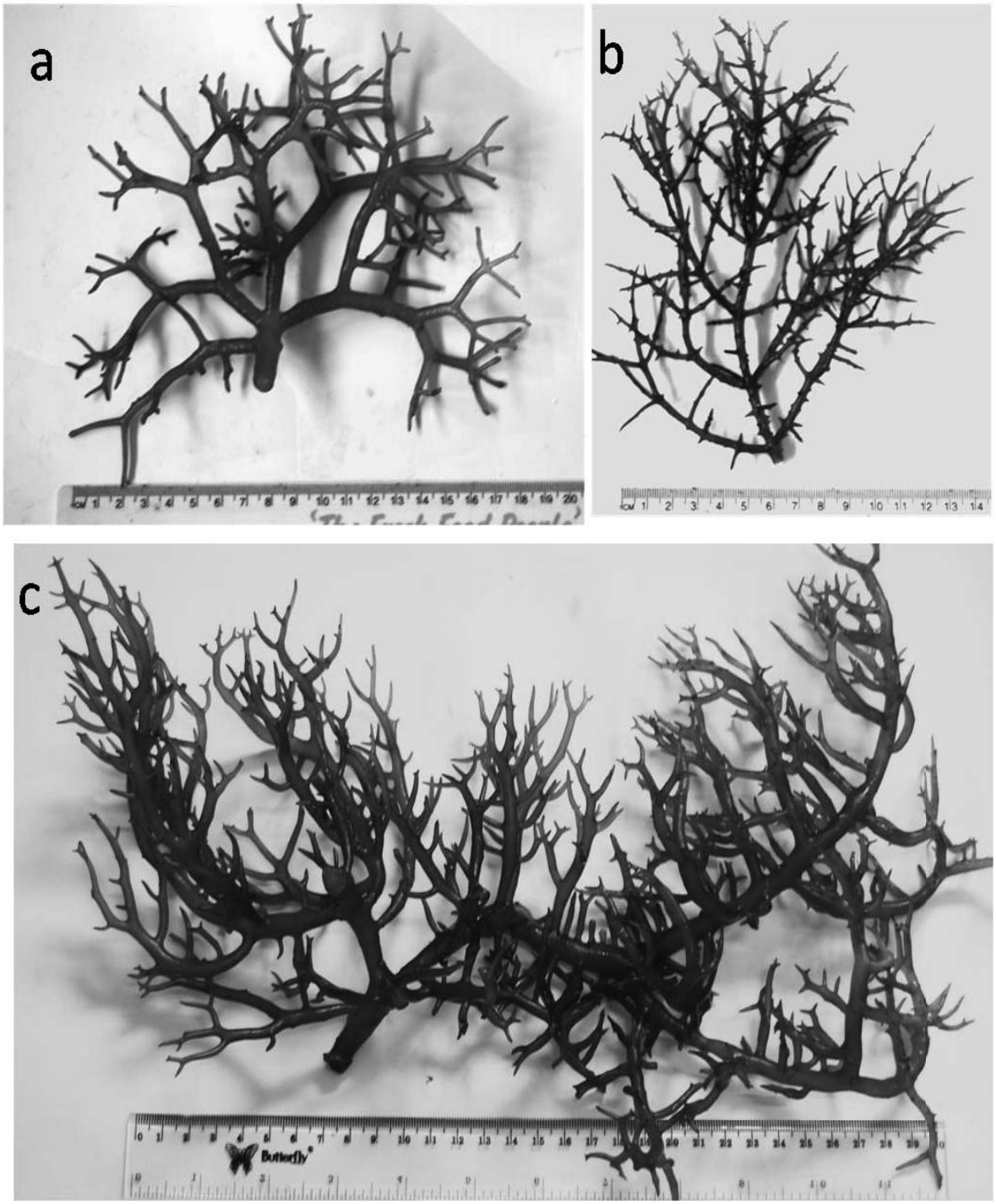

Figure 4. Three varieties of *Eucheuma and Kappaphycus* collected from cultivation areas in Serewe, East Lombok are *K alvarezii* Tambalang brown (a), *K. striatum* green (b), and (c) and *E. spinosum* (c).

