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RESEARCH ARTICLE

Population genetics information for the regional conservation of a tropical seagrass, *Enhalus acoroides*, around the Guimaras Strait, Philippines

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Abstract Seagrasses are marine angiosperms and play an essential ecological role in coastal ecosystems; however, seagrass meadows are threatened locally by anthropogenic disturbances. Understanding the dispersal patterns of seagrasses is essential for appropriate ecosystem management and establishment of marine protected areas (MPAs) in coastal ecosystems. In the Guimaras Strait in the Philippines, Banate (BAN) has been established as an MPA. However, there is a lack of information on the genetic diversity of seagrasses in BAN and the surrounding areas. In the present study, population genetics analysis of *Enhalus acoroides* was performed by using polymorphic microsatellite markers, for the estimation of genetic diversity, differentiation, and migration patterns of seagrasses within the regional geographical scale (~200 km) around the Guimaras Strait. The results showed that the genetic diversity of BAN is extremely low, although the Guimaras Strait is located in the tropical central habitat. Guimaras Island geographically divides the populations of *E. acoroides* into south and north. However, the genetic structure did not show any relationship between the geographical location and distance. 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 are derived from self-recruitment in the natal meadow. This study suggests that *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 in BAN. To maintain and enhance the genetic diversity of seagrasses within the MPA, the seagrass meadows in the surrounding areas should also be conserved.

Keywords Anthropogenic disturbance · Genetic diversity · Microsatellite · Recruitment · Seagrass meadow

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Introduction

Establishment of marine protected areas (MPAs) plays an important role in the conservation and management of coastal environments, such as the protection of biodiversity by preventing overfishing. The location of MPAs is commonly selected on the assumption that these reserves provide spillover benefits for fisheries productivity in surrounding areas (McClanahan and Mangi 2000). Furthermore, establishment of MPAs is considered as a useful method for maintaining coral cover, functioning as a

framework for reef ecosystems (Selig and Bruno 2010). However, MPAs site selection and spatial arrangement are often determined without considering the connectivity of associated-fauna among sites (Botsford et al. 2009). Understanding the dispersal patterns of organisms is essential for appropriate management and establishment of MPAs in coastal ecosystems under the threat of anthropogenic disturbances. Seagrasses are marine angiosperms that play an essential ecological role in coastal ecosystems, forming extensive meadows. Seagrass losses disrupt important linkages between seagrass meadows and other ecosystems, such as coral reefs or mangrove forests (Heck et al. 2008). Seagrasses are distributed in coastal areas throughout the world, except for the Antarctic, and are especially found in the tropical region of the Indo-Pacific (Short et al. 2007, 2011). Various types of herbivores inhabit seagrass meadows, including grazers that feed on live seagrass and consume epiphytic and benthic algae, as well as filter feeders that consume phytoplankton (Nakaoka 2005). Sexual reproduction, dispersal of seeds, as well as, a clonal reproductive strategy, known as rhizome elongation or splitting, facilitates maintenance of the local populations. Furthermore, long-distance dispersal may contribute to the expansion of seagrass meadows to other areas. However, the dispersal distance is mostly localized by geographical barriers and the complex biological and oceanographic factors (Muñiz-Salazar et al. 2005; Kendrick et al. 2012; Nakajima et al. 2014).

Genetic differentiation in seagrasses among various regions has been detected by population genetic analysis at large geographical ranges over a distance of 1000 km (Muñiz-Salazar et al. 2005; Serra et al. 2010; Nakajima et al. 2014; Arriesgado et al. 2015; Kurokochi et al. 2016). Significant differentiation has been found between seagrass populations even in regional or local geographical ranges, within a distance of 100 km (Tanaka et al. 2011; Ort et al. 2012; Campanella et al. 2013), compared to broadcast spawning invertebrates with high dispersal ability in the pelagic larval phase. This indicates that seagrass meadows could form the strong genetic structure among populations within a region. *E. acoroides* is one of the dominant seagrass species in the Indo-West Pacific and exhibits broad habitat tolerance, occurring on muddy sand, coral sand, and coarse coral rubble substrates (Brouns and Heijs 1986). This species is suitable for dispersal studies because sexual reproduction is dominant. Previous studies have estimated that the dispersal distance of released fruits and seeds of *E. acoroides* is limited (≤63.5 km, Lacap et al. 2002). Seeds and fruits are occasionally dispersed to long distances by strong sea currents, but clonal reproduction also occurs partially (Nakajima et al. 2014). Clonal reproduction influences the extension of seagrass meadows which causes competition with other, adjacent seagrass species by the long and ribbon-like leaves.

The genetic diversity and differentiation of *E. acoroides* across a distance of approximately 2100 km in the western Pacific has been investigated in the previous study (Nakajima et al. 2014). 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 *E. acoroides* by using polymorphic microsatellite markers was conducted in the MPA Banate Bay (BAN) and the surrounding area, within the regional geographical scale (~200 km) of the Guimaras Strait (Fig. 1). We hypothesized that geographical, oceanographic, and environmental factors influence the genetic diversity, differentiation, 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 (Short et al. 2007, 2011). The Guimaras Strait includes productive fishing grounds, and BAN is under fisheries pressure owing to the overexploitation of fisheries resources and the destruction of coastal ecosystems (Pomeroy et al. 2010). Furthermore, an oil spill accident on August 2006 caused distress on the environment and the people of southern Guimaras, and the effects of oil contamination may be more complex and long term for intertidal seagrass meadows (Nievales 2009). These multiple disturbances threaten the coastal ecosystems including seagrass meadows in this region.

