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Genetic Diversity in Slender Walking Catfish (*Clarias nieuhofii*) Populations: Implications for Population Management

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Abstract

Random amplified polymorphic DNA (RAPD) markers were applied to assess the genetic diversity and population structure of the slender walking catfish (*Clarias nieuhofii*, Clariidae) from 3 wild populations in peat swamp forests in southern Thailand (Surat Thani, Narathiwat, and Phatthalung). The selected 14 RAPD primers produced 105 RAPD bands, ranging from 6 to 11 bands per primer, and ranging in size between 400 and 3,000 bp. The percentage of polymorphic loci, gene diversity and Shannon's information index values were 75.00 %, 0.2252, and 0.3443 for Surat Thani; 86.59 %, 0.2982, and 0.4441 for Narathiwat, and 96.25 %, 0.3371, and 0.5049 for Phatthalung, respectively. Among the 3 populations, the highest genetic distance (0.2213) was found between the Narathiwat and Surat Thani populations. High genetic differentiation (G_{st} = 0.2815) was detected in 3 populations with low gene flow (*Nm* = 1.2762) among the overall populations. The clustering pattern obtained by the unweighted pair-group (UPGMA) method separated the *C. nieuhofii* samples into 3 groups, but 2 clusters. The results indicated a high level of genetic variation and genetic differentiation among *C. nieuhofii* from different populations in southern Thailand. This information would be useful to construct appropriate breeding programs, and could help conserve populations used as potential sources for stock management, restocking programs, and sustainable uses.

Keywords: *Clarias nieuhofii*, vulnerable species, population structure, genetic diversity, RAPD

Introduction

Slender walking catfish (*Clarias nieuhofii*, Clariidae) command a higher market price compared to other *Clarias*, because of its good taste and stunningly attractive appearance. It is beautifully patterned and eel-like in motion. It is reddish brown in color, with 13 or 14 vertical rows of white spots, which make it a valuable species for aquariums in Thailand. However, their populations and density have decreased significantly during the last 2 decades, mainly due to overexploitation, reduction in habitat area as a result of the reclamation of peat swamp forests, and the injudicious application of insecticides in paddy fields. They have been observed to be rare, and were identified as a vulnerable species by the Thailand Red Data Fishes in 2005 [1].

The vulnerability of the species requires conservation, recovery, and management strategies. Therefore, it is essential to understand the genetic composition of natural populations because the information on population structure and genetic variation of wild populations are of great importance to understanding variations in survival, reproduction, and growth rates [2]. The reduction of population size in the wild may lead to a decrease in genetic variation, and especially allelic diversity of populations. Adequate knowledge on genetic variation and population structure are necessary for the management of fisheries resources. High allelic and gene diversity are crucial factors to set up a founder population. This is the first step toward successful selection of a hatchery [3]. Furthermore, these factors are needed before

carrying out any restocking or stock enhancement project. Low levels of genetic diversity in founder populations lead to low potential for adaptation to changeable environmental conditions. Therefore, maintenance of both genetic diversity and population distinctiveness are major requirements for conservation management plans of overexploited species. Especially, the genetic diversity of *C. nieuhofii* is very limited.

Several methods are available to analyze genetic variability in natural fish populations. Random amplified polymorphic DNA (RAPD) is one of the best methods for the assessment of genetic variation among populations where there is limited genetic information [4]. This method is simple, rapid, and inexpensive. Only a small amount of DNA, and no prior knowledge of the genetic make-up, is required [5]. This technique has been previously successfully applied to population analysis of several catfish species [6-15]. Therefore, this study was conducted to explore the genetic diversity of 3 wild populations from southern Thailand. The objectives were to quantify the genetic variation, to understand the population structure of *C. nieuhofii* across its natural distribution range, and to apply this information for breeding and restocking programmes of this species in Thai peat swamp forest in order to maintain wild populations.

