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Population Genetic Structure of A Marine Pelagic Egg Producer and Popular Marine Aquarium Species, the Mandarinfish *Synchiropus splendidus*

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The mandarinfish *Synchiropus splendidus* is extensively collected in Southeast Asia (mainly in the Philippines) and highly favoured for the marine aquarium trade. Males are more popular than females for their large first dorsal fins and the fishery is not managed. To examine possible population replenishment dynamics arising as a result of selective fishing, the effects of sex-selective fishing on sex ratios and population connectivity were considered. This study determined the sex ratios and analyzed the population genetic structure from mandarinfish collected at six locations: one from Palau, where the species is not exploited, and five from Bohol in the Philippines, where the species has long been heavily fished. The findings reported very low male to female ratios (0.12 to 0.30) from four of the five locations in Bohol, with relatively more males to females in the specimens collected from Palau (2.3). The analyses from allozymes (43 alleles from 10 loci) and microsatellites (118 alleles from 5 loci) revealed that genetic connectivity was high among the five locations in the Philippines as well as with the specimens collected from the more-distant Palau. The genetic homogeneity observed across the geographical range considered is inconsistent with the hypothesized limited dispersal ability of the species and could be explained by recent species range expansion associated with sea level rise in the region. The results suggest that the present genetic structure, at least in the geographic region considered, may not be determined by current patterns of gene flow, but may, instead, be driven by recent sea-level changes associated with periods of glaciation. Caution is suggested to ensure that heavily localized fishing does not produce excessively biased adult sex ratios.

Key words: Dragonet, Aquarium trade, Bohol, Palau, Genetic structure, Sex ratio.

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BACKGROUND

Many marine fishes demonstrate significant connectivity even over broad geographic spatial scales. Combining high fecundity and long pelagic larval phases across open ocean they can spread their progeny widely and colonize large areas. Such connectivity is important in driving colonization of new habitats, increasing and spreading genetic diversity of isolated populations, and for replenishing exploited or depleted populations following overexploitation (Cowen and Sponaugle 2009). This dynamic capability is even more critical in the context of fragmented habitats, such as coral reefs. Even if wide dispersal is a rare event (Almany et al. 2017), over time long-distance dispersal of eggs and larvae can result in homogenization of gene pools across a broad region, as evidenced in many pelagic spawning reef fishes, such as the surgeonfishes *Naso brevirostris*, *N. unicornis* and *N. vlamingii*, as well as the demersal egg-producing clownfishes, *Amphiprion* species (Klanten et al. 2007; Horne et al. 2008; Simpson et al. 2014).

Nonetheless, population genetics studies have often found strong population structure in species with a pelagic life history phase, indicating restricted population connectivity in some marine fishes, for instance the three-spotted damselfish *Dascyllus trimaculatus* (Leray et al. 2010), Regal angelfish *Pygoplites diacanthus* and Sammara squirrelfish *Neoniphon samara* (DiBattista et al. 2013). Growing evidence suggests that many reef fish larvae exhibit strong homing behaviour, with parentage analysis revealing high levels of self-replenishment in some species such as the saddleback clownfish *A. polymnus* (Jones et al. 2005), the orange clownfish *A. percula*, and the Vagabond butterflyfish *Chaetodon vagabundus* (Planes et al. 2009; Almany et al. 2017). While many biotic factors, *e.g.*, pelagic larval duration (Bradbury et al. 2008), adult migration (Frisk et al. 2014) and physical factors (*e.g.*, oceanography, Pineda et al. 2007), have been evoked to explain the wide range of population connectivity levels observed in reef fishes, no single explanatory model pertains as the history and biology of each species is unique. With reef fishes increasingly threatened by anthropogenic activity and climate change, an understanding of the dynamics of population replenishment through population connectivity in this large group of taxa is becoming more pressing for improving conservation and developing spatially appropriate management measures (Lowe and Allendorf 2010).

