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Research Article

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Genetic Structure of The Dominant Tropical Seagrasses *Cymodocea rotundata and Enhalus acoroides* in Southern Philippines for Conservation Management

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Abstract

Large-scale genetic population study of the two dominant tropical species of seagrass is conducted in Mindanao, southern Philippines. The goal of the study was to understand population genetic status of the dominant tropical seagrass species, Cymodocea rotundata and Enhalus acoroides for appropriate management. Population genetics structure for the 15 sites was performed by using polymorphic microsatellite markers. The results showed that the clonal richness was high in E. acoroides than C. rotundata. The largest genet found in the study was at Rizal, northern Mindanao wherein throughout the sampling area (i.e. 300 x 40 m) only one genet was identified for C. rotundata. The mean FIS (coefficient of local inbreeding) values was positive (heterozygous deficit) with some sites deviated from Hardy-Weinberg Equilibrium. Isolation by distance (IBD) was detected in C. rotundata ($P<0.05$) but not in E. acoroides ($P=0.253$), with the Mindanao ocean currents influenced genetic connectivity and structure. Genetic differentiation did not show any relationship between the geographical location and distance exhibiting high FST values for E. acoroides (0.183) and C. rotundata (0.205). The floating, buoyant fruits of E. acoroides may play a role in their long-distance dispersal; however, such dispersal is not frequent. Almost all of the seeds and fruits of C. rotundata are derived from self-recruitment in the natal meadow. This study suggests that C. rotundata and E. acoroides populations possess a weak genetic connectivity, and that the persistence of the meadow is threatened due to the low genetic diversity and high degree of population isolation.

Keywords: Microsatellite Markers, Tropical Seagrass, Diversity Hotspot, Mindanao Currents, Flowering Plants

Introduction

Seagrasses are flowering plants living their full life cycle submerged in shallow oceanic and estuarine waters in all coastal areas of the world except Antarctic region [1]. They are currently divided into 5 families: Hydrocharitaceae, Cymodoceaceae, Posidoniaceae, Zosteraceae and Ruppiaceae including 12 genera, divided into approximately 60 species. About half of the species are tropical and half are temperate [2].

The Tropical Indo-Pacific is the region of the highest seagrass biodiversity, with many species often found in mixed meadows that have no clear dominant species [2]. The tropical seagrasses *Cymodocea rotundata* and *Enhalus acoroides* are dominant species and widely distributed in the Indo Pacific region. They are important, foundational seagrass species that can colonize diverse environments, and is often present in areas affected by coastal development and high anthropogenic activity. The abundance of each species differs from one area to another and is influenced by habitat and hydrodynamic regimes [3].

The Western Pacific Ocean is considered the evolutionary "center of origin" of seagrasses [4]. Seagrasses in this region are highly prolific and occur in mixed-species stands of great biodiversity and complexity [2]. Along this region, the Philippine Archipelago,

is the center of global marine shorefish diversity and is believed to be the area where seagrasses originally evolved and genetic structure are influenced by major ocean water currents [5].

It is becoming clear that ecological and genetic processes are inseparable when attempting to preserve the biodiversity of an ecosystem. Managing genetic structure can be vital to successful conservation and restoration efforts. Population genetic structure over a range of local to geographic scales provide useful indicators of natural history, contemporary changes and offer new projections under environmental disturbances [6]. Therefore, knowledge of the levels and distribution of genetic variation in population is a prerequisite for the establishment of effective and efficient conservation practices of each species.

The genetic approaches to answering ecological questions have become more efficient, powerful and flexible, and thus more widespread. On the other hand, in tropical regions, many studies on seagrass beds and mostly general mapping of their extent have been carried out in particular locations but knowledge and information on genetic aspects were meager and yet, for the tropical seagrasses C. rotundata and E. acoroides have remain unstudied in Southern Philippines. This present study examined the genetic distinctiveness of the two dominant species C. rotundata and E. acoroides populations living on the different habitats in the coastal areas to determine whether they are of particular conservation value in the context of environmental gradient-induced range shifts. To provide baseline genetic information for this foundational species, the microsatellite (SSR) markers were utilized to investigate the genetic diversity, genetic structure and extent of clonality spanning the southern Philippines.