Materials and methods

Study sites and sampling

Seagrass sampling sites, including the MPA BAN, were located around the Guimaras Strait, Philippines (8 sites; Fig. 1; Table 1). The Guimaras Island divides the sea into north and south regions. The maximum distance between KOT and LIN was ~200 km. All samples for this study were collected in September 2012 from approximately 200 m × 300 m areas, in the intertidal or subtidal zones, which were less than 3 m deep. A distance of over 10 m was kept between sampled shoots within each site, to avoid overestimating clonal diversity (Nakajima et al. 2014). A young leaf from each shoot was collected, desiccated with silica gel, and preserved until use.

DNA extraction and microsatellite analysis

Silica gel-dried leaves (<10 mg) from each shoot were ground using TissueLyser (Qiagen). Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Lian et al. 2001, 2003). Following

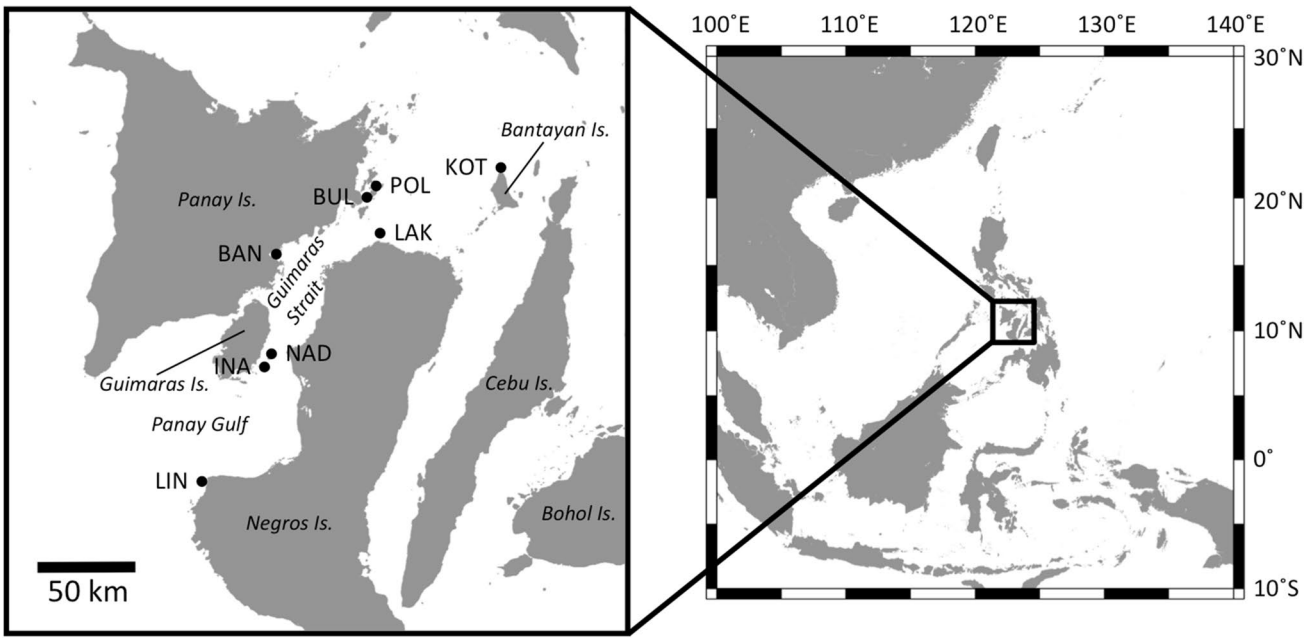

Fig. 1 Map of the eight sites of *Enhalus acoroides* in the Guimaras Strait, Philippines

Table 1 Sampling sites and indices of genetic data

Site	Code	Coordinate		*N*	*G*	*R*	*A*	A_R	P_A	H_O	H_E	F_{IS}	Bottleneck	
		Latitude (N)	Longitude (E)										IAM	TPM
Kota Park	KOT	11°18′03″	123°43′40″	13	13	1.00	3.25	3.25	0	0.375	0.389	0.015	0.422	0.922
Looc Polopinia	POL	11°12′55″	123°09′43″	47	45	0.96	4.88	4.07	1	0.539	0.554	0.052	**0.014**	0.156
Bulubadiangan Island	BUL	11°11′15″	123°09′32″	51	51	1.00	4.88	4.00	0	0.431	0.455	0.074	0.234	0.594
Lakawon Island	LAK	11°02′31″	123°12′14″	51	25	0.48	5.25	4.51	2	0.550	0.524	–0.051	0.055	0.469
Banate Bay	BAN	10°58′59″	122°47′14″	49	31	0.63	2.25	1.93	0	0.496	0.277	–0.570	0.219	0.578
Nadulao Island	NAD	10°30′20″	122°44′28″	34	28	0.82	3.75	3.31	1	0.433	0.430	–0.009	**0.039**	0.344
Inampulungan Island	INA	10°28′26″	122°41′40″	38	37	0.97	4.63	3.83	2	0.524	0.494	–0.055	**0.008**	0.055
Linawon	LIN	09°57′25″	122°26′47″	49	47	0.96	6.13	4.70	7	0.513	0.511	–0.030	0.289	0.813