The mandarinfish *Synchiropus splendidus* (family Callionymidae) is an attractive dragonet inhabiting the Indo-West Pacific. The species lives and spawns in sheltered, slow-moving, shallow, inshore waters and has one of the shortest larval durations (about 14 days) yet known among pelagic egg spawning marine fishes (Sadovy et al. 2001). Similar to clownfish in terms of larval period but with pelagic eggs (vs. demersal in clownfish), this species is predicted to show stronger population structure than those with long larval durations or with spawning modes that release eggs into open waters (Buston et al. 2007).

The mandarinfish is considered among the most attractive fishes by marine aquarists and is extensively collected in Southeast Asia for the international aquarium trade. It is caught in large numbers and, although locally abundant, there are concerns over the intensity and selectivity of collection activity in some areas. Individual populations or locations are possibly vulnerable to heavy, uncontrolled and spatially focused, fishing pressure which is selective for males due to their more spectacular first dorsal fin morphology (Chan and Sadovy 1998; Sadovy et al. 2001). The Philippines has been an important source of the species for the international aquarium trade for decades, particularly the centrally located island of Batasan (Sadovy et al. 2001). It is, therefore, of interest to better understand the population connectivity of this species in the Philippines, and in the neighbouring unexploited (for this species) region, to examine possible population replenishment dynamics. The possible outcome of sex-selective fishing on sex ratios of this species was also examined.

In this study, we used allozyme and microsatellite markers to examine the population structure and infer population connectivity among five mandarinfish collection locations from the central Philippines and, a sixth location, Palau. Results could shed light on possible dispersal limits of a short pelagic larval duration in a marine fish species, within the geographic region considered, and have possible conservation and management implications. We also compare sex ratios between Palau, where the species is not fished, and the Philippines, where it is heavily fished, and consider the implications for reproductive behavior.

MATERIALS AND METHODS

Sampling

A total of 323 individuals was collected in 2003 by specialized mini-spearfishing techniques (*i.e.*, using a needle attached to a chopstick to pin the fish by its tail for capture) used by fishers, from five adjacent localities within the central area of the Philippines archipelago, near Bohol island (Batasan Island, Inanoran Island,

Tagbilaran City, Handayan Island and Guindacpan Island) (Fig. 1A), and in 2002 fish were purchased in one additional location in Palau, about 1,170 km to the east (Fig. 1A) taken as an outgroup to the five locations in the Philippines. The five sampling locations around Bohol were chosen based on high catches of the mandarinfish with considerable distances among these locations. Sample size per location ranged from 47–56 individuals. Fish were kept alive before they were transported to the camp and preserved as full body in liquid nitrogen after anaesthetization. Sexes of all specimens were identified using the secondary sexual characteristic of their dorsal fins; males have the first spine of the first dorsal fin elongated after sexual maturation (Rasotto et al. 2010). The sex ratio per sampling location was presented as the number of males / the number of females (male: female).

Allozyme analyses

Individuals were dissected to isolate a piece of dorsal muscle (1 to 2 g) which was then homogenized at 4°C in an equal volume of Tris/EDTA/NADP buffer (pH 6.8) followed by centrifugation at 15,000 g for 30 min at 4°C and the supernatants were stored in a -80°C freezer for further analysis.

Allozyme variations were examined as described in Pasteur et al. (1987). A total of nine enzymes were processed, providing 10 loci: aspartate aminotransferase, EC 2.6.1.1 (AAT*; TC 8.0), esterase, E.C. 3.1.1.1. (EST*; TC 6.7), glucosephosphate isomerase, EC 5.3.1.9 (PGI-1* and PGI-2*; TC 6.7), isocitrate dehydrogenase, EC 1.1.1.42 (IDH*; TC 8.0), lactate dehydrogenase, EC 1.1.1.27 (LDH*; TC 8.0), malic enzyme, EC 1.1.1.40 (ME*; TC 8.0), peptidase leucine-glycine-glycine, E.C. 3.4.1.1. (PEP-LGG*; TME 7.4), peptidase phenylalanine-proline, E.C. 3.4.1.1. (PEP-PP*; TME 7.4) and phosphoglucomutase, EC 5.4.2.1 (PGM*; TC 6.7). Loci were scored according to enzyme nomenclature (Shaklee et al. 1990).