The genetic diversity and differentiation of C. rotundata, and E. acoroides across a distance of approximately 2100 km in the western Pacific has been investigated in the previous study [3,7]. However, a regional scale analysis is more useful for adaptive ecosystem management and the establishment of effective MPAs. In this study, population genetic analysis of C. rotundata and E. acoroides was conducted by using polymorphic microsatellite markers in the extant seagrass meadows in Mindanao (Fig. 1). It is hypothesized that geographical, oceanographic, and environmental factors influence the genetic structure and migration of seagrasses at a regional scale in the Philippines, the north area of the "coral triangle", center of seagrass habitat with abundant seagrass biomass and high species diversity [2]. The Mindanao Ocean Current which bifurcates from the North Equatorial Current (NEC) of the Western Pacific Ocean is believed to influence the seagrass genetic structure in their distributional ranges.

Materials and Methods

Sampling and assessment

Samples were collected from 10 provinces in southern Philippines namely: Misamis Oriental, Misamis Occidental, Zamboanga del Norte, Agusan del Norte, Davao del Norte, Davao Oriental, Surigao del Norte, Surigao del Sur, Sarangani and Tawi, comprising twenty (20) municipalities. Of the 20 sampling sites fifteen (15) extant seagrass meadows were considered for genetic analysis because in some areas the target species were very few forming patches (Figure 1). About 20-40 samples of vegetative shoots of target species were randomly collected by a minimum of 10 m to avoid overestimating of clonal diversity spanning the extent of ~200–300 m × 30–40 m for each collection site [6,8]. A young, fresh leaf from each sample shoot was selected and preserved using silica gel in a zippered plastic bag at room temperature until genetic analysis. In total, 525 samples were collected for each target species or 1,050 for the two species from 15 populations. DNA extraction and genetic analyses such as genetic diversity and genetic structure were performed in the University of Tokyo, Japan.

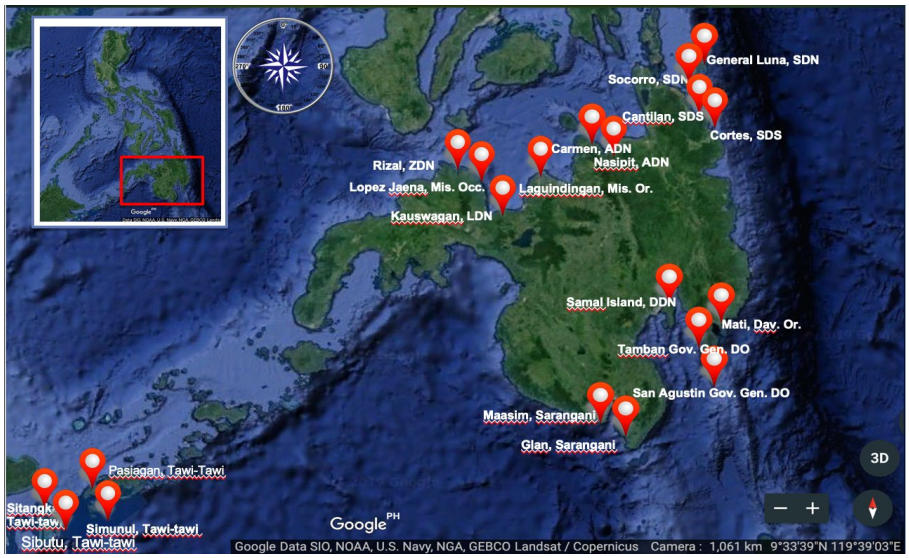


Figure 1: Map showing the twenty municipalities in southern Philippines of which fifteen sites of extant seagrass beds of the two-dominant species *Cymodocea rotundata* and *Enhalus acoroides* are considered and being sampled for genetic study.

DNA extraction and microsatellite analysis

Total genomic DNA extraction from the silica-gel-dried leaf tissue (< 10 mg) was performed using a modified cetyltrimethylammonium bromide (CTAB) protocol described by Lian et al (2003). Genotypes of each sample were analyzed and scored for seven microsatellite (SSR) markers for *C. rotundata* (CR004, CR015, CR027, CR032, CR039, CR040 and CR152) developed by Arriesgado et al (2014), and six SSR markers for E. acoroides (Eaco001, Eaco009, Eaco050, Eaco051, Eaco054 and Eaco055) developed by Nakajima et al (2012). The tailed primer method was used to perform the polymerase chain reaction (PCR), and was added with U19 (5′-GGTTTTCCCAGTCACGACG-3′) to the 5′ end of each primer pair [9]. Multiplex PCR amplifications was performed using a GeneAmp PCR 9700 thermocycler (Applied Biosystems). A 5-µL reaction mixture contained < 30-ng template DNA, 2× Type IT Mastermix (QIAGEN), and 0.2 µM (final concentration) of each of three primers for each locus: forward primer, reverse primer with U19 tail, and U19 primer fluorescently labeled with FAM, VIC, NED, or PET. Thermal cycling conditions were 95˚C denaturation for 5 min, followed by 32 cycles of 95˚C for 30 s, 58˚C for 90 s, 72˚C for 1 min, and a final extension of 60˚C for 30 min. The PCR products were diluted 5- to 20-fold with sterile water, and pooled for simultaneous fragment analysis in 9 µL of denatured formamide (HiDi; Applied Biosystems Corp) with size standard GS500-LIZ on an ABI PRISM 3130 xl genetic analyzer (Applied Biosystems). Allele sizes were scored using GENEMAPPERTM analysis software version 4.1 (Applied Biosystems), and visual validation.