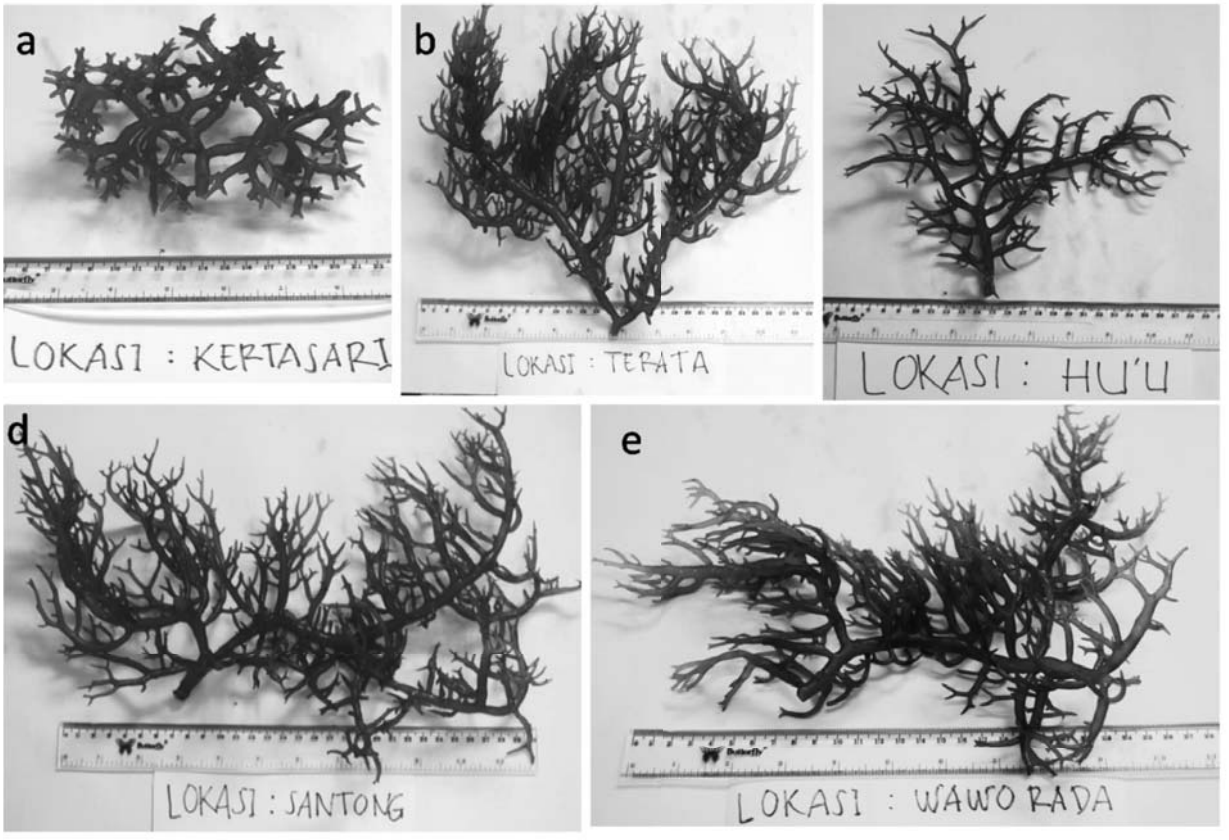

Figure 5. Three varieties of *Kappaphycus* collected from cultivation areas in Sumbawa Island are *K striatum* green (a; Kertasari, West Sumbawa), K. *alvarezii* Tambalang brown (b; Terata – Sumbawa), *K. alvarezii* Tambalang brown (c; Hu'u, Dompu), *K. alvarezii* Tambalang brown (d; Teluk Santong, Sumbawa), and *K. alvarezii* Tambalang brown (e; Teluk Waworada, Bima).

grouped with the ecotype obtained in Ekas Bay and subjected to molecular characterization.

Analysis of semi-refined carrageenan indicates that the carrageenan content of all samples are between 40 to 50% of dry-weight (Figure 9). Similar quantities of carrageenan was obtained from different variants of *K. alvarezii* Tambalang (brown and green), *K. alvarezii* Moumere, *K. striatum* (green and bown), *E. spinosum* (cultivated and wild). The carrageenan contents were not analyzed for *Eucheuma/Kappaphycus* sp. 1 and sp. 3 since there were inadequate samples available for the extraction at the time of this experiment.