Allele frequencies and genotypic variability parameters were computed using GENETIX version 4.05 (Belkhir et al. 2004). The deviation from Hardy-Weinberg equilibrium was assessed with the fixation index (F_{IS}) in each location. The multilocus F_{IS} was statistically tested using the Markov chain reaction implemented in Genepop 3.1d (Raymond and Rousset 1995). The genetic divergence between locations was assessed with the Wright's standardized variance in allelic frequencies (F_{ST}, Wright 1969) using (Weir and Cockerham 1984) algorithm in GENETIX. Significant differences between F_{ST} were tested for using the Fisher exact test implemented in Genepop. The F_{IS} and F_{ST} significance levels for statistical tests were adjusted for each population separately following a sequential Bonferroni (Rice 1989).

Microsatellite analyses

Tissue from the dorsal muscle of each individual was further dissected and subjected to DNA extraction using the Qiagen DNeasy Blood & Tissue Kit. Microsatellite primers were developed using a modification of the FIASCO-based method (fast isolation by AFLP of sequences containing repeats (Zane et al. 2002). Clones with 2 to 6 bp small tandem repeats were selected and primers were designed at the flanking regions using the online software NetPrimer (http://www.premierbiosoft.com/netprimer/index.html). The variability of the selected loci with tandem repeats was tested with the individuals from the six locations. The population differentiation of the six locations was evaluated using these microsatellite primers (Table S1).

Microsatellite genotypes were scored by using GeneMarker version 2.2.0 (Hulce et al. 2011). Observed and expected heterozygosity (H_O and H_E), and significant deviation from Hardy-Weinberg equilibrium were estimated by GenAIEx version 6.5 (Peakall and Smouse 2012). MICROCHECKER was used to detect potential scoring errors and null alleles. Genepop version 4.0 (Rousset 2008) was used to test for linkage disequilibrium by running Markov chain 100,000 iterations. Statistical significance was adjusted by using sequential Bonferoni. We calculated pairwise F_{ST} with and without using the ENA (excluding null alleles) method implemented in FreeNA (Chapuis and Estoup 2007) to correct for the bias due to null alleles. As FreeNA does not calculate *p*-value but instead provide 95% confident interval (CI) for F_{ST}, CIs above zero were considered statistically significant. FreeNA also provided estimates of null allele frequency across all loci and populations using the EM algorithm (Dempster et al. 1977). We tested genetic differentiation between locations more specifically between the Philippines and the distant population in Palau by AMOVA conducted in Arlequin version 3.5 (Excoffier and Lischer 2010). Global genetic variance among individuals was analyzed using a discriminant analysis of principal components (DAPC) (Jombart et al. 2010) that generate scatterplots of discriminant functions derived from the microsatellite genotypes with sampling location as prior. Genetic clustering was tested using the Bayesian approach implemented in STRUCTURE version 2.3.3 (Pritchard et al. 2000) using sampling location as prior. For each value of K (from 1 to 5), 20 replicates were run with 500,000 steps after 50,000 steps of burn-in. The Evanno method implemented in Structure Harvester (Earl 2012) was used to determine the best K

