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Original Article

Investigation of Population Genetic Structure of the Pink Anemonefish (Amphiprion perideraion) in the Southern Coast of Viet Nam

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Abstract *- Pink anemonefish (Amphiprion perideraion) has commercial value as a popular aquarium fish, and due to its collection from the wild, there are concerns for the conservation of the species. Six polymorphic microsatellites were used to reveal the population genetic structure of pink anemonefish from three locations (Quang Nam, Khanh Hoa – Ninh Thuan, and Phu Quoc) in Viet Nam. Analyses showed high levels of gene flow among populations sampled from the South China Sea (F_{ST} = 0.008, P > 0.05). the genetic homogeneity of A. perideraion in this region is likely due to the oceanographic-driven interaction of pelagic larvae in central waters off Viet Nam. in contrast, the strong genetic structure was evident among the South China Sea and Gulf of Thailand populations (F_{ST} = 0.131 – 0.146, P < 0.000). This high genetic differentiation between the South China Sea and Gulf of Thailand populations suggests that the sustainable management of this species in Viet Nam needs to be enacted according to indigenous needs as two separate stocks.*

Keywords *- Amphiprion perideraion, The coast of Viet Nam, Population genetic structure.*

1. Introduction

Amphiprion perideraion (Pomacentridae), also known as the pink anemonefish, are resident in tropical reefs across the Indo-Western Pacific region from the Gulf of Thailand, Australia, and New Caledonia. Pink anemonefish are sequential hermaphrodites, living in small based schools, each with a pair of adult fish, some pre-mature fish, and some juveniles. the largest adults in a size-based hierarchical group are female. When the dominant female dies or is displaced, the largest male in the group will undergo protandrous sex change into the female phenotype (Hattori, 2000). the species are obligatory symbionts with anemones, including those from the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) (Dohna et al., 2015). Anemones are used as the home of these fish, protecting them from predators. in contrast, the fish will protect the anemone against anemone-eating fish (Roopin et al., 2008) that might harm the anemone and provide nutrients to the anemones through excrement (Fautin,1991; Ricciardi et al., 2010).

Pink anemonefish have high economic value because of their desired shape and color patterns. As a result, they are extensively targeted for the global aquarium trade. However, due to the fish's relatively low reproductive potential (one pair of fish produces around 2,000 - 4,000 eggs per year) (Allen, 1991) and non-migratory life history, there are fears that overharvesting has altered the structure and viability of populations (Dohna et al., 2015). Posing an additional threat, the anemones the pink anemonefish have a symbiotic relationship with are also extensively harvested for the aquarium trade, thereby reducing available habitat.

Due to the species' commercial importance and heavy wild harvesting, fishery managers are interested in more effectively and sustainably managing the fishery. an important part of this process combining findings from parentage analysis or oceanographic modeling studies (Burgess et al., 2014) is the need to understand whether populations are interconnected through gene flow leading to continual recruitment from spawning populations elsewhere, or if extermination of the fish in one region will result in local extinction due to restricted gene flow.

As with much non-migratory marine fish, pink anemonefish exhibit two life stages; a pelagic phase with a duration of ~18 days before fish settle and a relatively sedentary life history associated with their host anemones (Coughlin et al., 1992; Fautin and Allen, 1992). in several fish species, a pelagic life stage is a driving factor in determining the genetic connectivity of populations (Pineda

et al., 2007). Because of their 18-day pelagic larval duration (Wellington et al., 1989), pink anemonefish larvae can potentially disperse widely, resulting in low levels of genetic structure. However, if biogeographical conditions create barriers to gene flow, larval dispersal may be impeded, thereby increasing genetic differentiation due to random genetic drift. *A. perideraion* genetic structure is evident among populations within the Indo-Malay Archipelago, particularly among Indonesian and Papua New Guinean populations (Dohna et al., 2015). However, little is known of how other populations of this species outside the Indo-Malay Archipelago are genetically connected.