Molecular Characterization

There is difficulty in characterization of the ecotypes based on morphological characters, therefore the use of mitochondrial *cox2-3* spacer molecular marker for identification of wild *Eucheuma/Kappaphycus* in NTB was attempted. DNA was isolated from eight samples of cultivated and wild *Eucheuma/Kappaphycus* in NTB including *E. spinosum* (cultivated Serewe), *E. spinosum* (wild Ekas), *K. striatum* green (cultivated Pengantap), *K. striatum* brown (cultivated Pengantap), *Eucheuma/Kappaphycus* sp. 2 (Jelenge, Sumbawa), *Eucheuma/Kappaphycus* sp. 3 (Jelenge, Sumbawa) and *Eucheuma/Kappaphycus* sp. 4 (Kaung, Sumbawa). The DNA (6 bands of ca. 300 bp) obtained (Figure 10) were sent for gene sequencing. The contiqs generated from sequences (Table) were blasted against NCBI nucleotide (nt/nr) database. Blast analysis of the tree sequences reveled that they have very high similarities (97-100%) with different isolates of *E. denticulatum* (ascension numbers JN980403.1; JX624081; JN234758; JN234756; JX624082; AY687429 and many more sequences of *E. denticulatum* in the GeneBank database).

The survey on *Kappaphycus* and *Eucheuma* in Nusa Tenggara Barat revealed that the cultivated variant in

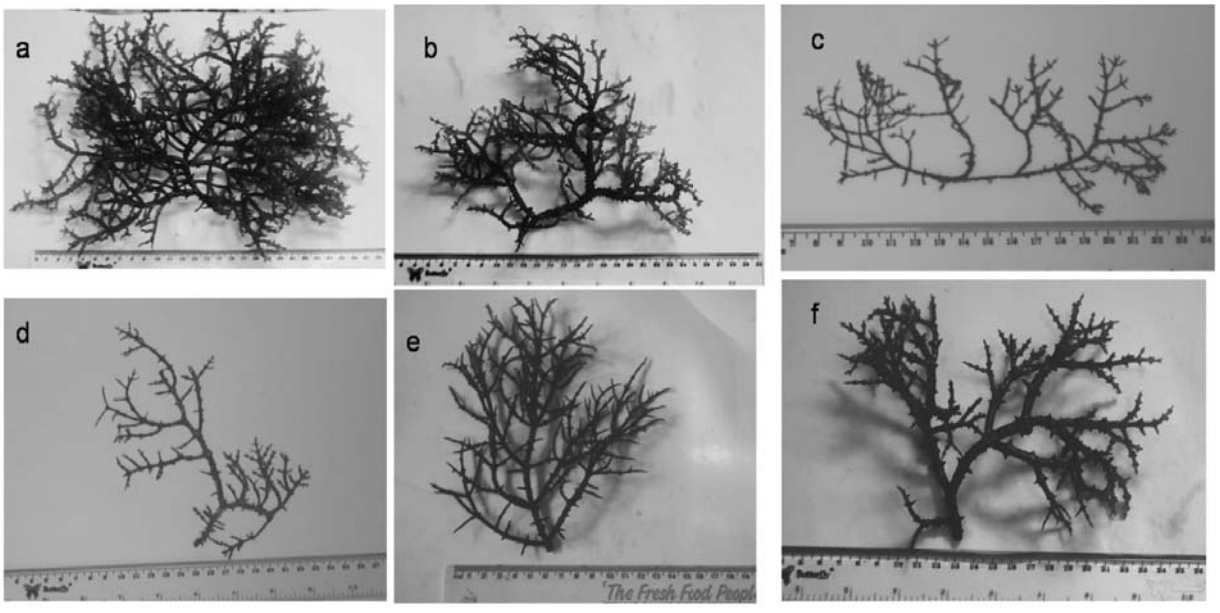

Figure 6. Six ecotypes of *E. spinosum* collected from Nusa Tenggara Barat, at Gili Indah, North Lombok (a), Pengantap,West Lombok (b), Bangko-Bangko, West Lombok (c), Gili Genting, West Lombok (d), Tanjung Ann, Central Lombok (e) and Teluk Ekas, Est Lombok (f)

