

## MITOCHONDRIAL DNA DIFFERENTIATION OF POPULATIONS OF *CLARIAS BATRACHUS* FROM SOUTH-EAST ASIA

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### Abstract

RFLP analysis of mitochondrial DNA (mtDNA) was used to study variation within 13 populations of *Clarias batrachus*, sampled respectively in Vietnam, Thailand and in the Indonesian Archipelago.

In this study an amplified region corresponding approximately to 2.3 kilobases of the Cytochrome-b and D-loop genes was digested using 8 restriction enzymes (HinfI, Hin6I, MvaI, MspI, HaeIII, BamHI, NdeII, DraI). 12 mtDNA haplotypes were found in 40 specimens.

Each sampling location was characterised by one haplotype, except Palembang (Sumatra, Indonesia) and Samarinda (Kalimantan, Indonesia) where 2 and 4 haplotypes were found respectively. The consensus tree calculated from 15 more parsimonious networks showed that mtDNA haplotypes are geographically distributed. Three well differentiated clusters were identified. The first cluster is composed by populations from both Thailand and Vietnam, the second cluster by two populations from West Sumatra and the last cluster by all other populations. High genetic divergence were observed in Sumatra between populations which come from highlands (altitude more than 1000 meters in Bukittingui, and 300 meters in Nias Island) and populations located in lowlands.

The significant genetic relatedness observed between populations from Sumatra (Jambi, Palembang, Muara Tebo, Teluk Kuantan) and populations from Java indicate a possible common origin which is probably in Kalimantan. This result is supported by the high diversity of haplotypes revealed in Samarinda (Kalimantan) and their intermediate position in the genetic network. The populations from highlands in West Sumatra which share a common haplotype are genetically more related to populations from Thailand and Vietnam than to populations from the rest of Indonesia. These populations seems to be relict populations from a first colonisation event which arise from the continental part of Asia. The fact that the highland haplotype is not observed in lowlands populations of Sumatra suggests that the colonisation way from Vietnam and Thailand is certainly ancient and that a strong bottleneck effect occurred in lowland area, probably due to a marine transgression. During a more recent marine regression, the present lowlands of Sumatra could have been newly colonised by flounders coming from Kalimantan.

### INTRODUCTION

The Clariidae is a large family of the Siluriformes, with about 15 genera inhabiting freshwaters from Africa eastward to India, south-east Asia and south-eastern East Asia (Menon, 1951; Nelson, 1976). The genus *Clarias* is the largest genus of the family with approximately 45 species (Garcia-Franco, 1993). *Clarias batrachus*, *C. macrocephalus*, *C. fuscus* and *C. gariiepinus* are still the main species used in aquaculture. These

species exhibit considerable intra-specific geographic variations in morphological and chromosomal characters, leading to overlaps and species misidentification (Garcia-Franco, 1993). This author demonstrated with a study on caryotypes that *C. batrachus* from India are closer to the species *C. fuscus* than to populations of *C. batrachus* from south-east Asia. As a consequence, the evolutionary history of these species remains to be clarified, as well as their specific status which remains unclear. In that way, a RFLP analysis of

mitochondrial DNA (mtDNA) was used in order to study genetic variation within populations of *Clarias batrachus* sampled respectively in Vietnam, in Thailand and in the Indonesian Archipelago.

## MATERIAL AND METHODS

### Sampling

Fish were collected in 13 locations (Fig. 1) respectively in Can Tho (Vietnam), So Phisat (Thailand), Samarinda (Kalimantan), Nias Island, Bukittingui, Teluk Kuantan, Muara Tebo, Jambi, Palembang, Tegineneng (Sumatra), Sukamandi, Solo, Brantas (Java).