Viet Nam has a diverse coral reef ecosystem, covering an area of 1,122 km^2 from north to south (Nguyen et al., 2014). in particular, in some places such as Cu Lao Cham, Khanh Hoa - Ninh Thuan, Con Dao, and Phu Quoc, coral reef ecosystems are concentrated with high density and are the main reefs where pink anemonefish are found. These places, however, have well-developed marine tourism activities and are heavily exploited for seafood and ornamental purposes, thus affecting reef ecosystems (Nguyen et al., 2014). Bleached coral reefs recorded in two areas of Phu Quoc in 2010 (Vo, 2013) and most recently in Con Dao in 2016 have resulted in severe habitat loss for many species of marine life and have indirectly affected anemonefishes. Accordingly, it is necessary to develop a plan for restoration, management, conservation, and sustainable exploitation of reef fish in these regions of Vietnam (Do and Nguyen, 2007; Vo, 2013). to date, there have not been any studies that have examined the genetic population connectivity of *A. perideraion* in Viet Nam. Therefore, this study was carried out to (1) determine the population genetic structure and levels of connectivity among Vietnamese *A. perideraion* and (2) provide basic information to inform conservation, management, and exploitation policies for this species in coral reef areas in Viet Nam.

2. Materials and Methods

2.1. Sampling and DNA extraction

Research divers collected *A. perideraion* species from two central Viet Nam sites in the coastal waters of the South China Sea (Quang Nam: 26 samples and Khanh Hoa - Ninh Thuan: 31 samples) and one site located in southern Viet Nam in the Gulf of Thailand (Phu Quoc: 33 samples) (Fig. 1). to minimize the impact, specimens were captured using a hand net from their anemone and placed in containers with seawater to enable sampling on a boat. Approximately 0.3 cm2 of caudal fin were cut from the specimens with scissors and stored in 96% alcohol. Specimens then were immediately released back to the anemone they were collected. Samples from central Viet Nam were in 2016 and 2017, while specimens from Phu Quoc were collected in 2017.

DNA extraction and Microsatellite Amplification DNA was extracted using the CTAB method (Adamkewicz and Harasewych 1996) and was diluted to 10–40 ng/µl for use as a template in preparation for amplification of microsatellites via PCR (Table 1). Microsatellites were individually amplified in 50 µl reaction volumes, including 10 µL of Bioline 2X buffer, 0.2 µL MyTaq HS Polymerase (Bioline), 0.4 µM of each primer, and 5 ng of the template DNA. Forward primers were marked by HEX or FAM fluorescence staining at the 5' end. PCR was performed on a BOECO Thermal Cycler (cycling parameters: 1 min at 95 °C, followed by 27 cycles of 95 °C for 15 s, 52 °C for 15 s, 72 °C for 10 s, before a final extension step of 60 °C for 30 min). the PCR products were then checked for consistent amplification by visualization on a 1.5% agarose gel.

PCR products were loaded on an ABI3100, using a 500 Rox size standard (Application Bios System) for size separation of alleles (service provided by Macrogen, Korea). Genemarker software (ver. 1.91 SoftGenetics, State College, PA, USA) was used to assign alleles into their allelic bins for each locus.

Fig. 1 Location of the three Vietnamese sites sampled for pink anemonefishes and the number of fish sampled from each site (numbers are circled)

Table 1. Details of the six microsatellites targeted for population genetic analyses in pink anemonefishes (Buston et al., 2007; Liu et al., 2007; Quenouille et al., 2004)