Materials and methods

Fish samples and DNA extraction

A total number of 149 *C. nieuhofii* specimens were collected from 3 wild populations (50 samples from Surat Thani; 50 samples from Narathiwat; 49 samples from Phatthalung) in southern Thailand (**Figure 1**). Tissue samples were clipped from the caudal fin of each fish and immediately preserved in an individual microcentrifuge tube containing 95 % ethanol and stored at 4 °C. The genomic DNA extraction was performed using a standard phenol/chloroform method [16] with slight modifications. In brief, approximately 50 mg of fin tissues was cut into small pieces and placed into a 1.5 ml microcentrifuge tube. The fin tissue was macerated in TNES-Urea buffer (10mM Tris HCl, 10 mM EDTA-2Na, 125 mM NaCl, 0.5 % SDS, 4M Urea) and 0.2 % Proteinase- K was added, mixed gently, and followed by incubation at 37 °C overnight. The digested products were extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1). The DNA was precipitated with ethanol, air dried, and then dissolved with TE buffer. The DNA samples were tested qualitatively on 1 % agarose gel electrophoresis (BIO-RAD SubcellGT) and visualized ethidium bromide stained bands under a UV transilluminator (VILBER LOURMAT ETX-40M) and quantified using a spectrophotometer (Biophotometer plus, Eppendorf, Germany). The concentration of extracted DNA was adjusted to 40 $ng.\mu l^{-1}$ for PCR amplification.

RAPD amplification

A total number of 70 RAPD primers (Operon Technologies) were initially screened in this study. Fourteen primers (**Table 1**) which produced clear and reproducible bands were selected for RAPD amplification in this study. The PCR reactions were performed on each DNA sample in a 20 µl reaction mix containing 1 µl of genomic DNA, 0.6 µl of 0.1 mM primer 100 pmol, 10 µl of Prime *Taq* Premix (GENET BIO) and 8.4 µl sterile distilled water. DNA amplification was performed in a C 1000 Thermal cycler (Bio-RAD). The reaction mix was preheated at 95 °C for 3 min, followed by 35 cycles consisting of 30 s denaturation at 95 °C, 30 s annealing at 30 °C, and 45 s extension at 72 °C. After the last cycle, a final step of 5 min at 72 °C (final extension) was added to allow complete extension of all amplified fragments, followed by holding at 4 °C.

The total volume of the amplified product (20 µl) of each sample was subjected to electrophoresis on 1.5 % agarose gel in a 1×TAE buffer system at 100 V for 45 min and stained with ethidium bromide (10 $\mu g.ml^{-1}$). The molecular sizes of the RAPD product were estimated, comparing them with a standard molecular size marker (1 kb DNA ladder, Invitrogen) which was run parallel to the amplified products in the gel. The amplified pattern was visualized on a UV transilluminator and photographed in a gel documentation system (Gel DOC ^{TM} XR^{+} Imaging System).

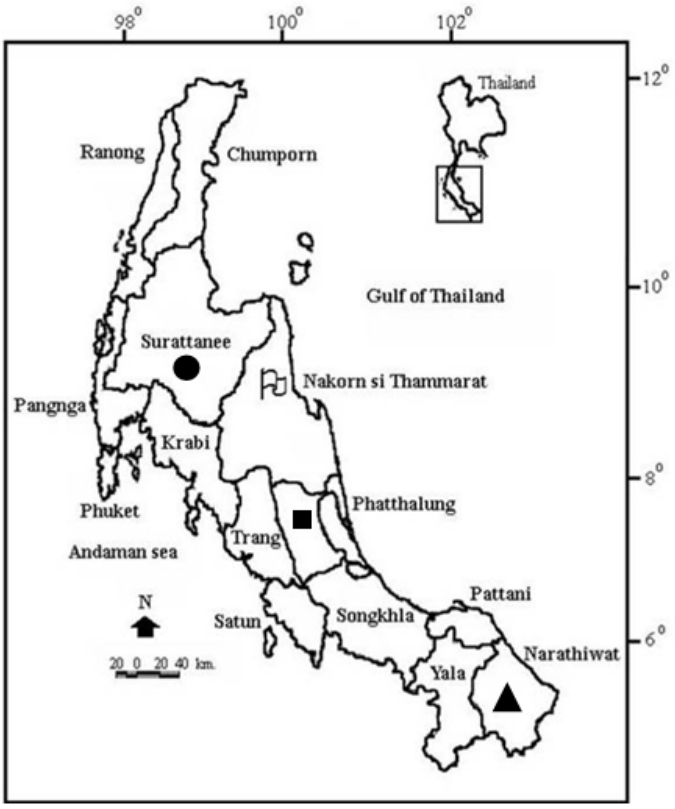

Figure 1 Sampling site of the 3 *Clarias nieuhofii* populations used in this study. Surat Thani is denoted as Surattanee in this map.