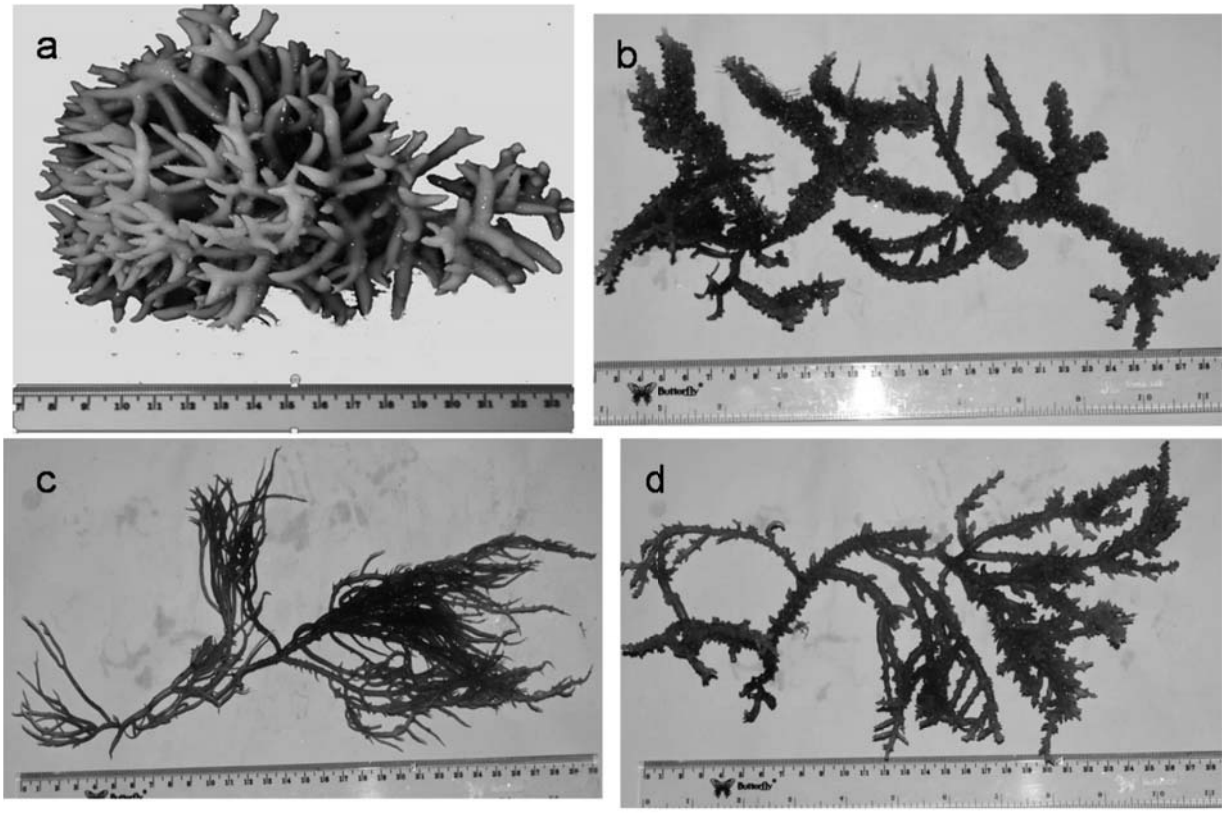

Figure 7. Four ecotypes of uncharacterized *Eucheuma/Kappaphycus* from Nusa Tenggara Barat are *Eucheuma/Kappaphycus* sp. 1 (a; Pulur, East Lombok), *Eucheuma/Kappaphycus* sp. 2 (b; Jelenge, West Sumbawa), *Eucheuma/Kappaphycus* sp. 3 (c; Jelenge, West Sumbawa), *Eucheuma/Kappaphycus* sp. 4 (d; Kaung, West Sumbawa).

Table 2. Ecotypes of *Kappaphycus* and *Eucheuma* varieties obtained in Nusa Tenggara Barat

No	Location	Species
1	Gili Indah (Gili Genting, Gili Meno and Gili Terawangan; West Lombok	*E. spinosum*
2	Gili Genting	*E. spinosum*
3	Bangko-bangko	*E. spinosum*
4	Tanjung Ann (Central Lombok)	*E. spinosum*
5	Teluk Ekas (East Lombok)	*E. spinosum*
6	Pulur (East Lombok)	*Eucheuma/Kappaphycus* sp.1
7	Jelenge (Sumbawa)	*Eucheuma/Kappaphycus* sp.2
		Eucheuma/Kappaphycus sp.3
8	Teluk Kaung (Sumbawa	*Eucheuma/Kappaphycus* sp.4

Table 3. Characteristics of ecotypes of *Kappaphycus* and *Eucheuma* varieties obtained in Nusa Tenggara Barat