No	Locus name	Code	PCR product length	Microsatellite repeat	Forward/ reverse primers	No. of nultiplex
1	Locus_55	55	453-471	(GT)16	TTAACTTCCACACCCAGTCT	1
					ACGCTGTGAGAGTCCATTAT	
2	Locus_626	Ac626	246-280	(TC)6(AC)20	CACACATGCACACACCTTGA	
					TAATTGAGGCAGGTGGCTTC	
3	Locus_120	120	467-501	(GT)18N20(GT)14	TCGATGACATAACACGACGCAGT	2
					GACGGCCTCGATCTGCAAGCTGA	
4	Locus_1578	Ac1578	270-274	(AC)9	CAGCTCTGTGTGTGTTTAATGC	
					CACCCAGCCACCATATTAAC	
5	Locus_137	Ac137	285-371	(AC)19	GGTTGTTTAGGCCATGTGGT	3
					TTGAGACACACTGGCTCCT	
6	Locus_915	Ac915	236-274	(AC)9	TTGCTTTGGTGGAACATTTGC	
					TCTGCCATTTCCTTTGTTC	

2.2. Data analysis

The reliability of each microsatellite locus for population genetic analysis in *A. perideraion* was assessed before statistical analyses. Evidence for the presence of null alleles was evaluated with MICRO CHECKER (Ver 2.2.3; Van Oosterhout et al., 2004). Summary statistics such as the number of alleles, private alleles, observed and expected heterozygosities were calculated for the microsatellites in GENALEX 6.1 (Peakall and Smouse, 2006), which was also used to test for deviations from Hardy-Weinberg Equilibrium (HWE). in all statistical analyses where multiple pairwise tests were performed, P-values were corrected according to Benjamini and Hochberg (1995) (also known as FDR values). GENEPOP on http://genepop.curtin.edu.au/ was used to test for linkage disequilibrium among microsatellite loci and calculate the inbreeding coefficient Fis according to Weir and Cockerham (1984) and Robertson and Hill (1984).

The level of the genetic structure of *A. perideraion* based on microsatellite markers was analysed using an Analysis of Molecular Variance (AMOVA) with 10,000 permutations to calculate global FST, as well as the calculation of pairwise FST comparisons between populations. Both steps were carried out with ARLEQUIN 3.5 (Excoffier et al., 2005).

The population structure of *A. perideraion* was studied using the model-based clustering method in STRUCTURE (Ver 2.3.4, Pritchard et al., 2000). This model applies a Bayesian approach to estimate the probability of correctly assigning all genotypes in the data in the number K of clusters. in this analysis, all fish specimens were identified by their sampling location. the burn time was set to 100,000 repetitions. Each K (1-10 clusters) was run for 10 repetitions, and the probability was calculated as the basis of the estimated average probability of the data. the results were then submitted to http://taylor0. biology.ucla.edu/ structureHarvester / to get the true K value (Earl and vonHoldt, 2012).

3. Results and Discussion

3.1. Genetics characteristics

Data analysis for the six microsatellites (Appendix 1) showed that samples possessed high observed heterozygosity (0.590 - 0.857) for all microsatellites, except for locus Ac1578 (0.207). No significant differences were observed, and expected heterozygosity for each locus (Table 2).

The highest alleles were found for locus Ac137 (n = 33). However, Microchecker indicated the possible presence of null alleles at this marker. in deciding whether or not to remove this locus in case it influenced genetic analyses, AMOVA and pairwise F_{ST} tests were performed that included and excluded this locus; no significant differences in variance statistics or F_{ST} values were evident between analyses, and thus this marker is included in the results provided below. Specimens of A. perideraion from Phu Quoc had a lower allelic diversity (53 alleles) than from Quang Nam and Khanh Hoa - Ninh Thuan (66 alleles) (Table 2).

3.2. Genetic structure

AMOVA analysis showed that ~10% of genetic variability was partitioned among the populations surveyed, with the remaining variation within populations (90%) (Table 3). the estimate of global F_{ST} indicated evidence of significant genetic structuring (F_{ST} = 0.10; P < 0.001) (Table 3). To examine the evidence of population genetic variants, pairwise F_{ST} tests were conducted, indicating that central Vietnamese *A.* perideraion exhibited no evidence of genetic structuring (Quang Nam and Khanh Hoa-Ninh Thuan) (F_{ST} = 0.008, P = 0.08). However, *A.* perideraion from Phu Quoc were significantly different from both central Vietnam populations (Quang Nam F_{ST} = 0.146, P < 0.01; and Khanh Hoa - Ninh Thuan, F_{ST} = 0.131, P < 0.01 (Table 4).