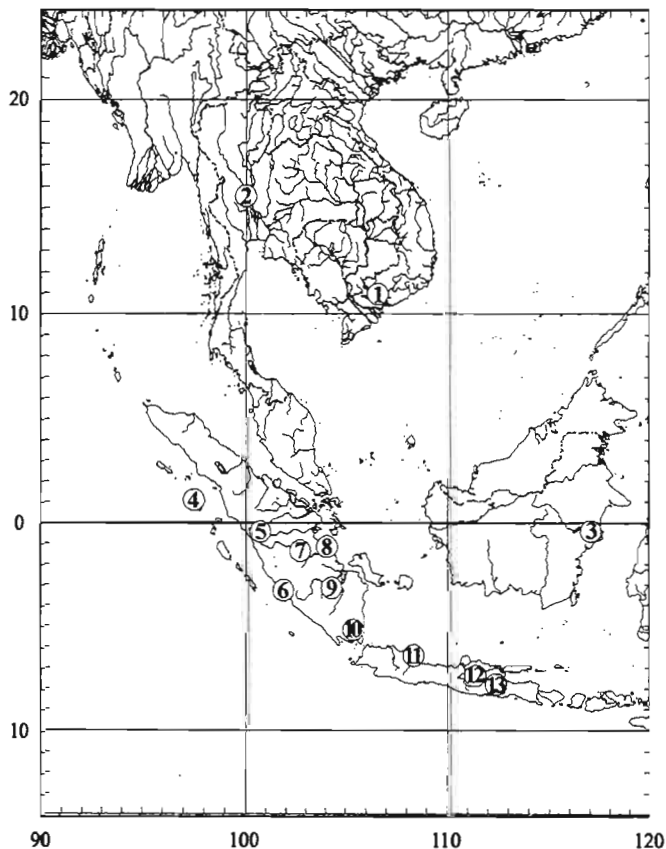


Figure 1: *Clarias batrachus* samples locations.

### DNA extraction

A piece of muscle (60 mg) from each specimen preserved at  $-20^{\circ}\text{C}$  was extracted using a standard Phenol-Chloroform protocol.

### Amplification of the Cyt-b-D-loop region of mitochondrial DNA

An amplified fragment of approximately 2300 bases was obtained using the following primers V-GLU (5' GACTTGAAGAACCACCGTTG) and

HN20 (5' GTGTTATGCTTTAGTTAAGC) identified by Bernatchez (comm. pers.). The amplification conditions were as follow: 3mM  $\text{MgCl}_2$ , 0.5 mM of each dNTP's, 0.2  $\mu\text{M}$  of each primer, 2 units of taq polymerase (Promega), 1x of taq Polymerase buffer and 5  $\mu\text{l}$  of DNA solution in a final volume of 50 $\mu\text{l}$ . The amplification program was:  $95^{\circ}\text{C}$  for 1 min,  $91^{\circ}\text{C}$  for 1 min,  $50^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 2 min 30 sec. The last three steps were repeated 35 times. A final elongation was performed at  $72^{\circ}\text{C}$  for 10 min.

### Collection of restriction enzymes

Eight 4-base recognition restriction endonucleases were used to digest the amplified mtDNA.

### Digestion of the amplified products

Five to seven  $\mu\text{l}$  of the PCR-amplified region was digested by 5 units of one of the restriction enzymes in a final volume of 20  $\mu\text{l}$  containing the appropriate buffer. Digestion were then done at  $37^{\circ}\text{C}$  overnight. The digestion products were separated and visualised in 2% horizontal ethidium bromide stained TBE agarose gel. Patterns of restriction digest products for enzyme *Hinf*I are presented in Figure 2.

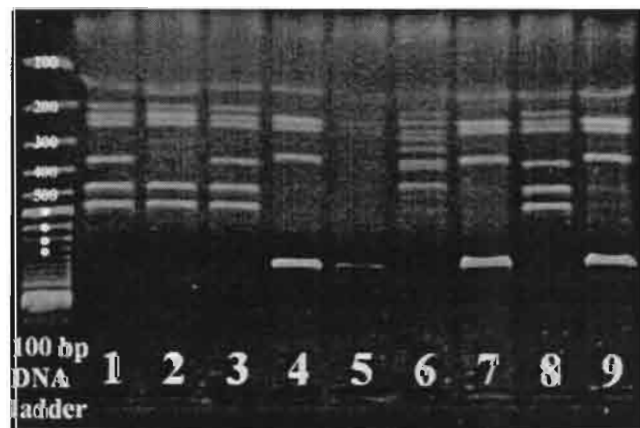


Figure 2: Patterns of restriction digest product for enzyme *Hinf*I to compose a haplotype definition of *Clarias batrachus* population.

Each column represent a digested sample. The column in left side is the 100 bp DNA ladder (mix of DNA markers from 100 bp to 2000 bp). On this picture specimen 1, 3, 8 share the same pattern of restriction. The specimen number 2 has a private haplotype which differs from previous haplotype by the acquisition of a new restriction site leading to the disappearance of a band in the middle part of the pattern (approx. 360bp) and apparition of new bands below 180 bp). Specimen 4, 7, 9 share an

other kind of pattern. The specimen with number 6 corresponds to an other species, *Clarias meladerma*.

#### Data analyses

Each restriction enzyme give a particular pattern of digested restriction fragments for one specimen. A collection of different patterns can be obtained with the same enzyme for different specimen if the number of restriction sites is variable. In that case each pattern is defined by a particular phenotype with a code letter.