No	Sample	Varian	Thallus morphology
1	*E. spinosum*	Spinosum (Gili Indah)	Thallus color is brown. It has robust, hard and cartilaginous thalli with pointed end. The thallus is cylindrical with irregular-type branches, has no nodule and pointed spines which later develop into branches. The diameter of primary and secondary thalli is less than 0.3 cm. The height of primary thalli is less than 10 cm. There are more than 15 and 25 spines in primary and secondary thalli respectively. The height of branches is less than 10 cm while the height of spine is between 0.4-0.5 cm and distance between each spine is more than 0.05 cm. Holdfast is present.
		Spinosum (Gili Genting)	Thallus color is brown. It has robust, hard and cartilaginous thallus with pointed end. The thallus is cylindrical with irregular-type branches, has rough surface, has pointed spine which later develop into branches. The diameter of primary and secondary thalli is less than 0.3 cm. The height of primary thalli is less than 10 cm.. There are more than 15 and 25 spine in primary and secondary thalli respectively. The height of branches is more than 10 cm while the height of spine is between 0.3-0.5 cm and distance between each spine is less than 0.05 cm . Holdfast is present.
		Spinosum (Bangko-Bangko)	Thallus color is light brown. It has robust, hard and cartilaginous thallus with pointed end. The thallus is cylindrical with verticillate branches, smooth surface, has pointed spines which later develop into branches. The diameter of primary and secondary thalli is less than 0.3 cm. The height of primary thalli is less than 10 cm.. There are less than 10 spines in primary thallus and more than 15 spines in the branch. The height of branches is more than 10 cm while the height of spine is between 0.3-0.5 cm, distance between each spine is less than 0.05 cm . Holdfast is present.

		Spinosum (Tanjung Ann)	Thallus color is light brawn with some dots, hard and cartilaginous thallus with pointed end. The thallus is cylindrical with verticillate branches, smooth surface, has pointed spines which later develop into branches. The diameter of primary and secondary thallus is less than 0.3 cm. The height of primary thalli is less than 10 cm.. There are less than 10 spines in primary thalli and more than 15 spines in the branch. The height of branches is more than 10 cm while the height of spines is between 0.3-0.5 cm, distance between each spine is less than 0.05 cm . Holdfast is present.
		Spinosum (Teluk Ekas)	Thallus color is greenish brown, hard and cartilaginous thallus with pointed end. The thallus is cylindrical with verticillate branches, smooth surface, has pointed spines which later develop onto branches. The diameter of primary and secondary thalli is less than 0.3 cm. The height of primary thalli is less than 10 cm. There are less than 10 spines in primary thalli and more than 15 spines in the branch. The height of branches is more than 10 cm while the height of spine is between 0.3-0.5 cm, distance between each spine is less than 0.05 cm. Holdfast is present.
2	*Eucheuma/ Kappaphycus sp-1*	Pulur (East Lombok)	Thallus color whitish brawn, hard and thallus with pointed end. The thallus is cylindrical with irregular-type branches appearing in the main thallus, rough surface and has no spines and sub-branches. The diameter of primary and secondary thalli is more than 0.5 cm. The height of primary thalli is less than 10 cm and secondary thalli is less than 5 cm. Holdfast is present.
3	*Eucheuma/ Kappaphycus sp-2*	Jelenge (West Sumbawa)	Cystocarpic, thallus color brawn, hard and cartilaginous thallus with blunt end. The thallus is cylindrical with irregular branching, few branches, rough surface and has blunt and dense spines. The diameter of primary and secondary thallus is more than 0.5 cm. The height of primary thallus is about 10 cm and secondary thallus is less than 7 cm. Holdfast is present.
	Eucheuma/ Kappaphycus sp-3	Jelenge (West Sumbawa)	Thallus color brown, hard and cartilaginous thallus with blunt or pointed end. The thallus is cylindrical with verticillate branches, smooth surface, has irregular spines and sub-branches. The diameter of primary and secondary thallus is less than 0.5 cm. The height of primary and secondary thalli is more than 10 cm. Holdfast is present.
	Eucheuma/ Kappaphycus sp-4	Teluk Kaung (Sumbawa)	Cystocarpic, thallus color brown, hard and cartilaginous thallus with blunt end. The thallus is cylindrical with irregular branching, rough surface, and has blunt spines, dense spines and sub-branches. The diameter of primary and secondary thallus is more than 0.5 cm. The height of primary and secondary thalli is about 10 cm. Holdfast is present.