Table 2. Genetic estimates for each microsatellite from three pink anemonefishes populations including

Population		Locus_ 55	Locus_ 626	Locus_ 120	Locus_ 1587	Locus_ 915	Locus_ 137	Average
Quang Nam	N	26	26	26	26	26	26	
	Na	9	15	11	3	6	22	11.00
	Pa	2	1	2	0	1	3	1.50
	Ho	0.89	0.85	0.77	0.15	0.42	0.69	0.63
	He	0.82	0.91	0.88	0.15	0.49	0.93	0.70
	HWE	0.934	0.115	0.445	0.944	0.944	0.069	
	HWE Significance	ns	ns	ns	ns	ns	ns	
	F_{ST}	-0.06	0.09	0.14	-0.04	0.16	0.27	0.09
Khanh Hoa – Ninh Thuan	N	31	31	31	31	31	31	
	Na	7	14	11	3	6	25	11.00
	Pa	0	2	1	0	2	4	1.50
	Ho	0.81	0.77	0.90	0.19	0.74	0.74	0.69
	He	0.76	0.89	0.85	0.18	0.67	0.95	0.72
	HWE	0.944	0.731	0.731	0.944	0.944	0.003	
	HWE Significance	ns	ns	ns	ns	ns	*	
	F_{ST}	-0.05	0.15	-0.04	-0.07	-0.09	0.23	0.02
Phu Quoc	N	33	33	33	33	33	33	
	Na	5	12	6	2	4	24	8.83
	Pa	0	2	1	0	0	3	1.00
	Ho	0.88	0.82	0.21	0.27	0.61	0.58	0.56
	He	0.70	0.75	0.20	0.24	0.63	0.92	0.57
	HWE	0.607	0.944	0.944	0.731	0.944	0.000	
	HWE Significance	ns	ns	ns	ns	ns	*	
	F_{ST}	-0.24	-0.07	-0.05	-0.14	0.05	0.39	-0.01
	N – total	30	30	30	30	30	30	
	Na – total	9	19	14	3	8	33	14.33
	Ho – mean	0.86	0.81	0.63	0.21	0.59	0.67	0.63
	He – mean	0.76	0.85	0.64	0.19	0.6	0.93	0.66

N: sample size; Na: number of alleles; Pa: number of private alleles; Ho: observed heterozygosity; He: expected heterozygosity*; HWE: Hardy-Weinberg equilibrium; ns: no statistical difference; *: significant difference compared to Hardy-Weinberg at 0.005 (FDR alpha value); F_{ST}: inbreeding coefficient.*

Table 3. Results of AMOVA analyses and examination of differences in genetic variation between Vietnamese populations of pink anemonefishes. (d.f, degrees of freedom)

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation
Among populations	2	30.584	0.22251	9.98
Within populations	177	271.287	2.00605	90.02
Total	179	299.983	2.22856	
Fixation index F_{ST} = 0.09984				

Table 4. Pairwise FST differences between Vietnamese pink anemonefishes populations (*, significance)

Populations	Quang Nam	Khanh Hoa – Ninh Thuan	Phu Quoc
Quang Nam	0.00000		
Khanh Hoa – Ninh Thuan	0.00763	0.00000	
Phu Quoc	0.14644*	0.13138*	0.00000

3.3. Structure analysis

The Bayesian analysis performed in STRUCTURE supported F_{ST} analyses and indicated the presence of two distinct genetic clusters. the approaches using L(K) (Fig. 2) as well as ΔK (Fig. 3) determined the best K is 2. *A. perideraion* from Phu Quoc had most of its genome assigned to one cluster (green cluster in Fig. 4),

While fish from Quang Nam and Khanh Hoa-Ninh Thuan had ancestry largely defined to the other cluster (red cluster, Fig. 4). Thus STRUCTURE analyses show that *A. perideraion* in Viet Nam consists of at least two distinct genetic stocks, one stock of the South China Sea and the second stock of the Gulf of Thailand.