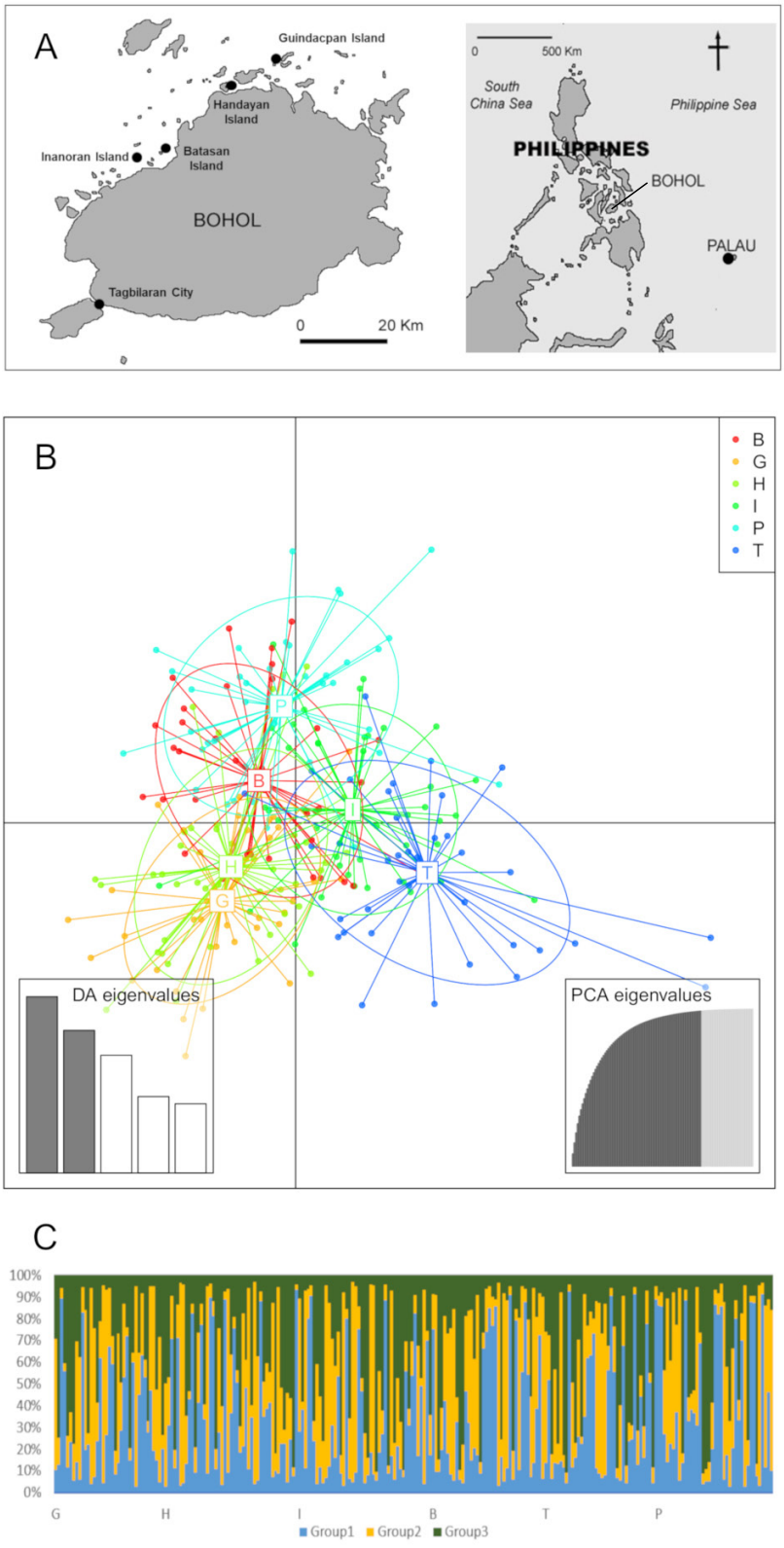

Fig. 1. (A) Maps showing the five collecting locations (Batasan Island, Inanoran Island, Tagbilaran City, Handayan Island and Guindacpan Island) around Bohol Island, the Philippines and positions of the Bohol, the Philippines and the Palau Islands. Black dots: collecting locations. (B) DAPC plot of six populations of mandarinfish. (C) SRUCTURE assignment plot of six locations of the mandarinfish. G: Guindacpan; H: Handayan; I: Inanoran; B: Batasan; T: Tagbilaran; P: Palau.

for the dataset and Clustering Markov Packager Across K (CLUMPAK) (Kopelman et al. 2015) was used to plot the clustering graph.

