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Spread beyond the border: Small Scale genetic structure of the introduced Banggai cardinalfish (*Pterapogon kauderni*) population in the Bali Strait

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Abstract

The living marine aquarium trade has been known to mediate various aquatic invaders to spread and established a new population outside of their natural home range. In most cases, the introduce species cause a cascaded effect which harm the native species and their ecosystem. The successful of the invasion event often related to the genetic properties of the introduced species. Therefore, using a molecular approach based on a mitochondrial DNA marker, the present work aimed to evaluate the population genetic structure of the introduced P. kauderni population in Bali Strait. Samples were collected from four sites in Gilimanuk Bay, Bali Strait. Our results showed a high haplotype diversity and low nucleotide diversity that might be a signal of a bottleneck. Overall, we found five haplotypes comprising two unique haplotypes and three shared haplotypes. Genetic structure was detected in some localities, which indicates Pterapogon kauderni might originated from various genetically distinct populations. However, we could not conclusively determine the observed patterns of the genetic structure within each site. These patterns are likely related to the multiple introduction event of P. kauderni in Bali Strait. Additional samples from both side (introduced and native habitat) combined with more rapidly evolving markers may describe the structure more clearly.

Keywords: *Aquarium Trade, Banggai Cardinalfish, Bali Strait, Control Region MtDNA, Exotic Species, Invasive*

Introduction

Invasion by alien species (non-indigenous organisms) in marine ecosystems has become a global challenge (Keith *et al*., 2016; Tsiamis *et al*., 2020) and the frequency of occurrence may continue to rise substantially over the coming decades (Levine and D'Antonio, 2003). Invasion can occur through shipping activities, the aquarium trade, and mariculture (Maceda-Veiga *et al*., 2013; Nunes *et al*., 2014; Venezia *et al*., 2018). A marine organism that invades a new habitat could disturb ecosystem function by introducing parasites and diseases (Blakeslee *et al*., 2012; Telfer and Bown, 2012), competition with local species (Silva *et al*., 2017; Balestri *et al*., 2018), changing food-web interactions in the ecosystem (Va'zquez-Luis *et al*., 2013), and can lead to the extinction of local species (Doherty *et al*., 2016; Gallardo *et al*., 2016; Ambariyanto, 2017). Cases of non-native marine species introduction have been widely documented in various taxa, including reef fishes (Azour *et al*., 2015; Bariche *et al*., 2015; Butterfield *et al*., 2015), crustacea (Heinonen and Auster, 2012; Koenders *et al*., 2012; McLay and Fowler, 2013; Lejeusne *et al*., 2014), molluscs (Ardura *et al*., 2015; Riquet *et al*., 2016; Dias *et al*., 2018) and some marine plants (Sghaier *et al*., 2011; Willette and Ambrose, 2012; Ceccherelli and Pinna, 2014).

The Banggai cardinalfish (*Pterapogon kauderni*) is an endemic species of the Banggai Islands which has been introduced to the Bali Strait (Allen and Erdmann, 2012) and other areas around Sulawesi (Erdmann and Vagelli, 2001; Moore and Ndobe, 2007). In at least one case, *P. kauderni* has become invasive (Erdmann and Vagelli, 2001). The introduction of *P. kauderni* to the Bali Strait probably occurred accidentally through activities related to the ornamental fish aquarium trade (Militz *et al*., 2016; Rhyne *et al*., 2017). *P. kauderni* has been traded by local people in Banggai Islands since 1992 and by 2000 there were around 118,000 fish traded every month (Lunn and Moreau, 2004). While aquaculture should be the main source of the fish production, recent data show that traded fish were reported as wild-caught (Rhyne *et al*., 2017; Ndobe *et al*., 2018). The scale of the trade, the limited distribution of the species, and its natural susceptibility to exploitation caused a continued population decline, which makes *P. kauderni* designated as an endangered species according to the World Conservation Union (IUCN RedList) (Allen and Donaldson, 2007).

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The Decree of the Minister of Marine and Fisheries (MMF) No:49/KEPMEN-KP/2018 has been announced to maintain *P. kauderni* populations around the Banggai Islands. This Decree aims to support the conservation and sustainable use of *P. kauderni* in Banggai Islands. Although, this species requires urgent protection due to over-exploitation within its home range, on the other hand, it has been recognized as an introduced fish in Bali, and some locations in Sulawesi (Moore and Ndobe, 2007; Vagelli, 2011). Moreover, the established populations in Lembeh Strait were potentially invasive (Molnar *et al*., 2008). High densities of *P. kauderni* may harm the benthic communities which are commonly associated with sea urchin and anemones such as other apogonid fishes and anemonefishes (Vagelli, 2011). Therefore, it is important to evaluate the *P. kauderni* populations in their native range and in introduced locations to provide an appropriate management for this species.