Bold value means $P < 0.05$

N number of samples; *G* number of multilocus genotypes; *R* index of clonal diversity calculated with the formula R = (G − 1)/(N − 1); *A* mean number of alleles per locus; A_R mean value of allelic richness calculated by FSTAT; P_A number of private alleles for eight loci; H_O and H_E mean observed and expected heterozygosities, respectively; F_{IS} mean inbreeding coefficient; *A,* P_A, H_O, H_E, and F_{IS} were calculated by GenAlEx; whether population had experienced recent bottleneck was estimated using BOTTLENECK

extraction, DNA pellets were dissolved in 100 µL sterilized water and kept at −30 °C until use. Eight polymorphic microsatellite markers (Eaco_001, Eaco_002, Eaco_009, Eaco_050, Eaco_051, Eaco_052, Eaco_054, and Eaco_055), developed by Nakajima et al. (2012), were used to score genotypes. The tailed primer method was used to perform the polymerase chain reaction (PCR) assay; U19 (5′-GGTTTTCCCAGTCACGACG-3′) or M13R (5′-CAGGAAACAGCTATGAC-3′) was added to the 5′-end of each reverse primer. PCR was performed using a Multiplex PCR Kit (Qiagen) in a 5-µL reaction mixture containing <30 ng of template genomic DNA, 2×Multiplex PCR Master Mix, and 0.2 µM (final concentration) of each of three primers for each locus: a forward primer, a reverse primer with a U19 or M13R tail, and a U19 or M13R primer fluorescently labeled with FAM, VIC, or NED. The PCR cycling conditions were: 15 min at 95 °C, followed by 32 cycles of 30 s at 94 °C, 90 s at 58 °C, and 60 s at 72 °C, with an extension of 30 min at 60 °C in the final cycle. The PCR products were identified and analyzed using an automated capillary DNA sequencer (ABI 3130xl Genetic Analyzer, Thermo

Fisher Scientific) and GeneMapper ver. 4.1 (Thermo Fisher Scientific).

Data analysis

Genotypic indices

The number of multilocus genotypes (MLGs) for each population was calculated with GenClone ver. 2.0 (Arnaud-Haond and Belkhir 2007). MLG considers whether two shoots belong to the same genet or to two different genets, based on the probability that identical MLGs are derived from distinct sexual reproduction events by chance (P_{SEX}). The P_{SEX} value was calculated by taking into account the inbreeding coefficient (F_{IS}) estimates in the data and was used to test for clonal identity and clonal propagation (Arnaud-Haond et al. 2005; Arnaud-Haond and Belkhir 2007). To eliminate duplicated clonemates from the analysis, identical MLGs at a given site were considered to be clonal replicates ($P_{SEX} < 0.01$). The index of clonal diversity (R) proposed by Dorken and Eckert (2001) was calculated with the formula $R = (G-1)/(N-1)$ in each population, where G indicates the number of MLGs and N indicates the number of shoots collected and genotyped. The mean number of alleles per locus (A) and allelic richness (A_R) of each population were estimated using GenAlEx ver. 6.501 (Peakall and Smouse 2006) and FSTAT ver. 2.9.3.2 (Goudet 1995), respectively. The observed and expected heterozygosity (H_O and H_E) and the number of private alleles (P_A) at each population were also calculated using GenAlEx. To evaluate whether populations had experienced recent bottlenecks, we adopted the Wilcoxon's signed-rank test with 1000 replications using BOTTLENECK ver. 1.2.02 (Piry et al. 1999) and applied assumptions for both the infinite allele mutation model (IAM) and the two-phase model (TPM: 30% IAM and 70% stepwise mutation model).

Genetic differentiation among populations

Genetic differentiation among populations was estimated using a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992) in different hierarchical levels (one region, two regions partitioned between BAN and NAD, and partitioned between INA and LIN). The genetic differentiation index between populations, pairwise F_{ST}, was calculated using GenAlEx, and a random effect model with 999 permutations was used to test the significance of each F_{ST} value. We estimated patterns of genetic differentiation derived from pairwise F_{ST} values among populations with a principal coordinates analysis (PCoA) using GenAlEx.

Genetic structure and migration patterns

The population genetic structure was inferred from microsatellite data using STRUCTURE ver. 2.3.3 (Pritchard et al. 2000). STRUCTURE analysis implements a Bayesian clustering algorithm to assign genotypes to clusters that minimize Hardy–Weinberg disequilibrium and linkage disequilibrium. Ten replicate runs were performed for each K between 1 and 10 without prior information using the admixture model and assuming correlated allele frequencies (Falush et al. 2003). Each run consisted of 1,000,000 Markov chain Monte Carlo (MCMC) replications after burn-in with 100,000 iterations. Optimal K was determined using the ΔK method of Evanno et al. (2005), as implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012). ΔK is an ad hoc quantity for predicting the number of possible genetic clusters (Evanno et al. 2005). Run data were merged and outputted using CLUMPP ver. 1.1.2b (Jakobsson and Rosenberg 2007) and DISTRUCT ver. 1.1 (Rosenberg 2004), respectively.