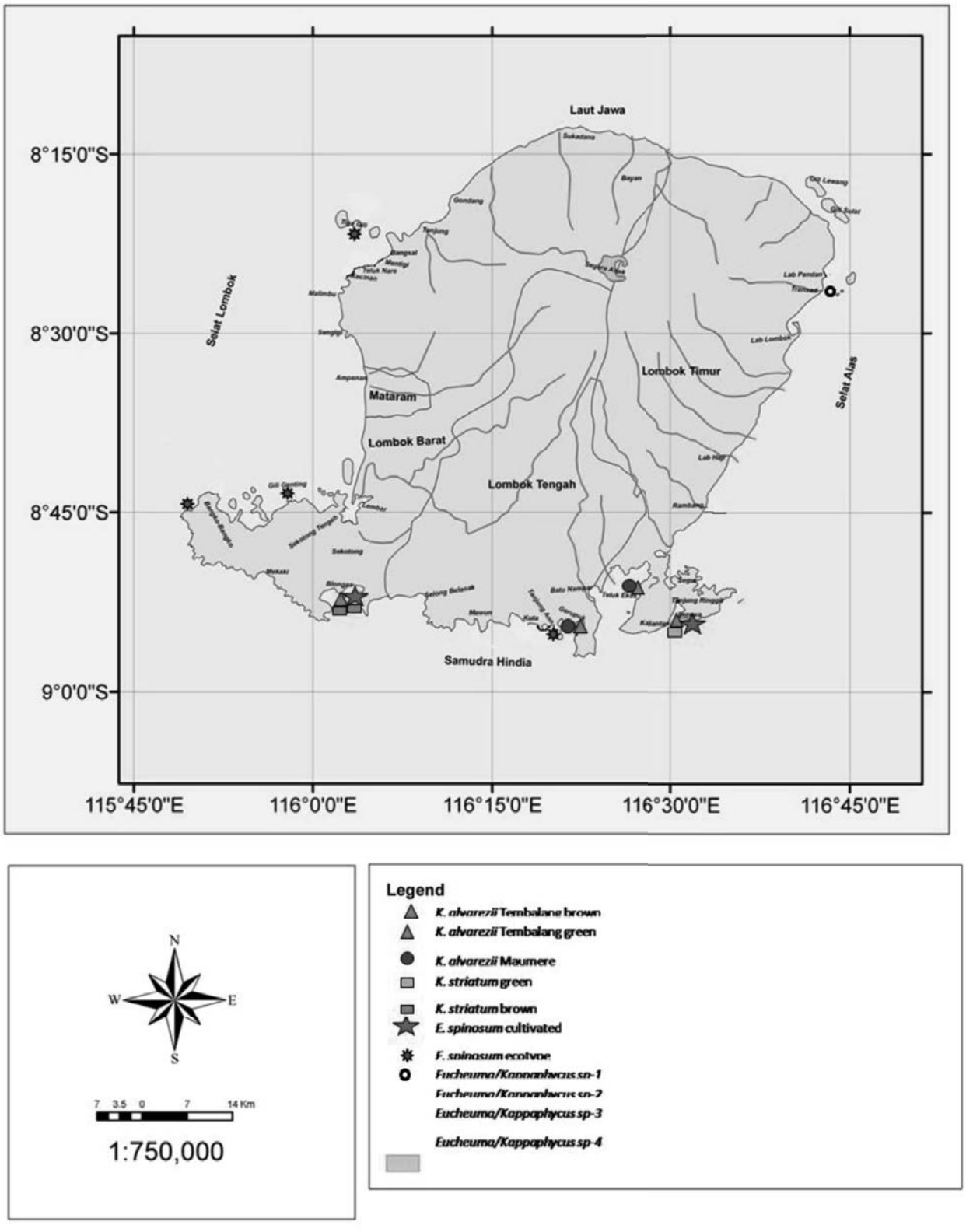

Figure 8. Distribution of of *Kappaphycus* and *Eucheuma* varieties in Nusa Tenggara Barat

Lombok Island is more variable than that in Sumbawa Island. The seaweeds farmed in Lombok Island comprise of *K. alvarezii* (Tambalang brown and green as well as well as Moumere), *E. stiatum (*green and brown), and *E. spinosum*. In Sumbawa Island, there is no *E. spinosum* cultivation. The morphology of cultivated *Kappaphycus alvarezii* strains are somewhat similar, with some variation in color, shape and size of thallus and clumps. The phenotypic plasticity of seaweed as a response to environment, including *Kappaphycus* and *Eucheuma*, have been well documented [6] [7]. However within the cultivated species, the *Kappaphycus* strain in Nusa Tenggara Barat can be easily identified as there are few morphological variations; this is probably due to quite similar environmental conditions in the cultivation areas. This is also reflected in the similar quantities of carrageenan produced by the species at different locations in Nusa Tenggara Barat. In addition, cultivated *Kappaphycus* can be separated from the *K. striatum* and *E. spinosum* due to distinct morphological