For one specimen, the set of phenotypes observed for the different restriction enzymes used is referred as an haplotype. A matrix of presence-absence of each restriction pattern could be assessed for each sample and used in order to implement the phylogenetic relationships among these samples. We used in this study the parsimony of Wagner (program MIX) and the bootstrapping re-sampling technique (program SEQBOOT). These programs are available in the PHYLIP software package (Felsenstein, v. 3.5).

## RESULTS

All the enzymes used cleaved the PCR mtDNA product. An identical multibanded phenotype among all samples of *C. batrachus* was observed with the enzyme *MspI*. Because this enzyme provide different patterns with the other species belonging to the *Clarias* genus, it was useful for assuming a good identification of *Clarias batrachus*.

The haplotypes collection is given in Table 1 for each analysed population. One haplotype was

found in each population, except in Palembang and Samarinda where 2 and 4 haplotypes were revealed respectively.

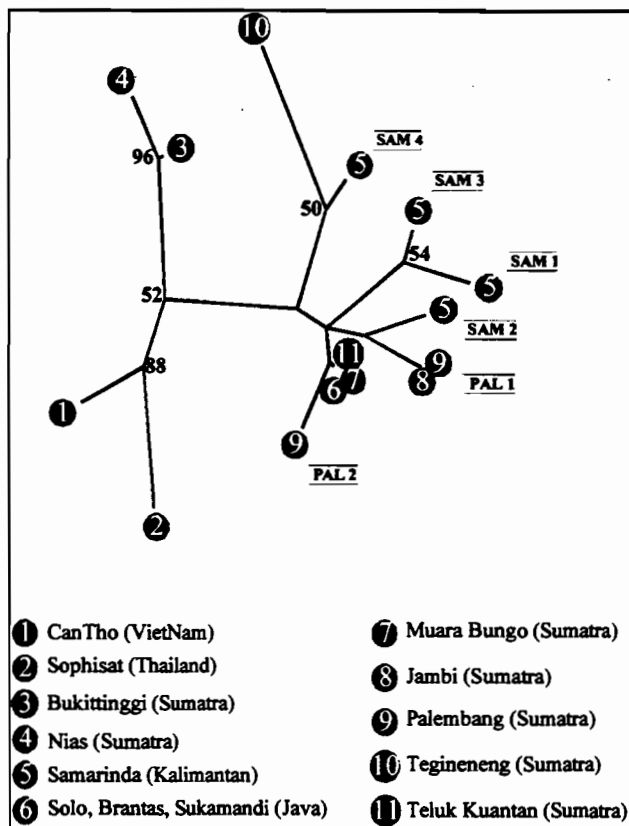
The corresponding genetic network calculated from the 15 more parsimonious trees is presented in Figure 3. MtDNA haplotypes are geographically distributed. Bootstrap up to 50% indicate the existence of three well differentiated clusters. The first one is composed by populations of Can Tho and Sophisat, the second one by populations of Bukittinggi and Nias island and the third cluster by all other populations. Populations which come from Sumatra are not monophyletic and do not share a common origin. The populations which come from highlands (mountains areas up to 1000 meters at Bukittinggi, up to 300 meters in Nias Island) in Sumatra are genetically related to populations from the continental part of Asia (Vietnam and Thailand). By contrast, all other populations in Sumatra which are located in lowlands (altitude between 10 and 200 meters) are genetically related to populations from Java and Kalimantan Islands.

## DISCUSSION

The distribution of species or populations and their genetic differentiation depend on biological, environmental and historical factors. Numerous works have shown that the genetic structure of freshwater fish populations was fashioned by fluctuations of sea level and alternative flowing and drying seasons during the past (see Hamilton, 1976 ; Maley, 1987, 1991, Maley et al., 1990 for more details). The last marine regression (-110 meters below actual sea level) which is dated

	Hinfl	HingI	MvaI	MspI	HaeIII	BamHI	Nde2	DraI
Tegineneng	A	B	C	A	C	B	C	B
Jambi	B	A	C	A	C	A	A	B
SoPhisat	C	A	A	A	A	A	A	A
Bukittingui	D	B	B	A	B	A	A	B
Nias	D	B	B	A	B	A	B	B
Telukkuantan	E	A	C	A	C	A	A	B
Palembang 1	B	A	C	A	C	A	A	B
Palembang 2	F	A	C	A	C	A	A	B
Samarinda 1	B	A	C	A	D	A	A	C
Samarinda 2	B	A	C	A	D	A	A	B
Samarinda 3	E	A	C	A	D	A	A	C
Samarinda 4	E	B	C	A	D	B	A	B
Can Tho	G	A	D	A	A	A	A	B
Java	E	A	C	A	C	A	A	B
MuaraTebo	E	A	C	A	C	A	A	B