The number of immigrants present in the first generation (F_0) was estimated with a likelihood-based assignment test using GeneClass2 (Piry et al. 2004). We assigned each genet to a source population using 10,000 re-sampling permutations with GeneClass2 as follows. First, a genet with ≥99% confidence of exclusion was excluded from the sampled population. Second, we assigned the individual as an immigrant to the highest-possibility population when the confidence of assignment was ≥10%. If a given genet had low probability with <10% confidence of assignment for all populations, the origin of this genet was considered unassigned. BayesAss ver. 3.0 (Wilson and Rannala 2003) was also used to determine directionality of migration patterns among populations by estimating recent migration rates per generation over the last few generations in the population level. The following settings were used: number of iterations 10,000,000, burn-in 1,000,000, and sampling frequency 1000.

Results

Genotypic data, genetic diversity, and bottleneck

Out of 350 shoots sampled from 8 sites around the Guimaras Strait, 332 were successfully genotyped with all primers used. Among these shoots, 277 MLGs were identified as genets that were raised from different sexual events based on P_{SEX} values calculated at each site. 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. Clonal diversity (R) is an indicator of the main

mode of reproduction as estimated by the ratio of the number of genotypes to shoots. The clonal diversity for all of the populations ranged from 0.48 to 1.00 (Table 1). The mean number of alleles (A) and allelic richness (A_R) per population ranged from 2.25 (BAN) to 6.13 (POL) and from 1.93 (BAN) to 4.70 (POL), respectively (Table 1). These results clearly showed that the population in BAN possessed low clonal diversity and allelic richness compared to those in other sites. In total, 13 private alleles (P_A) were detected within the 5 populations, and 7 out of the 13 were found in LIN (Table 1). The observed and expected heterozygosities (H_O and H_E) ranged from 0.375 to 0.550 and from 0.277 to 0.554, respectively, while inbreeding coefficients, F_{IS}, varied between −0.570 and 0.074 (Table 1). Three populations, POL, NAD, and INA appear to have undergone a recent bottleneck, resulting in a significant excess of heterozygosity compared with the heterozygosity expected at the mutation drift equilibrium under IAM assumptions ($P < 0.05$). However, no populations showed significant deviations from the mutation drift equilibrium under TPM assumptions ($P < 0.05$).

Genetic differentiation and migration patterns among populations

The hierarchical AMOVA detected genetic differentiation among populations in all hierarchical levels (one region, two regions partitioned between BAN and NAD, and partitioned between INA and LIN). A significant proportion of the observed genetic variation occurred among (15%) and within (85%) populations ($F_{ST} = 0.149$, $P = 0.001$) (Table 2). However, the proportion of genetic variance was not significantly partitioned among regions (partitioned between BAN and NAD: 0%, $F_{RT} = 0.002$, $P = 0.129$; partitioned between INA and LIN: 0%, $F_{RT} = -0.051$, $P = 1.000$) (Table 2). Pairwise F_{ST} values ranged from 0.061 to 0.365 (Table 3). Genetic differentiation based on F_{ST} was statistically significant among all pairs (all Ps = 0.001). The largest genetic differentiation was observed between BAN and NAD. High degrees of genetic differentiation between regions were apparent in the PCoA (Fig. 2).

The results of the STRUCTURE analysis based on Bayesian statistical model-based clustering indicated the existence of obvious population genetic structure among sites. When K was 3, the value of ΔK was the largest (mean LnP(D) = −4900.99, $\Delta K = 227.82$) (Fig. S1). In $K = 3$,

Table 2 Analysis of molecular variance (AMOVA) showing degrees of freedom (d.f.), sum of squares (SS), variance components (Var.), percentage of variances (%), and F-statistics among regions, among populations within regions, and within populations to estimate regional genetic structure for eight populations in different hierarchical levels

Source	d.f.	SS	Var.	%	F-statistic	P value
1 Region						
Among populations	7	170.565	0.330	15	$F_{ST} = 0.149$	0.001
Within populations	546	1033.631	1.893	85		
2 Regions (partitioned between BAN and NAD)						
Among regions	1	28.292	0.003	0	$F_{RT} = 0.002$	0.129
Among populations	6	142.273	0.328	15	$F_{SR} = 0.148$	0.001
Within populations	546	1033.631	1.893	85	$F_{ST} = 0.149$	0.001
2 Regions (partitioned between INA and LIN)						
Among regions	1	17.977	0.000	0	$F_{RT} = -0.051$	1.000
Among populations	6	152.588	0.366	16	$F_{SR} = 0.162$	0.001
Within populations	546	1033.631	1.893	84	$F_{ST} = 0.119$	0.001

Table 3 Pairwise F_{ST} values for all eight populations as an index of genetic differentiation between two populations

	KOT	POL	BUL	LAK	BAN	NAD	INA
POL	0.188						
BUL	0.129	0.113					
LAK	0.137	0.061	0.111				
BAN	0.351	0.220	0.209	0.216			
NAD	0.263	0.122	0.211	0.134	0.365		
INA	0.224	0.084	0.150	0.065	0.248	0.163	
LIN	0.157	0.079	0.102	0.100	0.167	0.119	0.124

All P values in the table are significant (Ps = 0.001)

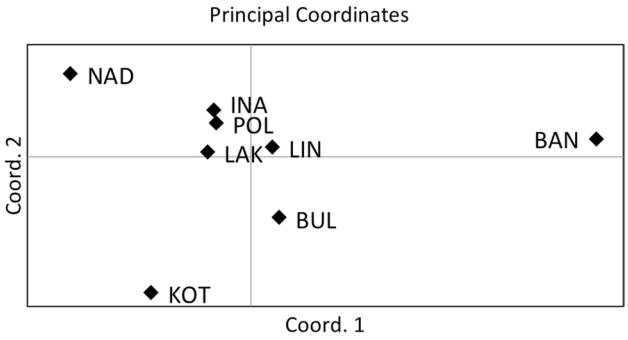

Fig. 2 Principal coordinates analysis (PCoA) from the covariance matrix output using GenAlEx based on pairwise F_{ST} values. The *first two axes* explained 61.47% of the variation (the *first axis* explained 34.60%, the *second axis*, 26.87%)