RESULTS

Male to female ratios

Variations in the male to female ratio (male: female) were observed among the 6 different locations (Table 1). The male to female ratios recorded are: Guindacpan Island - 0.24; Inanoran Island - 0.12; Tagbilaran city - 0.30; Batasan Island - 0.22; Handayan Island - 1.61; Palau - 2.29. Very low male to female ratios were recorded in four of the locations in the Philippines where the species has been particularly heavily fished. Highest ratio was recorded from Palau where the species is not commercially fished.

Allozyme results

Forty-three alleles were scored throughout the 10 loci with no significant multi-locus Hardy-Weinberg disequilibrium detected for any sampling locations. Single-locus analysis showed that Batasan Island had two loci with significant deviation from the Hardy-Weinberg disequilibrium, *i.e.*, PGM* showed significant homozygote deficiency, while PEP-LGG* showed significant heterozygote deficiency.

All six locations showed a low but not significant multi-loci F_{ST} value (0.0031). Locus by locus analysis demonstrated that PEP-LGG* expressed a significantly higher single locus F_{ST} value (0.0298) compared to others (Table 2). Pairwise multi-loci F_{ST} among locations showed low F_{ST} values only significant between Batasan Island and Handayan (F_{ST} = 0.0113, p = 0.025), and between Batasan Island and Guindacpan Island (F_{ST} = 0.0059, p = 0.006) (Table 3A).

Microsatellite results

Over the five polymorphic microsatellite loci, an overall total of 118 alleles were detected and screened for 242 individuals from the six locations (Table 4). The locus B59 displayed the highest allelic diversity with 30 alleles while the locus A20 only showed four alleles (data not shown). Mean number of alleles across loci ranged from 13.4 in Batasan to 15.2 in Inanoran. Only 26 private alleles among locations were detected, with Palau and Batasan having three private alleles each, and up to six private alleles in the specimens from Inanoran.

Table 1. Sex and sex ratio of the mandarinfish, *Synchiropus splendidus*, sampled at six locations

Location	Guindacpan Island	Inanoran Island	Tagbilaran City	Batasan Island	Handayan Island	Palau
Code	G	I	T	B	H	P
Female	21	25	33	23	18	14
Male	5	3	10	5	29	32
Uncertain	23	27	10	27	5	1
Male:Female	0.24	0.12	0.30	0.22	1.61	2.29

Table 2. F_{IS} and F_{ST} values for each of the ten polymorphic allozyme loci and overall loci for all the locations. *indicates significant values at the level of $p \leq 0.05$

Locus	F_{IS}	F_{ST}
PGM	-0.0064	-0.0034
PGI-1	0.0181	0.0060
PGI-2	-0.0316	-0.0032
ME	-0.0500	-0.0010
LDH	-0.0079	-0.0033
IDH	0.0873	-0.0061
PEP-LGG	0.1647*	0.0298*
PEP-PP	-0.0113	0.0052
EST	0.0247	0.0002
AAT	0.0138	0.0027
Multilocus	0.0266	0.0031

No significant linkage disequilibrium was detected (data not shown), but MICROCHECKER detected the presence of null alleles in three locations in locus A28, and in all locations in three loci (A29, B20, B59) (Table S2). All loci except A20 and A29 showed significant deviation from Hardy-Weinberg Equilibrium in three to six locations (Table S3), and all locations showed significant deviation from Hardy-Weinberg Equilibrium due to heterozygote deficiency, which is likely due to the presence of null alleles (Tables S2, S4). The inbreeding coefficient (F_{IS}) was generally high, ranging from 0.229 in Batasan to 0.411 in Guindacpan, with an overall value of 0.304 (Table 4), likely resulting from to the high frequency of null alleles (Table S4).