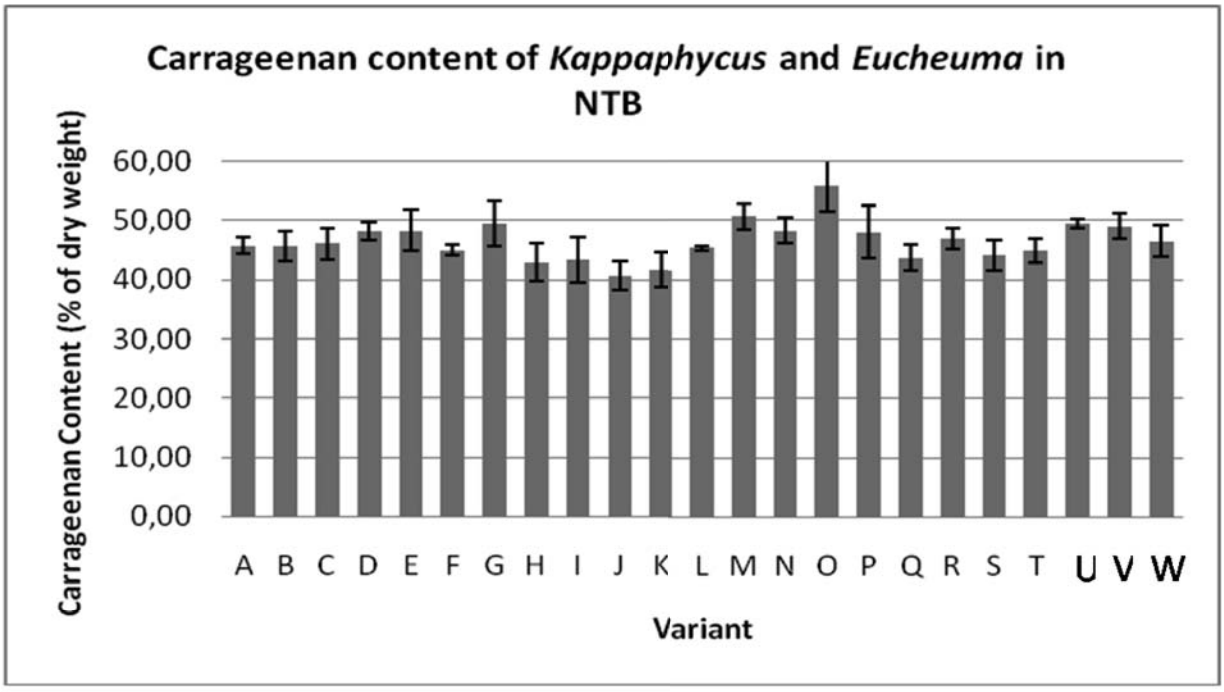

Figure 9. Amplified *cox2-3* spacer from cultivated/wild specimens of *Kappaphycus/Eucheuma* from NTB. Line 1: 1KB plus DNA ladder, line 2 to 9 *E. spinosum* and *K. striatum* samples from NTB.

differences between the three varieties. Difficulties in identification were experienced with the ecotypes (wild specimens). It was initially suggested that there are more than one species of *Eucheuma/Kappaphycus* obtained in Lombok Island, and the Lombok ecotype may differ from the Sumbawa ecotype as there is much variation in the morphology (Figure 8). However, molecular analysis of mitochondrial *cox 2-3* spacer reveals that the ecotype obtained in Lombok and Sumbawa Islands are essentially the same species of *Eucheuma denticulatum*. Interestingly, the ecotype and the cultivated strain (as cultivated in Serewe Bay and called "spinosum" by the locals) are essentially *Eucheuma denticulatum*. The presence of cultivated *Eucheuma denticulatum* in Lombok Island (in Ekas Bay and Serewe bay) has been reported previously [5], [8], [9], [10] and the sequence of *E. denticulatum* as reported in this paper is very similar to those previously reported by [5],[8],[9], [10]**.** However, to the best of our knowledge, the presence of the same species in Sumbawa Island has not been reported previously. Further study is now underway to assess the genetic diversity of other wild species of *Eucheuma* and *Kappaphycus* in Nusa Tenggara Barat.