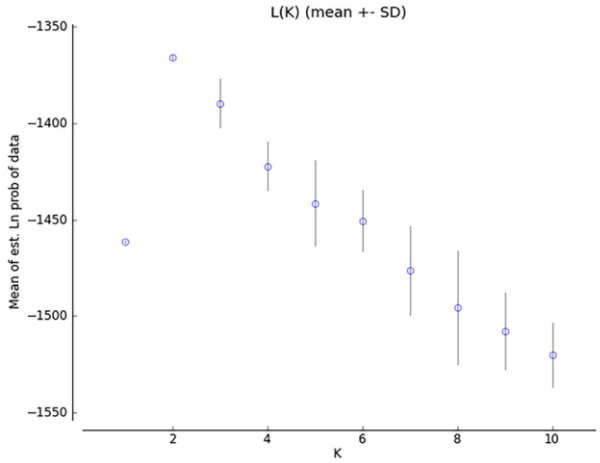

Fig. 2 the log-likelihood for each K, Ln P(D) = L(K). When K is approaching a true value (2), L(K) plateaus (or continues increasing slightly) and has a high variance between runs (Rosenberg et al., 2001)

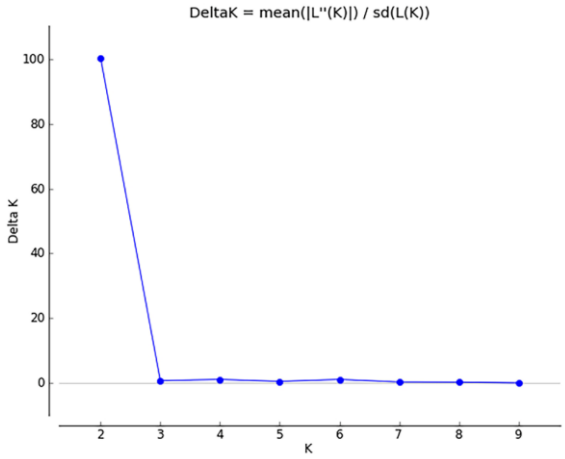

Fig. 3 Calculated based on the second-order rate of change of the likelihood (ΔK) (Evanno et al. 2005)**. the ΔK shows a clear peak at the true value of K =2**

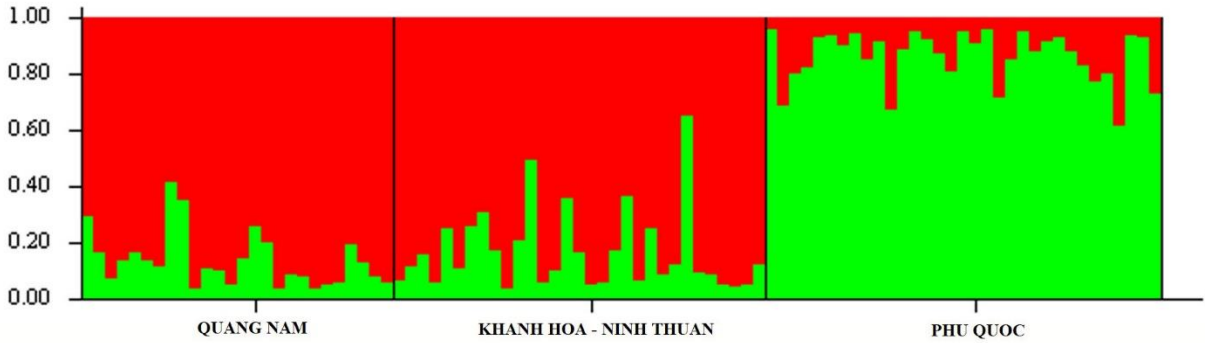

Fig. 4 Bayesian STRUCTURE analysis of pink anemonefishes from collected sites in Viet Nam. Data is represented as two genetic clusters. Bars represent the proportion of an individual fish's genome that is assigned to either the red cluster or green cluster