P. kauderni larvae show direct development and are presumed to have low dispersal capabilities (Vagelli, 1999). Juveniles of this species are usually found within the parental habitat, settles to particular microhabitats (e.g. seagrasses, sea urchin, anemones, corals, sponges, and mangrove roots), and exhibits low mobility and dispersing capability as adult (Kolm *et al*., 2005). The limited distribution and connectivity of this species is due to some of the biological aspects such as lack of pelagic dispersal ability, low fecundity, and their attachment to particular substrates in the coral reef (Vagelli, 1999). Recent work on the population structure of *P. kauderni* in the Banggai Islands revealed that populations separated by extremely short distances were genetically distinct (Hoffman *et al*., 2005). In addition, *P. kauderni* also shows a high level of self-reassignment which mean this species does not tend to spread over long distances (Vagelli *et al*., 2009).

Meanwhile, the genetic structure of *P. kauderni* that have been introduced to and become established in Bali Strait has not yet been identified. In Bali Strait, the species has been found in Gilimanuk Bay (Lilley, 2008) which is a semi-enclosed bay with an average depth of 2 meters. According to a previous survey, this species was found in a shallow area and associated with sea urchins, sponges, seagrasses, and corals (Arbi *et al*., 2019; Putra and Putra, 2019). Referring to several biological factors such as the short duration of pelagic larvae and the tendency for self-reassignment (Allen, 2000), we expected *P. kauderni* in Bali Strait would also show genetic isolation between populations. Therefore, this study aimed to determine the genetic population structure of the introduced *P. kauderni* population in Bali Strait. Understanding genetic structure of an introduced species is important to determine the ecological impact and the potential invasiveness of the species in order to protect local species and surrounded ecosystems.

Materials and Methods

Sample collections

Sample collection was conducted at four sites located in Gilimanuk Bay, Bali Strait, Indonesia (Figure 1). From each site, 5-8 *P. kauderni* specimens were collected using scoop nets while snorkeling in April 2019. A total of 25 specimens were collected and a small tissue sample from each specimen was preserved in 96% ethanol for subsequent analysis. According to the Decree of the Minister of Marine and Fisheries (MMF) No:49/KEPMEN-KP/2018, the specimen collection in this area could be completed without any special permit.

Molecular analysis

DNA was extracted from muscle or fin tissues by the Chelex 10% extraction method (Walsh *et al*., 1991). The control region of the mitochondrial genome (mtDNA) was amplified with forward primer CRA (TTC CAC CTC TAA CTC CCA AAG CTA G), and reverse primer CRE (CCT GAA GTA GGA ACC AGA TG) (Lee *et al*., 1995). DNA amplification reaction were made in 25 µl reactions with a final concentration of 2 mM $MgCl_2$, 0.8 mM of dNTPs, 0.5 µM of each primer,1X PCR Buffer, 14.5 µl ddH_2O, 2.5 U Taq DNA Polymerase, and 1 µl of DNA template. The PCR was conducted with the following profile: a denaturation step of 94°C for 45 sec, followed by an annealing temperature of 48°C for 45 sec and extension at 72°C for 60 sec, this cycle was repeated 35 times (Bernardi and Vagelli, 2004). All PCR products were evaluated on 2% agarose gel. Clear and strong PCR products were sent to 1st Base DNA Sequencing Service.

Data analysis

All sequences were initially aligned and edited manually using Mega 5 (Tamura *et al*., 2011). Multiple sequence alignment was done using ClustalW, as implemented in Mega 5 (Tamura *et al*., 2011). DnaSP ver. 6 (Rozas *et al*., 2017), was use to calculated the number of segregated sites (S), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π). In addition, neutrality tests including Tajima's D and Fu's Fs were estimated for each population using Arlequin Ver 3.5 (Excoffier and Lischer, 2010). To describe the