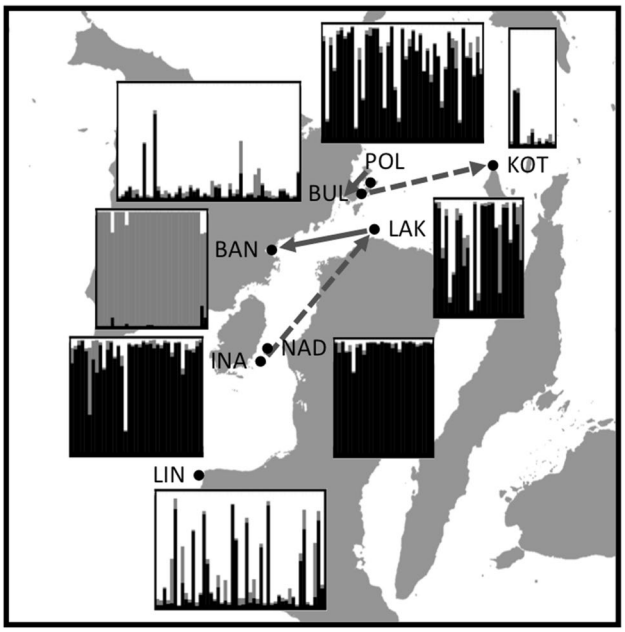

Fig. 3 *Bar plots* from a Bayesian clustering analysis implemented in STRUCTURE by clustering without prior information under the admixture model and assuming correlated allele frequencies. Assumed number of cluster is three ($K=3$). Bar plots of $K=2$ to 6 are shown in Fig. S2. *Solid arrow* shows direction of migrants detected by GeneClass2, and *broken arrow* shows direction with higher migration rates by BayesAss (Table 4)

three clusters were clearly identified, cluster 1 (POL, LAK, INA, and NAD), cluster 2 (BAN), and cluster 3 (KOT, BUL, LIN) (Fig. 3). The values of ΔK were also large in $K=2$ and $K=6$ (Fig. S1), and obvious population genetic structure was also shown among sites in other K values (Fig. S2). These outputs also suggested that the genetic structure was not associated with the geographical location. GeneClass2 suggested that five genets were immigrants from other populations (Table 4). Of these, two were assigned to populations we sampled while the origins of the remaining three were unassigned. BayesAss indicated the high migration rates (over 10%) from BUL to KOT and from INA to LAK in recent generations for each population (Table 4).

Discussion

Low genetic diversity in Banate Bay

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 (Diekmann and Serrão 2012). These are extremely important in seagrass conservation and serve as models for monitoring biodiversity in regions affected by anthropogenic disturbances and climate change (Olsen et al. 2004). 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. *E. acoroides* populations in Japan and China showed low genetic diversity (Nakajima et al. 2014), and decreasing genetic diversity in tropical species is usually found in the marginal ranges or high-latitude areas. However, the genetic diversity of BAN in the present study is extremely low, even though the Guimaras Strait is located in the tropical region. In our observations, seagrass species confirmed in BAN was only *E. acoroides*. BAN was not covered by seagrass meadows and the distribution of *E. acoroides* was dotted on the sediment covered by silts, though other sites analyzed appeared to be healthy meadows. The environment in BAN is not a suitable habitat for seagrass species; therefore, environmental selection may have occurred, and the seagrass population in BAN is likely to be endangered. Because seagrass meadows are often dominated by a single seagrass species, they are susceptible to pandemic disease outbreaks (Waycott et al. 2009). Decreasing genetic diversity of seagrasses may also correspond to the decrease of the resilience of meadows and the seagrass dwelling fish and invertebrates (Reusch et al. 2005).

In general, horizontal rhizome elongation is important for population maintenance in seagrasses (van Dijk and van Tussenbroek 2010). Further, previous studies have suggested the importance of sexual reproduction in *E. acoroides* from the Philippines (Rollón et al. 2003). This fact was also apparent from results of clonal diversity in Nakajima et al. (2014) ($R=0.47$–1.00) and this study ($R=0.48$–1.00). The minimum clonal diversity of *E. acoroides* tends to be higher compared to other seagrass species such as *Posidonia oceanica* ($R=0.10$–1.00, Serra et al. 2010), *Thalassia testudinum* ($R=0.09$–0.80,

Table 4 The results of assignment test by (a) GenClass2 and (b) BayesAss for estimation of immigrant patterns

(a)

	Exclude	KOT	POL	BUL	LAK	BAN	NAD	INA	LIN
KOT	0.001	–	0	0.006	0.041	0	0	0	0.003
BUL	0	0	**0.148**	–	0.008	0	0	0.001	0
BAN	0.002	0.004	**0.117**	0.008	**0.140**	–	0	0.006	**0.118**
NAD	0.002	0	0.014	0	0.002	0	–	0.001	0.004
INA	0.010	0	0	0	0.002	0	0	–	0

(b)