4. Discussion

Amphiprion perideraion populations in Viet Nam are under exploitation with notable concerns for the long-term sustainability of the wild fishery and a need for more effective management. This study examined the genetic structure of Vietnamese A. perideraion sampled from two South China Sea populations (Quang Nam and Khanh Hoa - Ninh Thuan) and one from the Gulf of Thailand (Phu Quoc). Genetic analyses clearly show regional genetic differentiation between the South China Sea and Gulf of Thailand populations. Accordingly, our results indicate that A. perideraion in Viet Nam should be managed as two distinct stocks. This finding aligns with the broader management recommendations Ablan et al. (2002) forwarded. They looked at the meso-scale transboundary fisheries management units in Viet Nam and proposed two fishery stocks should be considered as different regional management units.

The Quang Nam population should be classified as a north-central group (encompassing northwestern Taiwan, northern Vietnam, and the northwestern Philippines), and the Phu Quoc population should be classified as a southwestern group (comprising southern Viet Nam and the eastern coast of mainland Malaysia). the results of this study show that the Khanh Hoa-Ninh Thuan population belongs to the north-central group. However, this population was at the southern boundary of the north-central group and the northern boundary of the southwestern group. It can be explained by a range of coral reefs distributed in the north-central Viet Nam (Son et al., 2007) and current local patterns during the breeding season (Hu et al., 2000), which might support the

disperse of A. perideraion larvae in this area, while there are only a few coral reefs throughout southern Viet Nam.

Significant genetic structures among populations have also been found in the previous study on A. perideraion (Dohna et al., 2015) and other congeneric species of anemonefish, including A. ocellaris (Nelson et al., 2000; Timm et al., 2012), in which significant genetic differences were found between eastern, central, and western Indo-Malay Archipelago. the present study on A. perideraion is the first in the area and revealed the genetic differentiation between the regions, providing useful data to guide future studies and inform regional management. the genetic variation was also found in other Indo-Pacific coral reef species such as Chlorurus sordidus (Bay et al., 2004) and Tridacna crocea (DeBoer et al., 2008) or Dascyllus trimaculatus (Leray et al., 2010). These species have the same characteristics as the pink anemonefish in that they lay demersal eggs (Riginos et al., 2011), have a short larval development phase (PLD ~ 18 days), and adults, once settled, remain very to their resident sea anemone (Madduppa et al., 2014).

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APPENDIX 1: PCR product length of 06 microsatellites of each individual collected from three populations in Viet Nam

No	Sample ID	Populations	Locus_55		Locus_626		Locus_120		Locus_1587		Locus_137		Locus_915	
1	AP_001	QN	453	471	264	264	489	495	270	270	323	333	246	246
2	AP_002	QN	457	459	248	272	485	495	270	270	335	339	238	238
3	AP_003	QN	455	465	246	264	475	491	270	270	321	329	238	238
4	AP_004	QN	457	461	264	268	491	491	270	270	339	345	238	238
5	AP_005	QN	455	455	256	272	479	487	270	274	345	351	238	238
6	AP_006	QN	461	465	254	276	489	495	270	270	345	345	238	246