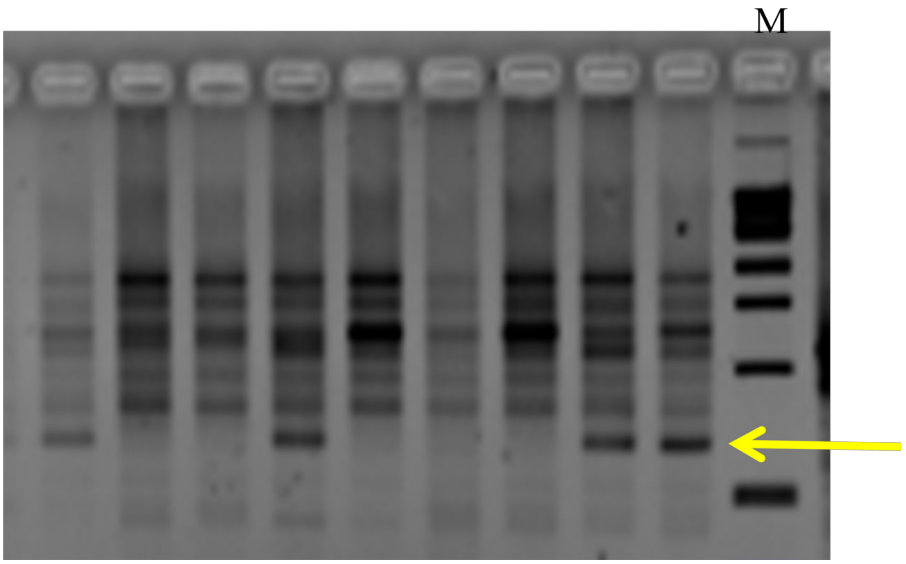

Figure 2 RAPD pattern of *Clarias nieuhofii* with primer AA-03. Arrow indicated polymorphic band in the sample. Lane M molecular marker size standard 1 Kb ladder.

Table 1 Random Amplified Polymorphic DNA primers, corresponding bands scored with their size ranges, and polymorphic bands amplified by PCR, from the samples of the 3 populations of *Clarias nieuhofii*.

Primer codes	Sequence (5' - 3')	No. of scorable bands	Size range (bp)	Polymorphic bands	Polymorphism (%)
AA 03	TTAGCGCCCC	10	700-2500	10	100
AA 10	TGGTCGGGTG	11	600-2500	11	100
AA 17	GAGCCCGACT	8	500-2500	8	100
AA 18	TGGTCCAGCC	7	500-1700	7	100
AA 19	TGAGGCGTGT	6	400-1250	6	100
AB 07	GTAAACCGCC	6	500-2000	6	100
AB 09	GGGCGACTAC	7	400-2000	7	100
AB 14	AAGTGCGACC	7	500-1750	7	100
AB 18	CTGGCGTGTC	8	500-1750	7	87.50
AC 03	CACTGG CCCA	6	800-2000	5	83.33
AC 04	ACGGGACCTG	9	500-3000	9	100
OPC 05	GATGACCGCC	6	500-1500	6	100
OPC06	GAACGGACTC	7	500-2000	7	100
OPE 11	GAGTCTCAGG	7	750-2000	7	100
Total		105		103	98.10

Table 2 Genetic variations in 3 wild populations of *Clarias nieuhofii*.