Statistical analysis

Marker resolution and genotypic indices

The number of alleles (NA), expected heterozygosity (HE) and observed heterozygosity (HO) were evaluated in FSTAT ver. 2.9.3.2 using SSR markers developed from Cymodocea rotundata and Enhalus acoroides. Deviation from Hardy-Weinberg equilibrium (HWE) was calculated using 1,000 permutations in FSTAT [10]. The combined exclusion probabilities of all SSR markers were determined using CERVUS ver. 3.0.3 [11]. If more than one copy of the same multilocus genotype (MLG) was identified, the null hypothesis of the same MLG being obtained repeatedly by chance was tested through sexual reproduction with Genclone ver. 2.0 [12]. This test is based on calculating the probability of obtaining MLGs from sexual events, taking into account the estimated FIS for the population. Clonal richness (R), a measure of the proportion of unique genotypes in the population, was calculated as R = (G-1)/(N-1), where G is the number of genotypes and N is the total number of genotyped samples [13]. In a monoclonal stand, R = 0, and R = 1 if each sample has a unique genotype.

Genetic diversity

After removal of clonal replicates, basic population genetic parameters, that is, the number of genotypes (G), the number of alleles (NA), observed (HO) and expected (HE) heterozygosity (HE; Nei 1987), allelic richness standardized to the smallest population size by rarefaction, and inbreeding coefficients (FIS) for each population using FSTAT were calculated [10,14]. The significance of FIS deviations from zero was tested in FSTAT using 1,000 random permutations [10].

Genetic structure and differentiation

The variation among populations in Mindanao regions was compared by analysis of molecular variance (AMOVA) using Arlequin 2.0 (Schneider et al. 2000). In addition, the genetic differentiation among populations was evaluated using pairwise FST values calculated from 1,000 random permutations in Arlequin. The patterns of isolation-by-distance (IBD) among populations was evaluated using a Mantel test between FST/(1-FST) and the natural logarithm of geographic distance, using GenAlEx ver. 6 [15]. The FST values on populations with more than one genet were calculated using FSTAT.

The partitioning of individuals and populations was assessed using the Bayesian-based clustering analysis in STRUCTURE ver. 2.3.2.1 [16]. Analyses were performed on distinct genotypes only, using the no admixture model for determining the ancestry of individuals, assuming free allele independent frequencies among populations [17]. The four-step method described by Evanno et al. (2005) was utilized, after performing 10 independent runs of ln (K) for K = 1 to 15 with 50,000 MCMC repetitions and a burn-in period of 50,000 iterations. The optimal value of K by calculating ΔK to identify the top level and salient peaks in the hierarchical structure was estimated.

Results

All SSR markers were highly variable with no loci deviated from Hardy-Weinberg equilibrium. Out of 446 shoots sampled for each target species from 15 sites across southern Philippines, 291 and 344 were successfully genotyped with all primers used for C. rotundata and E. acoroides, respectively. All shoots belonging to the same MLG were observed within identical populations. This indicates that MLG were not shared by shoots derived from different populations. The probability that individuals with the same MLG occurred by chance was low ($P < 0.001$). Therefore, it was considered that individuals with the same MLG were ramets of the same genet.

Genotypic data and genetic diversity

Genetic diversity ranged from 0.26 to 0.51 and 0.44 to 0.73 for C. rotundata and E. acoroides, respectively (Table 1). The number of alleles per locus ranged from 1.6 to 3.7 and 3.8 to 8.3 for C. rotundata and E. acoroides, respectively. Clonal richness (R) ranged from 0.6 to 1.0 and 0.9 to 1.0 in C. rotundata and E. acoroides, respectively. C. rotundata showed zero clonal diversity in RIZ population. Standardized allelic richness (Ar) across all loci ranged from 1.26 to 1.57 and 2.73 to 5.57 for C. rotundata and E. acoroides, respectively. All species populations revealed significant deviation from Hardy-Weinberg Equilibrium (HWE) in Laguindingan, Misamis Oriental. Most of the diversity hotspot populations did not significantly deviate from zero for the two species except for; LAG, LOP, MAA, and STA for C. rotundata and LAG for E.

acoroides, which exhibited positive FIS (heterozygote deficit).