No	Sample ID	Populations	Locus_55		Locus_626		Locus_120		Locus_1587		Locus_137		Locus_915	
7	AP_007	QN	461	463	266	274	479	499	270	274	333	343	236	238
8	AP_008	QN	459	459	248	272	479	497	270	270	333	333	236	238
9	AP_009	QN	457	459	254	264	479	489	270	270	323	345	238	246
10	AP_010	QN	453	457	266	280	491	497	270	270	349	357	238	238
11	AP_011	QN	453	457	256	270	479	485	270	270	335	341	238	250
12	AP_012	QN	455	457	254	264	493	497	270	272	309	321	238	238
13	AP_013	QN	455	457	256	268	487	493	270	270	285	285	238	238
14	AP_014	QN	457	459	256	266	479	493	270	270	331	343	238	238
15	AP_015	QN	457	457	264	280	479	493	270	270	297	309	238	246
16	AP_016	QN	459	461	266	278	489	489	270	270	333	333	238	244
17	AP_017	QN	457	463	254	278	485	489	270	270	333	333	238	238
18	AP_019	QN	461	463	256	268	495	495	270	270	309	333	238	238
19	AP_020	QN	455	457	256	272	491	491	270	272	307	315	238	274
20	AP_021	QN	453	455	274	274	475	489	270	270	311	311	236	236
21	AP_022	QN	455	463	270	280	491	493	270	270	307	307	238	238
22	AP_023	QN	455	457	260	262	489	495	270	270	351	351	238	238
23	AP_024	QN	455	467	248	248	489	489	270	270	357	369	238	238
24	AP_025	QN	455	463	254	272	479	491	270	270	335	347	236	238
25	AP_027	QN	457	463	248	270	467	467	270	270	337	345	236	238
26	AP_030	QN	455	457	268	268	493	497	270	270	337	341	236	250
27	AP_074	KH-NT	457	467	256	270	483	483	270	274	331	331	236	246
28	AP_075	KH-NT	457	461	256	264	489	491	270	270	335	335	238	238
29	AP_076	KH-NT	455	459	266	274	475	491	270	272	335	349	236	246
30	AP_077	KH-NT	457	457	256	264	489	497	270	270	357	357	238	238
31	AP_078	KH-NT	455	459	248	248	485	489	270	270	333	363	238	248
32	AP_079	KH-NT	455	457	254	266	479	497	270	270	315	315	238	240
33	AP_080	KH-NT	457	459	256	256	479	491	270	270	321	343	238	246
34	AP_081	KH-NT	455	461	248	274	491	499	270	270	305	323	238	246
35	AP_082	KH-NT	455	455	264	266	479	489	270	274	323	357	236	238
36	AP_083	KH-NT	457	457	254	274	489	491	270	274	317	345	238	238
37	AP_084	KH-NT	455	459	254	268	479	491	270	274	309	309	236	246
38	AP_085	KH-NT	459	459	252	264	479	489	270	270	323	333	238	246
39	AP_086	KH-NT	455	459	262	274	489	491	270	270	333	347	236	238
40	AP_087	KH-NT	457	459	264	268	493	495	270	270	341	371	238	238
41	AP_088	KH-NT	459	459	264	270	489	495	270	270	347	355	238	244
42	AP_089	KH-NT	455	459	256	256	479	495	270	270	323	335	236	238
43	AP_090	KH-NT	455	457	270	274	493	495	270	270	319	319	238	240
44	AP_091	KH-NT	459	459	256	280	493	495	270	270	331	343	236	240
45	AP_092	KH-NT	461	465	248	270	493	493	270	270	313	357	238	238
46	AP_093	KH-NT	455	459	270	274	479	491	270	270	329	329	238	244