Parameters	Populations			
	Surat Thani	Narathiwat	Phatthalung	Overall
No. of scorable bands	72	83	80	105
No. of polymorphic bands	54	71	77	103
Percentage of polymorphic bands	75	86.59	96.25	98.10
Gene diversity (*h*)	0.2252	0.2982	0.3371	0.3171
Observed no. of alleles (*A*)	1.7500	1.8659	1.9625	1.9810
Effective no. of alleles (*Ae*)	1.3826	1.5214	1.5759	1.5357
Shannon's Information Index (*I*)	0.3443	0.4441	0.5049	0.4797

Data recording and analysis

The RAPD patterns were visually scored and compared among the *C. nieuhofii* populations. All distinct bands were recorded as 1 or 0 for the presence or absence of fragments, respectively. The scores from all primers were pooled for constructing a single data matrix. These were used to estimate the number of polymorphic loci, percentage of polymorphic loci (%*P*), observed number of alleles (*A*), effective number of alleles (*Ae*), Nei's gene diversity (*h*), Shannon's Information index (*I*), Nei's gene diversity among populations (H_T), Nei's gene diversity within subpopulations (H_S), Nei's coefficient of gene differentiation (G_{st}, analogous to the fixation index *FST*), gene flow (*Nm*), and Nei's genetic distance (*D*) between different geographical populations, using the software POPGENE (Version 1.31) [17]. The UPGMA dendrogram of the population was constructed based on [18].

Results and discussion

Genetic diversity

The genetic monitoring of natural populations is fundamentally important for conservation, management of natural resources, and genetic improvement programmes. Therefore, it is necessary to assess the amount of genetic diversity and the structure of diversity in samples and populations. In the present study, among the 70 single decamer random primers, 14 primers were selected and used for the monitoring of genetic variation of *C. nieuhofii* populations collected from 3 wild stocks through RAPD. Fourteen selected primers generated a total of 105 bands, of which 103 were polymorphic (98.10 %). Most of the primers produced 100 % polymorphic bands, except AB 18 and AC 03 (**Figure 2**), which produced 87.50 and 83.33 %, respectively. The number and size of the fragments, together with the number of polymorphic bands produced by 14 RAPD primers in 3 populations of *C. nieuhofii*, are summarized in **Table 1**. The number of bands generated by each RAPD primer ranged from 6 to 11, with an average of 7.5 bands per primer. The number of bands is comparable to the results of studies carried out in *C. gariepinus.* With 10 primers, 6 to 12 bands were observed [19]. In contrast to the findings of the present study, the primer (OPC 06) generated a lower number of bands (6) and smaller band sizes (183 to 1452 bp) in butter catfish, *Ompok bimaculatus* (Bloch) [14]. A higher number of bands and size of PCR products in this study could either be attributed to comparatively lower annealing temperatures of 30 °C used for *C. nieuhofii*, as compared to *O. bimaculatus* (Bloch) (38 °C) [14], or difference in the genomes of the 2 distinct species.

The numbers of fragments amplified and polymorphic bands in each population are shown in **Table 2**. Among these 3 populations, Narathiwat showed a higher number of bands (83) than Phatthalung (80) and Surat Thani (72). Phatthalung showed a higher percentage of polymorphic bands (96.25) than Narathiwat (86.59) and Surat Thani (75.00). Among the selected primers, AA 10 gave the DNA profile with the highest number of bands. For 14 primers, the observed number of alleles (*A*), effective number of alleles (*Ae*), gene diversity (*h*), and Shannon's Information Index (*I*) were 1.9810, 1.5357, 0.3171, and 0.4797, respectively (**Table 2**). The *A* ranged from 1.7500 at Surat Thani to 1.9629 at Phatthalung. Values of *Ae* were less than those for *A* in every population, and ranged from 1.3826 at Surat Thani to 1.5759 at Phatthalung. The *h* ranged from 0.2252 at Surat Thani to 0.3371 at Phatthalung.

The percentage of polymorphic loci in the overall population of *C. nieuhofii* was relatively high (98.1 %) compared to other catfish; for example, 69.5 % in 6 populations of African catfish, *C. gariepinus* [19]; 64.98 % in *Mystus vittatus* (Bloch) [8]; 64.98 % in endangered yellow catfish, *Horabagrus brachysoma* [6], and slightly higher than those reported in the walking catfish, *C. batrachus*, 86.66 % [10]; Asian stinging catfish, *Heteropneustes fossilis,* 83.87 % [9]. Due not only to their specific and isolated habitats but also to their lack of commercial breeding appeal, *Clarias nieuhofii*'s wild population has a high genetic variation. However, the high percentage of polymorphic loci in *C. nieuhofii* was similar to that observed in yellow catfish, *M. nemurus*, 96.39 % [13]. The percentage of polymorphic loci in the 3 different populations of *C. nieuhofii* was low in Surat Thani (75.00 %) and high in Narathiwat (86.59 %) and Phatthalung (96.25 %). These results were similar to that observed in yellow catfish, *M. nemurus* [13] which was low in Khon Kaen (70.69 %) and high in Chiang Rai (87.10 %). The different status in each population may indicate that the populations of *C. nieuhofii* from different geographic area are relatively isolated from each other.