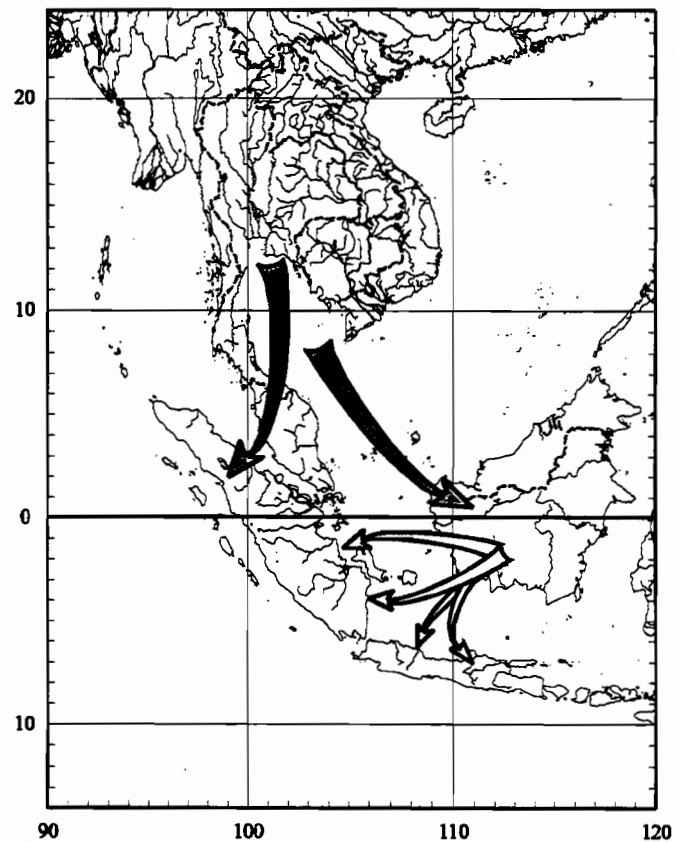
Table 1: Composite mtDNA haplotype definitions for *Clarias batrachus*. Letters refer to restriction fragment pattern that occur in these populations.



**Figure 3:** Genetic network calculation from 15 parsimonious trees of *Clarias batrachus* based on haplotype pattern.

20,000 years before present led to the disappearance of the South China sea and Java sea, meaning possible connections between river drainage which are presently independent and a possible dissemination of freshwater ichthyofauna. By contrast, the last marine transgression (6 meters above actual sea level) was responsible of the disappearance of many lowland areas and the decreasing of many freshwater populations, excepting in refuge areas as large river systems and highlands.

Considering on the one hand that Menon (1951), on the basis of evidences provided by paleontology and distribution records, stated that the Clariidae family originated from somewhere in South China during the early Pliocene period and, on the other hand that Garcia-Franco (1993) suggested that *Clarias batrachus* originated from North Burma, it is possible to reconstitute some of the past events that prevailed upon the current distribution of *C. batrachus* populations (Fig. 4). In these conditions, Kalimantan and Sumatra could have been colonised during a marine regression by populations coming from the continental part of Asia. Because colonisation events come with



**Figure 4:** Geographic colonisation assessed scenario of *Clarias batrachus* based on haplotype pattern.

bottleneck effects due to the small size of populations that generally go from one basin to another, the flounder populations which entered in Sumatra highlands had conserved only one haplotype. This haplotype is currently shared by populations of Nias and Bukittinggi. By contrast in Kalimantan, the bottleneck effects were not so high as in Sumatra probably because the colonised area was more accessible. In that way an important genetic diversity is observed (4 haplotypes). The fact that the haplotype from highlands in Sumatra is never observed in lowlands suggest a very poor gene flow between upstream and downstream in the same river basin (case of Batang Hari River) and that populations of *Clarias batrachus* were probably extinct in lowlands with marine transgression. The current populations sampled in low altitude areas in Sumatra (Jambi, TelukKuantan, Muara Tebo, Palembang, Tegineneng) show close relationships with population from Kalimantan. This is also the case for populations of Java which are characterised by only one haplotype also observed in Telukkuantan and genetically related with haplotypes of Kalimantan. These results suggest that Java Island

and Sumatra lowlands were colonised more recently from Kalimantan, possibly during the last marine regression that occurred 20,000 years ago.

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# THE BIOLOGICAL DIVERSITY AND AQUACULTURE OF CLARIID AND PANGASIID CATFISHES IN SOUTH-EAST ASIA



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