Genetic structure and differentiation

One of the populations of C. rotundata in Rizal was not included in the following analyses for AMOVA, pairwise FST, and IBD, because it was composed of only one genet after removal of clonal replicates. The AMOVA revealed the significant degree of differentiation ($P < 0.001$) among the two dominant seagrass species in Mindanao.

The 15 populations of the C. rotundata species were genetically differentiated with IBD $R = 0.29$, $P < 0.05$. However, there is no significant IBD in E. acoroides among populations, with $R=0.10$, $P=0.253$ (Fig. 2). Genetic differentiation based on FST was statistically significant among all pairs (all $P=0.001$). The largest genetic differentiation was observed between TON and RIZ for C. rotundata and COR and GLA for E. acoroides. The degrees of genetic differentiation between regions were also manifested in the PCoA (Fig. 3).

Table 1. Genetic indices of seagrasses in the fifteen (15) sites in Mindanao (Ns-Number of sample; Ng-Number of genotype; *R*-Clonal richness; He-Heterozygosity; Na-Number of allele; Ar-Allelic richness; FIS-Fixation index).

Sites	Code	Coordinate		*Cymodocea rotundata*							*Enhalus acoroides*						
		Long	Lat	Ns	Ng	*R*	He	Na	Ar(n =1)	FIS	Ns	Ng	*R*	He	Na	Ar(n=7)	FIS
Ayoke Island	AYO	126.05	9.39	25	24	1	0.38	2.1	1.38	-0.12	24	24	1	0.44	3.8	3.11	0.137
Cortes, Surigao del Sur	COR	126.19	9.25	24	20	0.8	0.39	2.6	1.39	0.06	23	23	1	0.44	3.8	2.73	0.161
Dahican, Davao Oriental	DAH	126.27	6.91	30	23	0.8	0.34	2.3	1.34	0.032	25	23	0.9	0.49	3.5	3.01	0.145
Glan, Sarangani	GLA	125.19	5.78	30	18	0.6	0.5	3	1.5	0.196	26	26	1	0.72	7.5	5.15	0.052
Kauswagan, Lanao del Norte	KAU	124.08	8.19	29	28	1	0.46	3.3	1.46	0.09	18	18	1	0.62	4.5	3.85	0.205
Laguindingan, Misamis Oriental	LAG	124.47	8.63	26	20	0.8	0.41	2.9	1.4	0.283*	22	22	1	0.68	5.3	4.46	0.277*
Lavigan, Davao Oriental	LAV	126.19	6.32	30	20	0.7	0.38	2.4	1.38	0.024	28	28	1	0.62	6.8	4.4	0.182
Lopez Jaena, Misamis Occidental	LOP	123.77	8.57	28	20	0.7	0.37	2.6	1.37	0.329*	27	27	1	0.62	6.7	4.22	0.065
Maasin, South Cotabato	MAA	125.09	5.88	29	27	0.9	0.51	3.7	1.51	0.208*	27	27	1	0.68	5.7	4.15	-0.03
Pasiagan, Bongao, Tawi Tawi	PAS	119.75	5.01	29	25	0.9	0.36	3	1.36	0.061	22	22	1	0.71	7	5.09	0.056
Rizal, Zamboanga del Norte	RIZ	123.54	8.63	21	1	0	NA	1.6	1.57	NA	23	20	0.9	0.65	5.7	4.23	0.087
Sanipaan, Davao del Norte	SAN	125.68	7.16	30	22	0.7	0.33	2.9	1.32	0.118	25	24	1	0.49	5.2	3.7	0.146
Siargao Island	SIA	126.16	9.78	26	22	0.8	0.42	2.9	1.42	0.039	28	26	0.9	0.55	4.8	3.56	0.003
Santa Ana, Agusan del Norte	STA	125.33	8.97	28	23	0.8	0.32	2.1	1.32	0.314*	27	27	1	0.48	3.8	2.88	0.081
Tongusong, Simunul, Tawi Tawi	TON	119.82	4.92	23	19	0.8	0.26	2.6	1.26	0.058	26	25	1	0.73	8.3	5.57	0.132