No	Sample ID	Populations	Locus_55		Locus_626		Locus_120		Locus_1587		Locus_137		Locus_915	
47	AP_094	KH-NT	457	465	248	278	489	491	270	270	307	313	236	238
48	AP_095	KH-NT	455	457	256	256	479	483	270	270	323	351	238	246
49	AP_096	KH-NT	455	463	264	270	475	499	270	270	339	363	238	238
50	AP_097	KH-NT	455	457	260	266	479	489	270	270	333	347	236	238
51	AP_113	KH-NT	455	457	248	248	479	491	270	270	341	355	238	246
52	AP_114	KH-NT	459	465	255	268	489	489	270	270	331	349	236	246
53	AP_115	KH-NT	457	459	248	266	483	495	270	274	349	357	236	238
54	AP_116	KH-NT	455	459	262	280	495	497	270	270	343	363	236	236
55	AP_117	KH-NT	457	463	270	270	489	491	270	270	335	335	236	236
56	AP_118	KH-NT	459	463	262	270	489	501	270	270	335	361	236	238
57	AP_119	KH-NT	455	459	274	274	491	493	270	270	305	309	238	246
58	AP_041	PQ	459	461	248	248	479	479	270	270	347	347	244	246
59	AP_042	PQ	455	455	248	256	479	479	270	270	321	331	238	238
60	AP_043	PQ	455	461	248	262	479	493	270	272	345	345	246	246
61	AP_044	PQ	459	461	264	272	479	495	270	272	337	347	238	246
62	AP_045	PQ	459	459	248	268	479	479	270	272	345	355	246	246
63	AP_046	PQ	455	461	264	270	479	479	270	272	345	351	244	244
64	AP_047	PQ	455	461	248	266	479	479	270	270	335	345	246	246
65	AP_048	PQ	459	461	248	248	479	479	270	272	333	333	238	246
66	AP_049	PQ	459	461	248	248	474	479	270	272	309	309	238	246
67	AP_050	PQ	459	461	248	262	479	479	270	272	327	361	246	246
68	AP_051	PQ	455	459	248	268	475	479	270	270	327	327	238	246
69	AP_052	PQ	455	459	248	264	479	479	270	270	317	325	238	238
70	AP_053	PQ	459	461	248	264	479	479	270	270	361	361	246	246
71	AP_054	PQ	455	461	248	272	479	479	270	270	339	345	238	244
72	AP_055	PQ	455	461	248	276	479	479	270	270	345	345	244	246
73	AP_056	PQ	455	455	248	258	479	479	270	270	341	351	246	250
74	AP_057	PQ	455	461	264	264	479	479	270	270	327	341	246	246
75	AP_058	PQ	455	459	264	272	479	479	270	270	329	335	238	246
76	AP_059	PQ	455	461	248	264	479	479	270	272	311	315	244	244
77	AP_060	PQ	455	461	248	260	479	495	270	270	315	321	238	246
78	AP_061	PQ	457	459	270	272	479	479	270	270	349	349	244	246
79	AP_062	PQ	455	459	248	264	479	479	270	270	345	363	244	246
80	AP_063	PQ	457	461	248	264	479	479	270	270	337	349	238	244
81	AP_064	PQ	457	461	248	264	479	479	270	270	331	345	246	246
82	AP_065	PQ	459	461	264	264	479	479	270	270	345	345	238	246
83	AP_066	PQ	459	461	248	270	479	479	270	270	343	343	244	250
84	AP_067	PQ	455	459	260	264	479	479	270	270	327	341	238	246
85	AP_068	PQ	455	455	248	267	479	479	270	270	327	341	238	246
86	AP_069	PQ	455	461	256	266	479	479	270	270	353	353	246	246

No	Sample ID	Populations	Locus_55		Locus_626		Locus_120		Locus_1587		Locus_137		Locus_915	
87	AP_070	PQ	455	463	248	248	475	479	270	270	345	345	238	244
88	AP_071	PQ	459	461	248	270	479	479	270	270	311	321	244	246
89	AP_072	PQ	459	461	248	268	479	479	270	270	353	353	246	246
90	AP_073	PQ	459	461	258	264	479	483	270	272	323	323	238	246

Notes: QN: Quang Nam; KH-NT: Khanh Hoa - Ninh Thuan; PQ: Phu Quoc