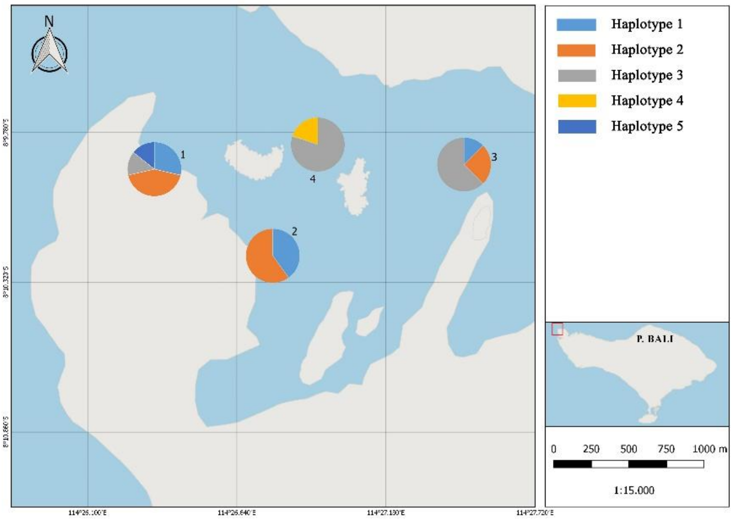

Figure 1. Location of the four *P. kauderni* sampling sites in Gilimanuk Bay, Bali Island, Indonesia. Pie charts show the mtDNA haplotype distribution of individuals sampled from each site (sites are coded with 1, 2, 3, and 4).

relationship among haplotypes, a Minimum Spanning Network (Bandelt *et al*., 1999) was use to constructed a haplotype network as implemented in PopART version 1.7 (Leigh and Bryant, 2015). To determine between site population structure, Φ_{ST} was estimated between each population pair using the software package Arlequin Ver 3.5 (Excoffier and Lischer, 2010).

Results and Discussion

Genetic diversity

Sequences obtained from 25 individuals of *P. kauderni* collected from four sites in Gilimanuk Bay were aligned and analysed (Table 1). Among these 25 individuals, five haplotypes were observed, involving four polymorphic nucleotide sites. Genetic diversity indices showed high levels of haplotype diversity but low nucleotide diversity within the *P. kauderni* population at each sampling site. Haplotype diversity ranged from 0.60 in the population from site 2 to 0.81 in site 1. Meanwhile, nucleotide diversity ranged from 0.003 to 0.004 (Table 1). The overall number of haplotypes (H) was 5, haplotype diversity (Hd) was 0.72, nucleotide diversity, πT was 0.003. Neutrality tests produced positive values and none of these tests were significant, indicating that the DNA sequence evolving in random process (neutrally) (Table 1).

Endemic species are often characterized by a low abundance (Weir *et al*., 2011; Baker *et al*., 2013), making them susceptible to genetic drift which causes decreased in genetic diversity. In contrast, our research found that the introduced *P. kauderni* population in Gilimanuk Bay had high haplotype (Hd > 0.5) and low nucleotide (π < 5%) diversity. These results suggest that this *P. kauderni* population may have experienced a population bottleneck, which may be associated with the founder effect. Although further analysis using Tajima's D, and Fu's Fs tests could not confirm the results since these tests were not significant. The founder effect occurs when a new population is formed by individuals from a larger population; this can result in changes to allele frequencies relative to the source population, due to the significant reduction in population size (Johnson *et al*., 2016; Morim *et al*., 2019). *P. kauderni* has been spread outside of its native range, mediated by the living marine aquarium trade (Militz *et al*., 2016; Rhyne *et al*., 2012). Introduced population sites was found within Sulawesi Island (Vagelli, 2011) and Bali (Allen and Erdmann, 2012). According to trade route data from early 2001, at least 1,000-8,000 *P. kauderni* were traded by exporters or employer's company in Bali (Lunn and Moreau, 2004). Therefore, this species may possibly have been released in Gilimanuk Bay over several years (Lilley, 2008; Ndobe *et al*., 2018).

Since the *P. kauderni* population in Gilimanuk Bay is an introduced population, we could expect that this population would have a low level of genetic diversity compared to the population(s) of origin in the Banggai Islands. Contrary to prior expectations, we found that the haplotype diversity of *P. kauderni* in Gilimanuk Bay was similar to that reported for native populations in the Banggai Islands. For instance, the present study found a Hd range of 0.60-0.81, while a previous study conducted in the Banggai Islands reported a Hd range of 0.00-0.82 (Bernardi and Vagelli, 2004). The increased genetic

Table 1. Sample collection site, number of samples (n), number of segregated sites (S), number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), Tajima's D (D), and Fu's Fs (Fs) of *P. kauderni* collected from four sites in Gilimanuk Bay. ns= not significant

Site	Latitude, longitude	n	S	H	Hd	π	D	Fs
1	-8.16491, 114.44025	7	3	4	0.81	0.004	1.459^{ns}	-0.538^{ns}
2	-8.16951, 114.44699	5	2	2	0.60	0.003	1.459^{ns}	1.688^{ns}
3	-8.16415, 114.45550	8	3	3	0.61	0.003	0.204^{ns}	0.562^{ns}
4	-8.16415, 114.45550	5	1	2	0.72	0.003	-0.816^{ns}	0.090^{ns}
Total		25	4	5	0.72	0.003	0.95^{ns}	0.105^{ns}

Table 2. *P. kauderni* population Φ_{ST} values (below diagonal) and Φ_{ST} P values (above diagonal) derived using Arlequin ver. 3.5.