*significantly larger than 0

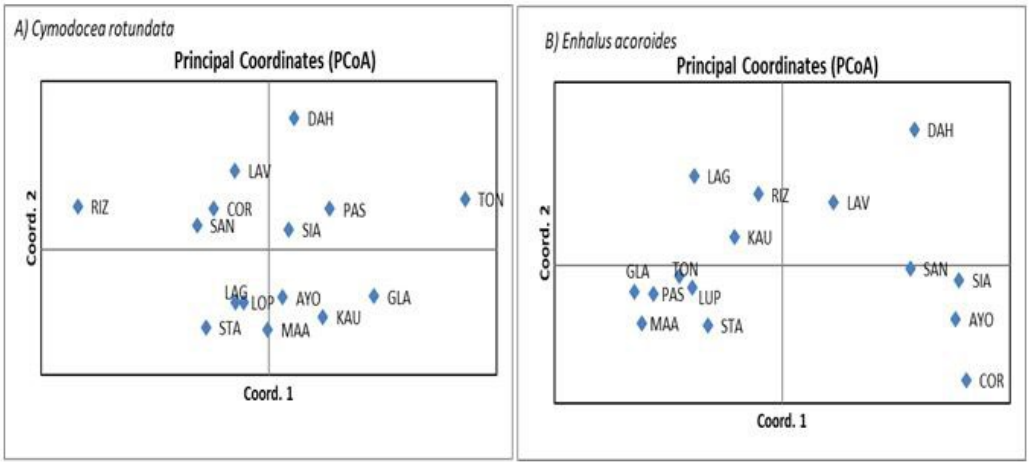


Figure 2: Relationship between pairwise differentiations described by FST vs the logarithm of distance (km) among populations in Mindanao: A) *C. rotundata* and B) *E. acoroides*.

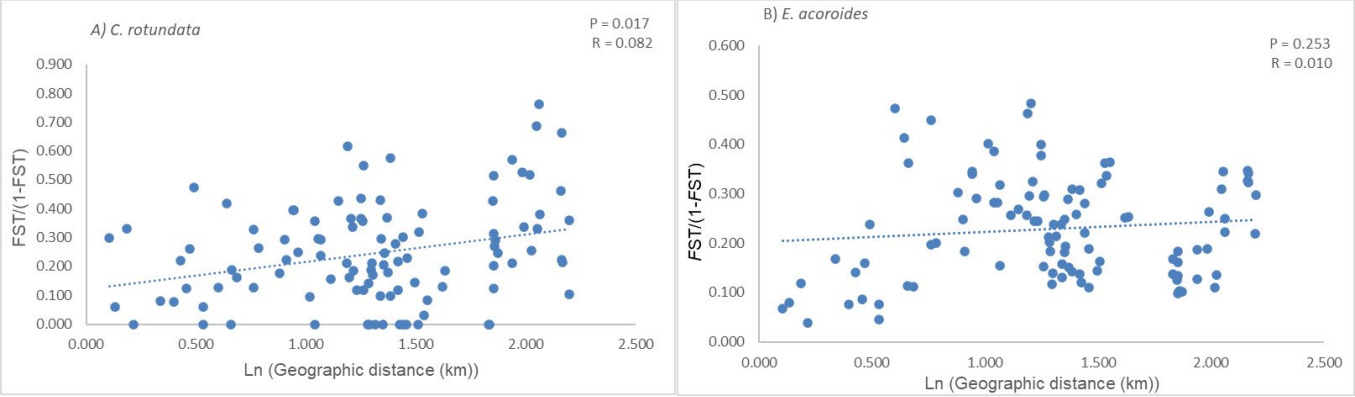
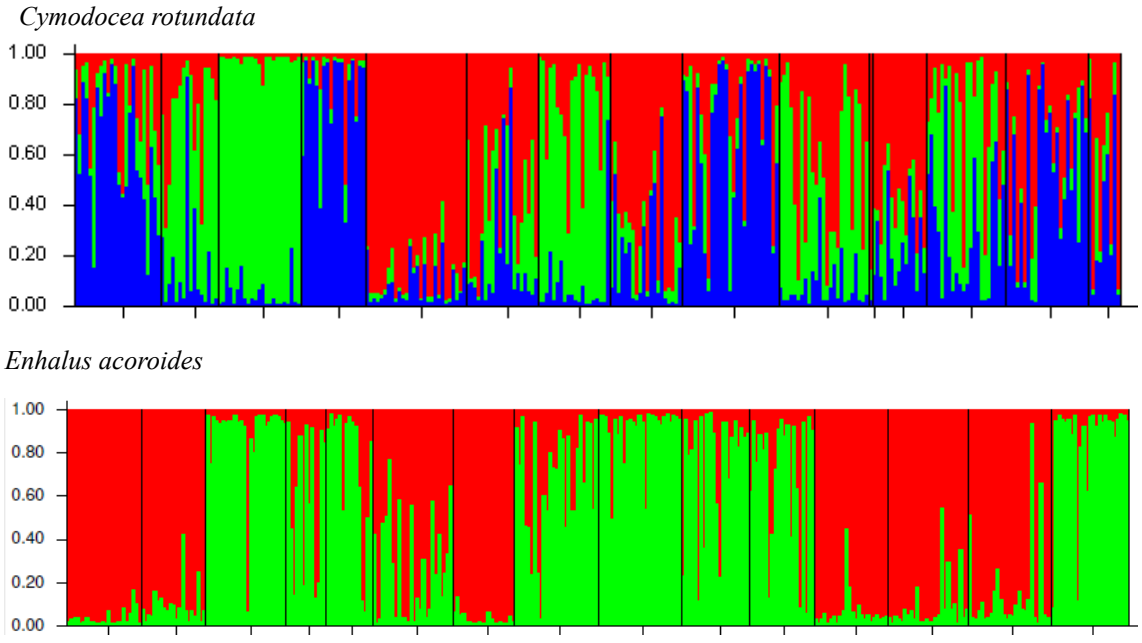