In/From	KOT	POL	BUL	LAK	BAN	NAD	INA	LIN
KOT	***0.712***	0.016	**0.179**	0.031	0.014	0.014	0.018	0.016
POL	0.007	***0.915***	0.017	0.009	0.015	0.009	0.017	0.011
BUL	0.006	0.023	***0.932***	0.009	0.007	0.006	0.008	0.008
LAK	0.012	0.028	0.038	***0.729***	0.030	0.012	**0.134**	0.018
BAN	0.009	0.010	0.010	0.009	***0.936***	0.009	0.009	0.010
NAD	0.009	0.012	0.011	0.010	0.009	***0.927***	0.012	0.010
INA	0.009	0.013	0.014	0.008	0.017	0.011	***0.915***	0.013
LIN	0.007	0.041	0.012	0.008	0.014	0.021	0.010	***0.888***

In GenClass2, the genet was assigned as an immigrant to the highest-possibility site, when genet was excluded ($P \leq 0.01$). Bold site and P values in brackets in assigned column show the most likely site as the recruitment source ($P \geq 0.1$). When the probability was low in all populations ($P < 0.1$), the genet was not classified. BayesAss shows recent migration rates per generation. Bold site shows the likely site as the possible recruitment source (over 10%). Italics indicate self-recruitment rates for each population

van Dijk et al. 2009), and *Zostera marina* ($C = 0.37$–1.00, Tanaka et al. 2011; $R = 0.00$–1.00; Diekmann and Serrão 2012), although sampling and analysis strategies are different in every study. In this study, relatively low values of clonal diversity were found in LAK ($R = 0.48$) and BAN ($R = 0.63$). The low clonal diversity at the meadow level may reflect the large, long-lived nature of these populations. For both populations of low clonal diversity, the clonal diversity of BAN is relatively higher but the genetic diversity is lower than those of LAK. This distinctive feature may be caused by the endangered population in BAN and the long-lived meadow in LAK, respectively. This fact also appears to show evidence of differences in meadow formation (selection or long-lived meadow).

Strong genetic structure and geographical factors

We hypothesized that the population genetic structure is divided into south and north because of the existence of Guimaras Island. However, the genetic structure did not show a relationship between geographical location and distance; the strong and irregular genetic structure of *E. acoroides* in seagrass meadows around the Guimaras Strait could not be explained by location. POL and BUL are close to each other (~3 km). However, the genetic clusters based on the results of STRUCTURE are different from each other. Although only one migrant per generation is sufficient to break the population genetic structure (Spieth 1974), genetic breaking may occur even with low values of the genetic differentiation index (Hedgecock et al. 2007). Therefore, the existence of strong restrictions in dispersal was suggested for *E. acoroides* around this region. Nakajima et al. (2014) suggested that strong sea currents contribute to maintain the same main genetic cluster and migrants from east Philippines to Japan (~1100 km). Though sexual reproduction occurs via the simultaneous annual flowering and fertilization, *E. acoroides* seeds and fruits in a regional scale can hardly disperse to distant meadows; almost all of the seeds and fruits will derive from self-recruitment in the natal meadow. Therefore, it is likely that weak currents can hardly contribute to the transportation of *E. acoroides* seeds and fruits.

The irregular genetic structure may be explained by the historical and geological events. Fluctuations in the sea level are generally considered as one of the factors that can cause genetic differentiation, and it is often not explained by current geographical distance and location. Disagreements between geographical distance and genetic differentiation in regional scales could be influenced by historical fluctuations in coastlines and basin boundaries, since the Pleistocene. Sea levels during the Pleistocene were ~120 m lower than the present levels in our target region (Voris 2000). This low sea levels might result in the division of this region. This division

appears to have acted as a physical barrier for dispersal, as indicated by the pattern of genetic differentiation in seagrass, which showed the multiple genetic clusters in the Visayas, central region of the Philippines (Nakajima et al. 2014). Thus historical event is likely to have caused the difference among populations in meadow formation. However, this is one of the possible hypotheses and does not directly explain the pattern of genetic structure. Further studies are needed to elucidate the mechanisms accurately.

Migration patterns among populations around the Guimaras Strait

The present study found high genetic differentiations among populations, and therefore credibility of assignment tests by GeneClass2 and BayesAss are likely to be high (see Cornuet et al. 1999; Meirmans 2014). GeneClass2 indicated migrations from POL to BUL and from LAK to BAN as individual based analysis. This assignment test enables to determine the first-generation migrants (Piry et al. 2004). On the other hand, BayesAss indicated migrations from BUL to KOT and from INA to LAK. Assignment method in BayesAss enables to detect migration rates past several generations (Meirmans 2014). The differences between software may be caused from methods and generations (see "Materials and methods" section).

Around the Guimaras Strait, sea current flows from northeast to southwest in the dry season, but conversely from southwest to northeast in the rainy season by the Westerlies (May et al. 2011). The flowering season of *E. acoroides* is between November and February in the northwestern Philippines (Duarte et al. 1997; Agawin et al. 2001), which is the dry season. Two migrants estimated by GeneClass2 showed the direction of sea currents during the dry season. On the other hand, migration rates by BayessAss (>10%) show the direction of sea currents during the rainy season. This can cause the differences in the focusing generations as above, and dispersal in the latest generation may be influenced by the sea currents, though the detected migrants are limited. Infrequent buoyant floating fruit will play a role in the long-distance dispersal of migrants. Long-distance dispersal of vegetative fragments is also thought to be possible in seagrass species (Kendrick et al. 2012), though there was no common genet derived from clonal replicate among populations in our samples collected. Studies with other marine species, including seagrasses, will enable to resolve the influence of the relationship between migrant direction and sea currents.