Genetic variability was comparable among locations. Expected heterozygosity (H_E) per locus varied from 0.383 (locus A20 at Tagbilaran) to 0.928 (locus A29 at Palau) (Table S3). Observed heterozygosity (H_O) was the lowest at locus A28 at Handayan (0.222) and maximum at locus A29 at Palau (0.846) (Table S3). Mean H_E across loci ranged from 0.764 Tagbilaran to 0.821 in Inanoran, while mean H_O across loci ranged from 0.471 in Guindacpan to 0.615 in Batasan (Table 4).

Pairwise F_{ST} only indicated very weak, if any, genetic differentiation among the locations, both with and without ENA correction (Table 3B). Pairwise F_{ST} were all below 0.022, and all but two of the pairwise F_{ST} were insignificant (between Handayan and Tagbilaran, and between Palau and Tagbilaran with CIs above zero). Both standard and locus-by-locus AMOVA showed no significant partitioning between the individuals collected from the Philippines and Palau, with most of the genetic variation observed within location (> 98.92%) (Table S5). The DAPC analysis did not show any significant difference among locations although several individuals from Tagbilaran were clearly separated from the distribution of all others (Fig. 1B). The best number of cluster K = 3 was determined for STRUCTURE analysis (Fig. S1), but STRUCTURE did not indicate any geographic pattern in the distribution of genetic variation with K = 3 (Fig. 1C), nor with other K values from two to five (Fig. S2).

DISCUSSION

High population connectivity in the mandarinfish

Both allozyme and microsatellite analyses indicated high genetic homogeneity of the mandarinfish among the specimens collected in the different locations in the central Philippines, as well as with the specimens from the more distant location, Palau. Such congruence suggests significant gene flow between the Bohol area within the Philippines, and also with Palau which is approximately 1,170 km away. Because of the low genetic differentiation, quantitative estimates of migration rate from programs like BAYESASS (Wilson and Rannala 2003) could not be reliably obtained

Table 3. (A) Pairwise F_{IS} between locations based on ten polymorphic allozyme loci, with levels of significant difference denoted as * for $p \leq 0.05$ and ** for $p \leq 0.01$. (B) Pairwise F_{ST} based on five microsatellite loci without ENA correction (below diagonal) and with ENA correction (above diagonal) calculated by FreeNA, with significant genetic differentiation (95% CI above zero) in italic

(A) Locations	Guindacpan Island	Handayan Island	Inanoran Island	Batasan Island	Tagbilaran City	Palau
Guindacpan Island	-	0.0082	-0.0039	0.0059**	-0.0022	-0.0005
Handayan Island	-	-	0.0028	0.0113*	0.0059	0.0051
Inanoran Island	-	-	-	0.0069	-0.0053	0.0025
Batasan Island	-	-	-	-	0.0001	0.0026
Tagbilaran City	-	-	-	-	-	0.006

(B) Locations	Guindacpan Island	Handayan Island	Inanoran Island	Batasan Island	Tagbilaran City	Palau
Guindacpan Island	-	-0.004	0.005	-0.002	0.010	0.002
Handayan Island	-0.007	-	*0.009*	0.004	0.016	0.001
Inanoran Island	0.006	*0.012*	-	0.003	0.007	0.007
Batasan Island	-0.005	0.004	0.004	-	0.011	0.006
Tagbilaran City	0.011	0.019	0.008	0.013	-	*0.020*
Palau	-0.001	-0.001	0.008	0.005	*0.022*	-

(Faubet et al. 2007).

The apparent absence of genetic structure is not unusual in marine organisms but appears surprising for a sedentary and short larval life (14 days) species that spawns in sheltered shallow inshore waters (*i.e.*, relatively limited water flow). The mandarinfish is a small (maximum size circa. 7.0 cm total length) demersal species which is sedentary after settlement and with no record of adult migration (Sadovy de Mitcheson unpubl. data). The species is one of the smallest pelagic egg spawners with small batch size (12–205 eggs released per spawn) (Sadovy et al. 2001; Rasotto et al. 2010). It was reasonable to hypothesize, therefore, that, considering recent evidence of high levels of local retention, such as in the saddleback clownfish which has a short pelagic larval phase, comparable in duration to that of the mandarinfish (Jones et al. 2005; Almany et al. 2007; Planes et al. 2009; Almany et al. 2017), we might expect similarly local differentiation. By contrast, however, we observed genetic homogeneity both at local and large-scale spatial levels, and irrespective of the markers used.