Figure 3: Principal coordinates analysis (PCoA) from the covariance matrix output using GenAlEx based on pairwise FST values. A) *C. rotundata:* The first two axes explained 62.04% of the variation (the first axis explained 46.67%, the second axis, 23.38%). B) *E. acoroides:* The first two axes explained 65.25% of the variation (the first axis explained 50.42%, the second axis, 28.72%).

In the STRUCTURE analysis, salient peaks of ΔK were exhibited at K=3 and K=2 for C. rotundata and E. acoroides, respectively. Under these conditions, the STRUCTURE result illustrated the genetic clusters and admixtures among populations of the two dominant seagrass species, the northern Mindanao and southern Mindanao pooled with most of the range edge populations, but that it was permeable to gene flow being some individuals sampled from the southern populations could probably mixed their alleles with the northern sites (Fig. 4). The grouping of the southern populations was genetically varied but dominated with individual genotypes for the two species revealing admixtures in some populations of central Mindanao and the southernmost part of Mindanao which is Tawi. Remarkably, there was evident of an intermingled populations or genetic admixtures across Mindanao populations among the two-seagrass species as detected in the Structure (Fig. 4). The results of the STRUCTURE analysis based on Bayesian statistical model-based clustering indicated the existence of population genetic structure among sites.

One of the populations in northeastern part of Mindanao, the Siargao Island (SIA), (9° 46' 48" N; 126° 9' 36" E) is exhibiting mixtures of several alleles. Apparently, the southernmost population of Tawi Island (PAS; 5° 0' 36" N; 119° 45' 0" E) was bearing the genetic footprints typical of the Siargao Island population. Genetic relationship among populations assessed by the principal coordinates analysis (PCoA) supports STRUCTURE and revealed varied and differentiated populations in the Mindanao populations of both C. rotundata and E. acoroides seagrass species (Fig. 3) with intermingling populations forming admixture but dominated with individual genotypes (Fig. 4).



Population code														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AYO	DAH	GLA	KAU	LAG	LAV	LOR	LUP	MAA	PAS	RIZ	SAN	SIA	STA	TON

Figure 4: Graphical summary of Bayesian clustering analysis implemented in STRUCTURE by clustering without prior information under the admixture model and assuming correlated allele frequencies. Assumed number of clusters is three (K=3) for *C. rotundata* and two (K=2) for *E. acoroides* [18].

Discussion

The present study is the first report of genetic diversity, and genetic structure, among populations of the two dominant seagrass species Cymodocea rotundata and Enhalus acoroides spanning the diversity hotspot and range edge populations in Mindanao, (southern Philippines) using microsatellite (SSR) markers. Conforming novel genetic records for these two species of seagrass in the tropical areas and detection of high levels of polymorphism underlined impressively that genetic markers are powerful tools for assessing genetic diversity in seagrass.