A high level of genetic variation for the overall populations was found in this study, due to *C. nieuhofii*'s fragmented habitats and rare current situation of *C. nieuhofii*'s culture. The observed number of alleles (*A*), effective number of alleles (*Ae*), Nei's gene diversity (*h*), and Shannon's information index (*I*) were 1.9810, 1.5357, 0.3171, and 0.4797, respectively. From the values of all indicators (**Table 2**), genetic variation of *C. nieuhofii* populations in southern Thailand should be concluded as high level, compared with African catfish, *C. gariepinus*, [19]; *M. vittatus* (Bloch) [8]; endangered yellow catfish, *H. brachysoma* [6]; walking catfish, *C. batrachus* [2,10], and Asian stinging catfish, *H. fossilis* [9].

Genetic diversity within population is highly important for adaptation to changeable environments. Generally, vulnerable species show less genetic diversity and heterogeneity within or among populations. In the present study, several significant findings were high in relation to the genetic diversity of fish

populations. High levels of genetic variation within each population were also observed in *C. nieuhofii*. Higher genetic diversity was shown within the Phatthalung population (% P = 96.25, h = 0.3371, I = 0.5049), and lower genetic diversity was found for the Surat Thani population (% P = 75.00, h = 0. 0.2252, I = 0.3443). In general, it is predicted that genetic variation within species should positively correlate with population size. Therefore, one possible reason for lower genetic diversity in the Surat Thani population may be impact from the increase of population density, which causes damage to the habitat and when overexploited results in a low population size of *C. nieuhofii*.

Genetic differentiation

Genetic differentiation of *C. nieuhofii* populations were estimated by H_T, H_S, and G_{st} values (analogous to F_{st}). There are shown in **Table 3**. Our results showed high H_T (0.3161), H_S (0.2271) and G_{st} (0.2815) which indicates very great genetic differentiation [20]. These results were confirmed with low values of gene flow (Nm = 1.2762). It is important to point out that there was genetic differentiation among the 3 populations of *C. nieuhofii*. Similar results were observed in *C. batrachus* [21] and *H. brachysoma* [6]. As a result, genetic differentiation in this study could be explained by the restricted gene flow which resulted from limited movement of *C. nieuhofii* across geographic barriers. The limitation of gene flow, reduction of population size, and increase in endogamy resulted in a greater probability of differentiation.

Table 3 Genetic differentiation and population genetic structure for overall populations.

RAPD primers	H_T	H_S	G_{st}	N_m
AA 03	0.3438	0.1527	0.5558	0.3996
AA 10	0.2791	0.2027	0.2736	1.3272
AA 17	0.3906	0.3067	0.2148	1.8280
AA 18	0.3384	0.2635	0.2214	1.7585
AA 19	0.3877	0.3484	0.1014	4.4332
AB 07	0.3740	0.3130	0.1630	2.5678
AB 09	0.3847	0.2946	0.2343	1.6342
AB 14	0.2353	0.2013	0.1447	2.9553
AB 18	0.2550	0.1925	0.2450	1.5409
AC 03	0.3281	0.1984	0.3951	0.7655
AC 04	0.2476	0.1252	0.4945	0.5112
OPC 05	0.2558	0.1772	0.3074	1.1265
OPC 06	0.3285	0.2478	0.2455	1.5364
OPE 11	0.3056	0.2492	0.1845	2.2107
Overall primers	0.3161	0.2271	0.2815	1.2762

Table 4 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) using RAPD markers in *Clarias nieuhofii*.