Environmental issues and conservation of seagrass meadows

Loss of seagrass habitat and degradation of seagrasses is threatening the status of seagrass species, especially in areas with low seagrass diversity or with limited seagrass distribution, and can have severe impacts on marine biodiversity and the health of other marine ecosystems (Short et al. 2011). However, even in the central habitat of seagrasses such as the "coral triangle", anthropogenic disturbances caused from activities such as overfishing and development are serious problems from the viewpoint of natural conservation of coastal ecosystems. Multiple coastal ecosystems are strongly associated each other; seagrass meadows are linked to coral reefs and mangroves in tropical regions (Heck et al. 2008). This study suggests that populations of *E. acoroides* possess weak genetic connectivity and the maintenance of seagrass meadows in BAN has been threatened because of low genetic diversity and isolated genetic cluster. Although the cluster is likely to be influenced by the recruitment from LAK, constant recruitment cannot be expected. Considering the low number of migrants from external meadows, each meadow should be considered as a separate protected unit and effective management rules should also be implemented based on the meadow size and abundance of seagrass-associated fauna, and species diversity. 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.

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References

Agawin NSR, Duarte CM, Fortes MD, Uri JS, Vermaat JE (2001) Temporal changes in the abundance, leaf growth and photosynthesis of three co-occurring Philippine seagrasses. J Exp Mar Biol Ecol 260:217–239

Arnaud-Haond S, Belkhir K (2007) GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. Mol Ecol Notes 7:15–17

Arnaud-Haond S, Alberto F, Teixeira S, Procaccini G, Serrão EA, Duarte CM (2005) Assessing genetic diversity in clonal organisms: low diversity or low resolution? Combining power and cost efficiency in selecting markers. J Hered 96:434–440

Arriesgado DM, Kurokochi H, Nakajima Y, Matsuki Y, Uy WH, Fortes MD, Campos WL, Nadaoka K, Lian C (2015) Genetic

diversity and structure of the tropical seagrass *Cymodocea serrulata* spanning its central diversity hotspot and range edge. Aquat Ecol 49:357–372

Botsford LW, White JW, Coffroth M-A, Paris CB, Planes S, Shearer TL, Thorrold SR, Jones GP (2009) Connectivity and resilience of coral reef metapopulations in marine protected areas: matching empirical efforts to predictive needs. Coral Reefs 28:327–337

Brouns JJWM, Heijs FML (1986) Production and biomass of the seagrass *Enhalus acoroides* (L.f.) Royle and its epiphytes. Aquat Bot 25:21–45

Campanella JJ, Bologna PAX, Smalley JV, Avila DN, Lee KN, Areche EC, Slavin LJ (2013) An analysis of the population genetics of restored *Zostera marina* plantings in Barnegat Bay, New Jersey. Popul Ecol 55:121–133

Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000

Diekmann OE, Serrão E (2012) Range-edge genetic diversity: locally poor extant southern patches maintain a regionally diverse hotspot in the seagrass *Zostera marina*. Mol Ecol 21:1647–1657

Dorken M, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of clonal plant, *Decodon verticillatus* (Lythraceae). J Ecol 89:339–350

Duarte CM, Uri JS, Agawin NSR, Fortes MD, Vermaat JE, Marba N (1997) Flowering frequency of Philippine seagrasses. Bot Mar 40:497–500

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Heck KL Jr, Carruthers TJB, Duarte CM, Hughes AR, Kendrick G, Orth RJ, Williams SW (2008) Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems 11:1198–1210

Hedgecock D, Barber PH, Edmands S (2007) Genetic approaches to measuring connectivity. Oceanography 20:70–79

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806

Kendrick GA, Waycott M, Carruthers TJB, Cambridge ML, Hovey R, Krauss SL, Lavery PS, Les DH, Lowe RJ, Mascaró i Vidal O, Ooi JLS, Orth RJ, Rivers DO, Ruiz-Montoya L, Sinclair EA, Statton J, van Dijk JK, Verduin JJ (2012) The central role of dispersal in the maintenance and persistence of seagrass populations. Bioscience 62:56–65

Kurokochi H, Matsuki Y, Nakajima Y, Fortes MD, Uy WH, Campos WL, Nadaoka K, Lian C (2016) A baseline for the genetic conservation of tropical seagrasses in the western North Pacific under the influence of the Kuroshio Current: the case of *Syringodium isoetifolium*. Conserv Genet 17:103–110

Lacap CDA, Vermaat JE, Rollon RN, Nacorda HM (2002) Propagule dispersal of the SE Asian seagrasses *Enhalus acoroides* and *Thalassia hemprichii*. Mar Ecol Prog Ser 235:75–80

Lian CL, Miwa M, Hogetsu T (2001) Outcrossing and paternity analysis of *Pinus densiflora* (Japanese red pine) by microsatellite polymorphism. Heredity 87:88–98

Lian CL, Oishi R, Miyashita N, Nara K, Nakaya H, Wu B, Zhou Z, Hogetsu T (2003) Genetic structure and reproduction dynamics of *Salix reinii* during primary succession on Mount Fuji, as revealed by nuclear and chloroplast microsatellite analysis. Mol Ecol 12:609–618