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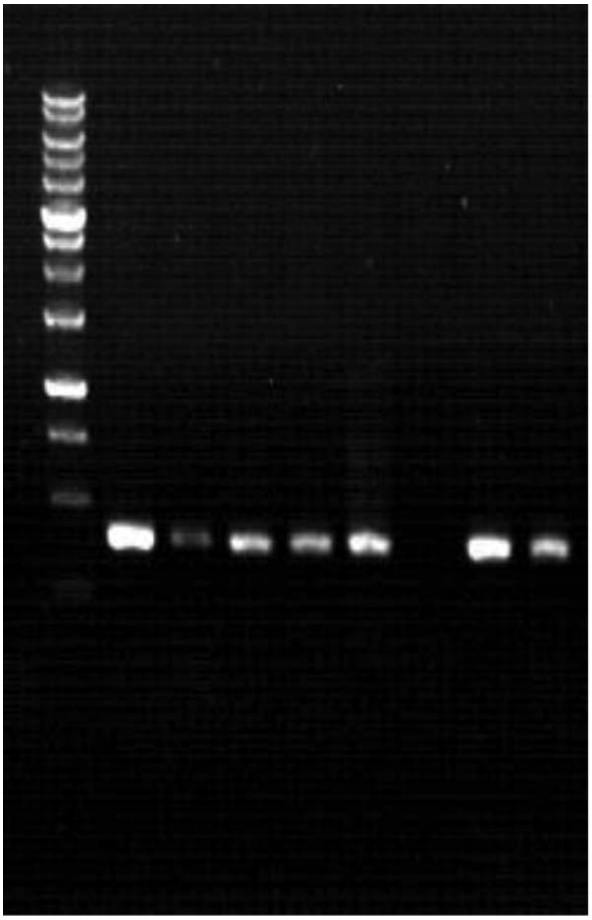

Figure 10. Amplified *cox2-3* spacer from cultivated/wild specimens of *Kappaphycus/Eucheuma* from NTB. Line 1: 1KB plus DNA ladder, line 2 to 9 *E. spinosum* and *K. striatum* samples from NTB

Table 4. Characteristics of cultivated *Kappaphycus* and *Eucheuma* varieties obtained in Nusa Tenggara Barat

No	Sample	Varian	Thallus morphology
1	*E. spinosum*	Spinosum (Pengantap)	Thallus color is light brown. It has robust, hard and cartilaginous thallus with pointed end. The diameter of primary and secondary thallus is less than 0.5 cm. There are more than 10 branches in primary thallus and primary thallus height is less than 10 cm. The thallus is cylindrical with fverticillate branches, has no nodule and blunt spines which later develop into branches. The height of branches is less than 10 cm while the height of spine is between 0,4-0,5 cm and distance between each spine is more than 0.05 cm . Holdfast is present.
		Spinosum (Serewe)	It has a dark brown, cartilaginous, cylindrical, robust and hard thallus with pointed-end. The thallus is cylindrical with rough spinal surface, verticillate branches, has no nodule and blunt spines which later develop into branches. The diameter of primary and secondary thallus is less than 0.5 and 0.2 cm respectively. Each bunch is 21-25 cm wide and 16-20 cm in height. There are about 11 to 15 spines in primary thallus and primary thallus height is between 11 to 20 cm. The height of branches is less than 11-20 cm while the height of spine is between 0.2-0.3 cm and distance between each spine is between 0.05 to 0.3 cm. Holdfast is absent.
2.	*K. alvarezii*	Tambalang green (Pengantap)	It has a dark green, cartilaginous, cylindrical, robust and smooth thallus with pointed-end. The thallus is cylindrical with irregular-type of branching, has no spine but has nodule and holdfast. The diameter of primary and secondary thallus is more than 1 cm . Each bunch is 16-20 cm in wide and 16-20 cm in height. The height of primary thallus is between 16 and 20 cm. The height of branches is between 11-20 cm.
		Tambalang green (Gerupuk)	The thallus is light green, cartilaginous, cylindrical, robust and smooth thallus with pointed-end. The thallus is cylindrical with irregular-type of branches, has no spine but has nodule and holdfast. The diameter of primary and secondary thallus is more than 1 cm. Each bunch is 16-20 cm wide and 16-20 cm in height. The height of primary thallus is between 20 and 30 cm. The height of branches is between 11 and 25 cm.
		Tambalang brown (Gerupuk)	It has light brown, cartilaginous, cylindrical, robust and smooth thallus with pointed-end. The thallus is cylindrical with irregular-type of branches, has no spine but has nodule and holdfast. The diameter of primary and secondary thallus is more than 1 cm. Each bunch is 11 - 15 cm wide and 16-20 cm in height. The height of primary thallus is less than 20 cm. The height of branches is between 11 and 25 cm.