The approach used in the present study is different from that used in the aforementioned clownfish studies because it is based on allelic frequencies and individual variance, and not on parentage analysis. It was shown previously that only a small amount of gene flow is sufficient to homogenize allelic frequencies (Pinsky et al. 2017). Therefore, the present results suggest that the quantity of long-distance dispersal in this species is sufficient to homogenize allele frequency. Consistent with previous studies of genetic differentiation (Puebla et al. 2012) this result shows no differences across at least part of the western Pacific (Planes and Fauvelot 2002). A similar pattern was also recorded in intertidal decapods, such as in fiddler crabs and ghost crabs, for which species populations in the western Pacific were found to be genetically homogeneous (Ma et al. 2019; Shih and Poupin 2020). However, since the species is distributed from the Ryukyu Islands, Japan, in Southeast Asia and Micronesia to Australia and New Caledonia, it is still possible that more distant locations, or those unlikely connected by oceanographic conditions, may show population separation.

Although the mandarinfish inhabits inshore waters and its larval dispersal may be restricted by local inshore currents, it spawns frequently year-round (Randall et al. 1990; Sadovy de Mitcheson, Rasotto, de Mitcheson pers. obs. for Palau), and extensively across inshore waters, factors that might increase the chance of larvae becoming picked up in large-scale oceanographic currents that carry them to distant habitats. The westward flowing Equatorial Current connecting Palau and the Philippines, for example, could facilitate larval dispersal between these two areas (Monismith et al. 2018). Consistent with this hypothesis, the genetic homogeneity indicated by microsatellite loci implies some gene flow in a contemporary time-scale. Conversely, while many factors can determine connectivity, limited locations and timing of spawning events, as indicated in some aggregating groupers, could influence, including reduce, connectivity (Ma et al. 2018).

Another factor possibly contributing to high population connectivity is that the species underwent recent demographic growth and range expansion. The same hypothesis has been suggested in other studies of coral reef fishes such as for butterfly fishes and damselfishes (Fauvelot et al. 2003). Global sea levels rose and fell due to numerous glacial cycles occurring around 2.5 million to 10,000 years ago (Haq et al. 1987). During the Last Glacial Maximum, (LGM), around 19,000 years ago, the sea level retreated down to about 130 m below the present level (Yokoyama et al. 2000). The Sunda Shelf, as well as the Bohol area, was above sea level during the LGM (Hanebuth et al. 2000). In the later phase of the Ice Age, the sea level rose again following deglaciation, and the Sunda Shelf became flooded about 14,600 years ago. Colonization of species could have proceeded as the seawater advanced and,

Table 4. Sampling sizes and overall genetic diversity of five microsatellite loci, including number of individuals (N), number of alleles (Na), mean observed heterozygosity (H_O) and expected heterozygosity (H_E), and fixation index (F_{IS})

Locations	N	Na	Mean H_O	Mean H_E	F_{IS}
Guindacpan Island	37	13.6	0.471	0.788	0.411
Handayan Island	45	14.2	0.501	0.787	0.368
Inanoran Island	45	15.2	0.608	0.821	0.270
Batasan Island	38	13.4	0.615	0.777	0.229
Tagbilaran City	38	14.4	0.574	0.764	0.262
Palau	39	14.8	0.599	0.804	0.269
All	242	23.6	0.561	0.790	0.304

regardless of dispersive capability, resulting in a rapid expansion of the species' range. Lourie et al. (2005) hypothesized on the influence of range expansion in two seahorse species which resulted in sharing of common haplotypes between sites inside and outside the Sunda Shelf. The homogeneous genetic structure of mandarinfish from Bohol to Palau may be a result of this recent range expansion event. Moreover, the wide range of homogeneity of the mandarinfish suggests either that founding populations were large and diverse, or that successive and contemporary colonizations occurred from large and diverse source populations.