May PW, Doyle JD, Pullen JD, David LT (2011) Two-way coupled atmosphere-ocean modeling of the PhilEx Intensive Observational Periods. Oceanography 24:48–57

McClanahan TR, Mangi S (2000) Spillover of exploitable fishes from a marine park and its effects on the adjacent fishery. Ecol Appl 10:1792–1805

Meirmans PG (2014) Nonconvergence in Bayesian estimation of migration rates. Mol Ecol Resour 14:726–733

Muñiz-Salazar R, Talbot SL, Sage GK, Ward DH, Cabello-Pasini A (2005) Population genetic structure of annual and perennial populations of *Zostera marina* L. along the Pacific coast of Baja California and the Gulf of California. Mol Ecol 14:711–722

Nakajima Y, Matsuki Y, Lian C, Fortes M, Uy W, Campos W, Nakaoka M, Nadaoka K (2012) Development of novel microsatellite markers in a tropical seagrass, *Enhalus acoroides*. Conserv Genet Resour 4:515–517

Nakajima Y, Matsuki Y, Lian C, Fortes MD, Uy WH, Campos WL, Nakaoka M, Nadaoka K (2014) The Kuroshio Current influences genetic diversity and population genetic structure of a tropical seagrass, *Enhalus acoroides*. Mol Ecol 23:6029–6044

Nakaoka M (2005) Plant–animal interactions in seagrass beds: ongoing and future challenges for understanding population and community dynamics. Popul Ecol 47:167–177

Nievales MFJ (2009) Some structural changes of seagrass meadows in Taklong Island National Marine Reserve, Guimaras, western Visayas Philippines after an oil spill. Publications of the Seto Marine Biological Laboratory. Special Publication Series, 9, 37–44

Olsen JL, Stam WT, Coyer JA, Reusch TBH, Billingham M, Boström C, Calvert E, Christie H, Granger S, La Lumiere R, Milchakova N, Oudot-Le Secq M-P, Procaccini G, Sanjabi B, Serrão E, Veldsink J, Widdecombe S, Wyllie-Echeverria S (2004) North Atlantic phylogeography and large-scale populations differentiation of the seagrass *Zostera marina* L. Mol Ecol 13:1923–1941

Ort BS, Cohen CS, Boyer KE, Wyllie-Echeverria S (2012) Population structure and genetic diversity among eelgrass (*Zostera marina*) beds and depths in San Francisco Bay. J Hered 103:533–546

Peakall R, Smouse PE (2006) GenAlEx6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Piry S, Luikart G, Cornuet JM (1999) Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536–539

Pomeroy R, Garces L, Pido M, Silvestre G (2010) Ecosystem-based fisheries management in small-scale tropical marine fisheries: emerging models of governance arrangements in the Philippines. Mar Policy 34:298–308

Pritchard JK, Stephens P, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc Natl Acad Sci USA 102:2826–2831

Rollón RN, de Ruyter van Steveninck ED, van Vierssen W (2003) Spatio-temporal variation in sexual reproduction of the tropical

seagrass *Enhalus acoroides* (L.f.) Royle in Cape Bolinao, NW Philippines. Aquat Bot 76:339–354

Rosenberg (2004) DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138

Selig ER, Bruno JF (2010) A global analysis of the effectiveness of marine protected areas in preventing coral loss. PLoS One 5:e9278

Serra IA, Innocenti AM, Di Maida G, Calvo S, Migliaccio M, Zambianchi E, Pizzigalli C, Arnaud-Haond S, Duarte CM, Serrao EA, Procaccini G (2010) Genetic structure in the Mediterranean seagrass *Posidonia oceanica*: disentangling past vicariance events from contemporary patterns of gene flow. Mol Ecol 19:557–568

Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: a bioregional model. J Exp Mar Biol Ecol 350:3–20

Short FT, Polidoro B, Livingstone SR, Carpenter KE, Bandeira S, Bujang JS, Calumpong HP, Carruthers TJB, Coles RG, Dennison WC, Erftemeijer PLA, Fortes MD, Freeman AS, Japtag TG, Kamal AM, Kendrick GA, Kenworthy WJ, La Nafie YA, Nasution IM, Orth RJ, Prathep A, Sanciangco JC, van Tussenbroek B, Vergara SG, Waycott M, Zieman JC (2011) Extinction risk assessment of the world's seagrass species. Biol Conserv 144:1961–1971

Spieth PT (1974) Gene flow and genetic differentiation. Genetics 78:961–965

Tanaka N, Demise T, Ishii M, Shoji Y, Nakaoka M (2011) Genetic structure and gene flow of eelgrass *Zostera marina* populations in Tokyo Bay, Japan: implications for their restoration. Mar Biol 158:871–882

van Dijk JK, van Tussenbroek BI (2010) Clonal diversity and structure related to habitat of the marine angiosperm *Thalassia testudinum* along the Atlantic coast of Mexico. Aquat Bot 92:63–69

van Dijk JK, van Tussenbroek BI, Jiménez-Durán K, Márquez-Guzmán GJ, Ouborg J (2009) High levels of gene flow and low population genetic structure related to high dispersal potential of a tropical marine angiosperm. Mar Ecol Prog Ser 390:67–77

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr 27:1153–1167

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL Jr, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL, Paine RT (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc Natl Acad Sci USA 106:12377–12381

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191