Site	1	2	3	4
1	0	0.789	0.358	0.008*
2	-0.104	0	0.124	0.008*
3	0.035	0.245	0	0.213
4	**0.390**	**0.636**	0.107	0

Significant Φ_{ST} values marked by bold number with *P< 0.05.

diversity in aquatic invaders is not surprising as reported in some papers (Lejeusne *et al*., 2014; Roman, 2006; Guardiola *et al*., 2016; Arnaud-Haond *et al*., 2017; Negri *et al*., 2018). Several mechanisms may work simultaneously to dilute the founder effect (Roman, 2006; Geller *et al*., 2010). For example, both population growth rates and the probability of establishment could be increase by a large number of released individuals (Lockwood *et al*., 2005; Johnston and Purkis, 2016). Alternatively, repeated temporal introduction events from genetically distinct source populations could also compensate for the lack of genetic diversity in any one particular introduction event (Lejeusne *et al*., 2014; Rius *et al*., 2015). Previous studies have found that *P. kauderni* in the Banggai Islands showed a restricted gene flow and high genetic structuring (Hoffman *et al*., 2005; Vagelli *et al*., 2009). Thus, our results suggest that the *P. kauderni* population in Gilimanuk Bay was most likely established through repeated temporally distinct introductions from different areas in the Banggai Islands.

Population structure

The Minimum Spanning Network is presented in Figure 2. A loop was present in the network, indicating possible alternative relationships among haplotypes. Network analysis showed there were two (40%) unique haplotypes (Hap_4 and Hap_5) and 3 shared (60%) haplotypes (Hap_1, Hap_2, and Hap_3) (Figure. 1.). The most common haplotypes were shared by 10 individuals (Hap_3) and this haplotype was found in all sites except site 2. Haplotype distribution showed there was no shared haplotype between sites 2 and 4 (Figure 2.) These results indicate a low level of gene flow between these two sites, a conjecture further supported by the high Φ_{ST} values (Table 2). Similarly, sites 1 and 2 also showed a low level of gene flow with just one haplotype shared between these two sites.

According to the Φ_{ST} analysis, genetic structure was detected (P<0.05) in some localities (Table 2.). The Φ_{ST} values range from -0.104 to 0.636 and the highest Φ_{ST} values was between site 2 and site 4 (Table 2.). A previous genetic study on *P. kauderni* revealed a low level of gene flow around the Banggai Islands (Hoffman *et al*., 2005) and on small scale areas in Bangkulu Island (Vagelli *et al*., 2009). A strong genetic structure was observed in population separated over a distance of km (Hoffman *et al*., 2005). Consistent with previous works, our results also found significant genetic structuring within a small-scale area. The limited dispersal potential maybe related to the lack of pelagic larval duration (PLD) (Allen, 2000) and adult philopatric behaviour (Kolm *et al*., 2005). These are suspected to limit the gene flow in *P. kauderni* such found in some other fish species like blennioid fish (Riginos and Victor, 2001), and anemonefishes (Madduppa *et al*., 2014; Timm *et al*., 2017). In fact, coral reef fishes with long-distance dispersal potential can also show strong genetic partitioning (Ravago-Gotanco and Juinio-Meñez, 2010; Ackiss *et al*., 2013; Pertiwi *et al*., 2019).