Conservation implications

The homogeneous genetic structure over the western central portion of the geographic range of the mandarinfish suggests that the species could be managed as part of a regional unit, at least across the Central Pacific of its range which was the focus of this study. While the high population connectivity detected in this study using allozyme and microsatellite analyses implies that populations in the study area are interdependent in terms of population replenishment, our data could not provide sufficient resolution to determine source-sink dynamics. In theory, if the source population is overfished, it could have an extensive impact on the sink populations (Tittler et al. 2006). Research in future could identify key source populations in this species to prioritize conservation actions. This may become important if most individuals of this species marketed in the international aquarium trade continue to come from the wild. Sampling of fish from more extreme locations in its geographic range (*e.g.*, western Indonesia, southern Japan, New Caledonia, etc.) is needed to further test the initial dispersal hypothesis.

Moreover, the selective focus of fishers on males (due to the spectacular nature of their elaborated dorsal fins), which may account for the more female-skewed sex ratios in the more heavily exploited Philippines study locations compared to the unfished Palau site, could potentially negatively influence reproduction or sexual selection in future. For example, since females prefer to mate with large rather than small males, there is a possibility for local impacts on reproduction and reproductive output in this species as sex ratios become heavily female-skewed (Rasotto et al. 2010). While the central Philippines-Palau area could be managed as a single unit, since heavily female-skewed sex ratios may affect reproduction male bias could be minimized by avoiding high male removals in localized areas.

CONCLUSIONS

The mandarinfish is a pelagic spawner exploited for the aquarium trade, with males being particularly heavily harvested. Our study found a much lower male to female ratio from the more exploited populations in the Philippines than in Palau, which is likely the result of sex-selective fishing. Contrary to our expectation for a species with possible limited dispersal ability, both allozymes and microsatellites revealed high genetic connectivity across the Central Pacific. It could not be determined whether Palau is a possible source area for the Philippines. However, the detected connectivity pattern indicated may be driven by recent sea-level changes associated with periods of glaciation. This study provides the first population genetics and sex ratio analyses of the mandarinfish which have implications for conservation and management.

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Authors' contributions: YSM, SP and ML conceived and designed the study. ML collected materials and all authors contributed to analysis. The first draft of the manuscript was written by PTYL and KYM, and all authors commented on all versions of the manuscript.

Competing interests: PTYL, KYM, ML, SP and YSM declare that they have no conflict of interest.

Availability of data and materials: Allozyme and microsatellite data are available at https://doi.org/10.6084/m9.figshare.c.5123381.v1.

Consent for publication: All authors give their consent to publish.

Ethics approval consent to participate: Not applicable.

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Supplementary Materials

Table S1. Information of the five polymorphic microsatellite loci for the mandarinfish, *Synchiropus splendidus*. (download)

Table S2. The presence of null alleles based on MICROCHECKER. Please refer to Table 1 for location codes. (download)

Table S3. Summary of chi-square tests for Hardy-Weinberg equilibrium. * and ** indicate sequential-Bonferroni-adjusted $p < 0.05$ and $p < 0.01$, respectively. H_O: observed heterozygosity; H_E: expected heterozygosity. (download)

Table S4. Estimates of null allele frequency. (download)

Table S5. AMOVA based on microsatellite markers, where all samples were included in two groups: the Philippines vs Palau. (download)

Fig. S1. Plot of Evanno's delta K as a function of K, over 20 replicates. (download)

Fig. S2. SRUCTURE assignment plot of six locations of the mandarinfish with K = 2 to K = 5. 1: Guindacpan; 2: Handayan; 3: Inanoran; 4: Batasan; 5: Tagbilaran; 6: Palau. (download)