Populations	Surat Thani	Narathiwat	Phatthalung
Surat Thani	****	0.8015	0.8136
Narathiwat	0.2213	****	0.8710
Phatthalung	0.2063	0.1381	****

Genetic distance and phylogenetic relationships

Nei's genetic distances (D) between pairs of *C. nieuhofii* populations with RAPD analysis ranged from 0.1381 to 0.2213. The lowest genetic distance values among *C. nieuhofii* populations was shown between Narathiwat and Phatthalung, whereas the highest values was found between Surat Thani and Narathiwat, because of separate geographical distance. Our result agreed with Chandra *et al*. [22], which stated that geographical isolation, limited dispersal, and phylopatric behavior of populations should promote genetic differentiation, particularly in freshwater habitats. Moreover, the greatest genetic identity (0.8710) was observed between the Phatthalung and Narathiwat populations. The UPGMA dendrogram clustered into 2 clusters, but 3 groups (**Figure 3**). The Surat Thani population alone belonged to one cluster, whereas the Narathiwat and Phatthalung populations were in another cluster, which were in agreement with the geographical locations. The results supported the influence of geographic barriers over the gene flow of *C. nieuhofii* in Thailand. This observation clearly indicates that the 3 populations of *C. nieuhofii* have separated gene pools.

The depletion of *C. nieuhofii* populations has been a continuous process, attributable chiefly to habitat degradation**.** The present results show that *C. nieuhofii* populations are genetically different from each other, shown by the high level of genetic structure. In the present situation, the recovery of *C. nieuhofii* populations depends on 3 main implementation approaches. The first is to conserve, or even rehabilitate, habitat, because conservation of habitat may help increase population size and lower risk in vulnerability of the species in the future. The second approach is to promote stock enhancement into local areas where populations were overexploited. For example, the result indicated that the Phatthalung population had the highest genetic diversity because the percentage of polymorphic loci was 96.25, gene diversity was 0.3371, and Shannon's information index value was 0.5049. Therefore, this population was suitable for promoting stock enhancement. Brood stock will be selected from this population to breed in a hatchery, and restocking will be done to maintain population in the original location. The last approach is to culture *C. nieuhofii* for commercial purposes, against fishing pressure. The results suggested that brood stocks of the culture should be selected from the Surat Thani and Narathiwat populations, because of the high genetic distance between both populations (0.2213).

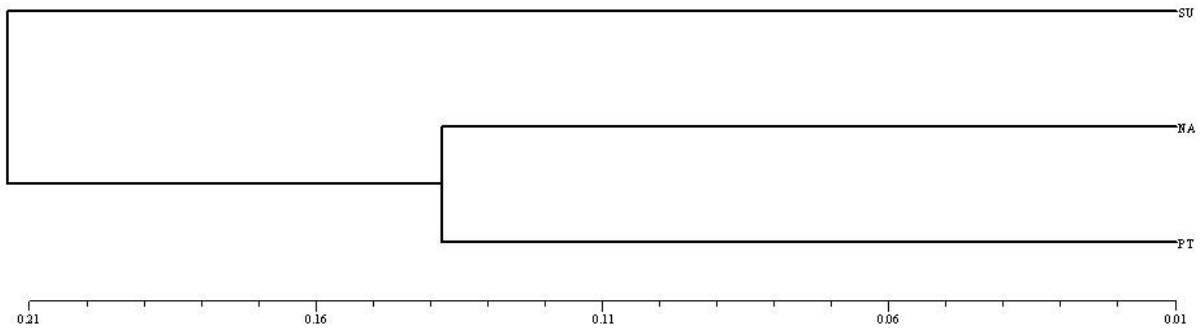

Figure 3 UPGMA dendrograms using Nei's unbiased genetic distances of *Clarias nieuhofii* populations. Population in Surat Thani province (SU), population in Narathiwat province (NA), and population in Phatthalung province (PT).

Conclusions

The results of this study give a preliminary view of genetic variation both within and between these populations. The high level of genetic variation and genetic differentiation among *C. nieuhofii* from different populations in southern Thailand were unquestioned. This information would be useful for

constructing appropriate breeding programs, and could help protect populations that might be used as potential sources for stock management, future restocking programs in nature, and sustainable uses of wild resources. To successfully implement our study, the Conservation Division of the Fisheries Department should apply all 3 approaches to all areas in southern Thailand. An immediate conservation programs for this species, *C. nieuhofii*, is a crucial process to use in order to save the environment and benefit people nationwide.

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