Our data could not definitely determine the genetic structuring within each site based on the observed patterns. Larger sample sizes combine with more rapidly evolving molecular markers might help address this question. Population differentiation was observed between sites 1 and 4 and between sites 2 and 4 (Table 2.), all of which are located quite close to each other. There are no obvious geographical barriers (*i.e.* strong currents, deep range) between these sites that could promote

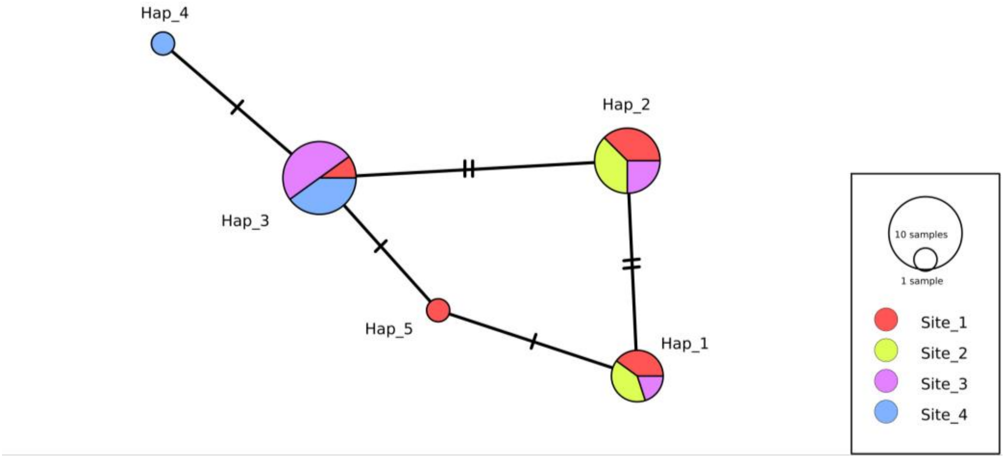

Figure 2. A minimum-spanning network of the control region haplotypes of *P. kauderni*. The size of each circle indicates the haplotype's frequency and each circle represents a unique haplotype. Colours in this case were used to assign haplotypes to four sampling localities (Site_1, 2, 3, 4). Lines on bars that separate haplotypes represent the mutational steps between one haplotype and another.

such differentiation, because Gilimanuk Bay is a semi-enclosed bay with an average depth of 2 meters (Macklin *et al*., 2019). In the absence of a geographical barrier, we consider that the most reliable explanation for the observed patterns is the origin of the four introduced populations sampled. The fish released at each site probably have come from different populations (sites) in the Banggai Islands. A comprehensive survey conducted in Banggai and nearby islands found that *P. kauderni* was recorded in 34 islands encompassing areas approximately 5000 km^2 (Vagelli, 2011). Two previous studies in the Banggai Islands found that each *P. kauderni* population sampled was genetically distinct (Bernardi and Vagelli, 2004; Hoffman *et al*., 2005). Our data indicate that the introduced population in Gilimanuk Bay seems to have come from several different source populations. Some of the main fish exporters in Bali are known to be based in Gilimanuk Bay and have actively traded *P. kauderni* since 1992 (Lunn and Moreau, 2004). Each company probably has a specific collection site in Banggai Islands, thus affecting the founder population composition and the observed genetic structure. However, further research using larger sample sizes from both the introduced population and potential source locations would be needed to confirm this hypothesis.

In recent years, genetic data have been use broadly for marine biodiversity conservation (Palsbøll *et al*., 2006; Von Der Heyden *et al*., 2014; Venegas-Li *et al*., 2016). Genetic approaches have also very useful to determine, trace, and mitigate biological invasion (Hänfling *et al*., 2011; Bariche *et al*., 2015; Negri *et al*., 2018). To the best of our knowledge, this is the first genetic data available for *P. kauderni* in Bali. Thus, we believe that this present work could be useful for management of *P. kauderni* in Bali, particularly for stock delineation. Furthermore, protection of genetic diversity and stock delineation is one of the key priorities for *P. kauderni* conservation and management in Banggai Islands (Moore *et al*., 2017; Ndobe *et al*., 2019). Regarding to the introduction of *P. kauderni* in Gilimanuk Bay, recent works have been showed that *P. kauderni* has been found in the entire bay occupying various microhabitat (*e.g*., sea urchin, seagrasses, hard coral, and sponges) and the populations continue to flourish (Arbi *et al*., 2019; Putra and Putra, 2019). Therefore, regular monitoring for the *P. kauderni* populations status is important to determine whether they are invasive or not to the surrounding ecosystems.

Conclusion

Despite the low samples sizes collected during the present study, our results show that the *P. kauderni* population in Gilimanuk Bay, Bali Strait, exhibits high haplotype diversity and low nucleotide diversity. Significant genetic structuring was detected in some localities, indicating low gene flow between some sites. The genetic structure observed within this study might also implied that the founder population of *P. kauderni* in Bali Strait, may have originated from several genetically distinct populations in Banggai Islands. Given the high level of genetic diversity and rapid establishment of *P. kauderni* in Bali Strait, it is important to monitor the populations regularly to prevent this species from harming the native organisms and the rest of ecosystems.

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