Genetic diversity and connectivity

The species richness and structure of seagrass across regions is believed to be influenced by hydrodynamic regimes due to the intersection of favorable major and minor ocean currents meandering the extant seagrass communities [19,20]. Seagrasses have the potential to disperse over long distances via ocean currents during various life-history stages [21]. Population genetic studies in combination with hydrodynamic models have increased understanding of the role/potential of connectivity in natural seagrass meadow recovery [22,23]. A combined understanding of the dispersal mechanisms and reproductive biology of seagrasses will add to the overall understanding of spatial and genetic connectivity.

Genetic diversity is related to adaptive potential, and a loss in genetic diversity can increase the possibility of population extinction. Genetic diversity hotspots are located in a central area within the distribution range of a focal species [24]. These are extremely important in seagrass conservation and serve as models for monitoring biodiversity in regions affected by anthropogenic disturbances and climate change [25]. The Philippines is likely to function as a genetic diversity hotspot influenced by populations of tropical seagrasses, owing to its location in a central habitat. Genetic diversity of C. rotundata and E. acoroides populations in Mindanao are ranging from low to high, and decreasing genetic diversity is usually found in the marginal ranges especially for C. rotundata. Upon observations, seagrass species confirmed in RIZ was dominated with E. acoroides and C. rotundata was intermittently found in shallow areas. The single genet of C. rotundata found in this meadow manifested that sexual reproduction is not apparent in this area and this is alarming for the conservation of this species in the area since anthropogenic activities are recurring with observations on the proliferation of the fast-growing algae that inhibited the penetrations of sunlight for photosynthesis. The environment in RIZ is not very suitable habitat for a seagrass species with short leaves therefore, environmental selection may have occurred, and the seagrass population in RIZ is likely to be endangered. In seagrass meadows often dominated by a single seagrass species, they are susceptible to pandemic disease outbreaks Waycott et al (2009). It was further implicated by Reusch et al (2001) that decreasing genetic diversity of seagrasses may also correspond to the decrease of the resilience of meadows and the seagrass dwelling fish and invertebrates.

The overall allelic richness of E. acoroides (4.01) and C. rotundata (1.40) is higher compared with the previous studies of Nakajima et al (2017) for E. acoroides in the Guimaras Strait, Visaya, Philippines, and comparable with Arriesgado et al (2016) for C. rotundata (1.64 Norther Philippines, 1.78 Central Philippines and 1.94 Ryukyu Island), respectively. Furthermore, allelic richness of the two dominant seagrass species in Mindanao was comparable with some seagrass species; Halophila ovalis (1.56) in the Western Pacific Ocean Cymodocea nodosa (2.20) in the Atlantic regions and Zostera marina (2.74) in San Juan Archipelago, Washington, USA [26-28].

The decreased allelic richness in some populations is possibly the effect of drift because of small population size, as a result of reduced habitat and low gene flow and/or natural selection across life stages of some clonal species (e.g. C. rotundata) which favor clonal reproduction for environment fitness. Genetic diversity is related to adaptive potential, and a loss in genetic diversity can increase the possibility of population extinction. Genetic diversity hotspots are located in a central area within the distribution range of a focal species [24]. These are extremely important in seagrass conservation and serve as models for monitoring biodiversity in regions affected by anthropogenic disturbances and climate change [25]. The Philippines is likely to function as a genetic diversity hotspot influenced by populations of tropical seagrasses, owing to its location in a central habitat. However, in the present study even though Mindanao is located in the tropical region, the genetic diversity in some areas is low. The observation of low genetic diversity in some sites was also manifested in Japan and Hainan China in E. acoroides and C. rotundata [6,7]. Decreasing genetic diversity of seagrasses may also correspond to the decrease of the resilience of meadows and the seagrass dwelling fish and invertebrates [29]. In general, horizontal rhizome elongation is important for population maintenance in seagrasses, which was evidenced in RIZ population [30].

Previous study suggested the importance of sexual reproduction in seagrass in the Philippines [31]. This fact was also apparent from

results of clonal diversity in Nakajima et al. (2014) for E. acroides (R=0.47–1.00), Jahnke et al (2019) for T. hemprichii (R=0.26–0.95), Arriesgado et al. (2016) for C. rotundata (0.21–1.0) and this study for E. acoroides (R=0.9–1.00) and C. rotundata (0.7–1.0), respectively. The remarkable high genetic diversity of E. acoroides as compared to C. rotundata was explained by a hypothetical scenario of the possibility that because of the dispersal mechanisms exhibited by E. acoroides, of which the Mindanao Ocean currents is perhaps continuously transporting drifting flower in the surface water providing supply of new genotypes into the different seagrass meadows in the region (Fig. 3). This was also asserted in the previous study manifesting that the Kuroshio current greatly influenced the genetic diversity and structure of C. rotundata in the Ryukyu Island as propagules were carried and drifted by the water currents from the Philippines [3]. This was even confirmed by some studies on scleractinian corals who found that the strong Kuroshio Current with high sea surface temperatures greatly influenced their distribution and genetic diversity across the Kuroshio triangle [32,33].

Genetic differentiation

The results of AMOVA and FST indicated pronounced genetic differentiation among C. rotundata and E. acoroides populations in the Mindanao region, suggesting limited gene flow. This is not surprising for C. rotundata considering the low dispersal capability of this clonal species which develop fruits at the base of shoots and seeds attach to the rhizome, which are frequently buried under the substrate [4,34]. This characteristic of seed production inhibits seed dispersal, resulting in significant genetic differentiation among populations. On the other hand, the lower genetic differentiation of E. acoroides populations may be explained by the dispersal mechanism of this species influenced by major and minor currents in Mindanao. For instance, the hydrodynamics in Siargao Island is greatly influenced by the uninterrupted winds and currents coming from the Pacific Ocean, which was further intensified by the Mindanao Ocean currents running westward through the Siargao Strait. This has probably influenced the genetic connectivity among populations in Siargao and the southernmost seagrass meadows in Tawi. Furthermore, the significant isolation by distance (IBD; Fig. 2) manifested by C. rotundata is likely a consequence of habitat fragmentation as seen in other studies [3,24,35] Assis et al (2013). The dominance of large genets indicates that these meadows are the result of ecological and evolutionary processes integrated over long time scales. Range edge populations are typically small and restricted to particular habitat islands within a matrix of unsuitable landscapes [36]. These populations have persisted for longer time periods in relative isolation, which resulted in reduced genetic diversity [37]. Thus, their isolated life results in remarkably high population differentiations even at small geographical distances, which leads to extraordinary levels of regional genetic diversity [37,38]. A large genetic structure across spatial gradients was observed as a result of successive colonizations of the population having formed mosaics of genets to colonize space through vegetative elongation and/or produce seedling through reproduction among flowers of the same genet or its relatives, recruiting closed to the maternal plants. Selection for local adaptation to their environment is suggested to play an important role, which may result in the development of distinct ecotypes.

Implications for conservation

The finding of this study has important implications for conservation issues. The water currents play an important role in the recruitment and establishment of populations of this species in Mindanao, southern Philippines. The region was considered of extreme importance for conservation objectives and could be proposed as a model for monitoring biodiversity. It is important to conserve diversity in some range edge populations because such locally adapted genotypes uphold important evolutionary potential in face of future environmental change.

Genetic diversity and connectivity can inform decision-making and help to prioritize management actions. For example, connectivity estimates can be used to identify areas that are more likely to recover naturally following decline (e.g., areas that have steady supply of propagules from non-local sources) and areas that have limited recovery potential due to recruitment limitations (e.g., isolated areas expected to receive minimal or no propagule recruitment from non-local sources). Habitat enhancement and ecological engineering to encourage settlement would become priority management actions for areas showing limited signs of recovery despite expected propagule supply. In contrast, translocations (e.g., physical planting) in combination with habitat restoration investments would be needed in areas with limited propagule supply to ensure population establishment.

The level of genetic diversity of source and recipient seagrass meadows is also an important factor to consider when augmenting remnant seagrass meadows or establishing new meadows. Seagrass meadows at the edge of their range may have lower genetic diversity and higher levels of clonality or have reduced seed production as a result of pollen limitation. Small isolated populations often have similar issues [21]. Overall genetic diversity is positively associated with population fitness, and standing genetic variation within populations is closely tied to adaptive capacity and resilience to environmental change [39,40]. Consequently, selecting genetically diverse meadow as a donor source is important for maximizing restoration success. Local extinction, especially of organisms with low dispersal ability, in the coastal ecosystems is anticipated in the near future. This region should be monitored to conserve the coastal ecosystems and fisheries resources.

In general, seagrasses are in a vulnerable state and continuously declining. Effective management should equally espouse awareness factor through information, education and communication. This could be motivated by promoting and spreading public awareness about seagrasses and the importance of maintaining healthy seagrass habitats to the general public, environmentalists and policy makers which can help protect further loss and decline of these important habitats. If protected, healthy seagrass meadows will continue to support the many valuable and important creatures liv-

ing within the meadows, and the biota of coral reefs and mangrove forests maintaining healthy interconnectivity of these three ecosystems as well [41-45].

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Statements & Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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