

## PHYLOGENETIC RELATIONSHIPS AMONG PANGASIID CATFISH SPECIES (SILURIFORMES, PANGASIIDAE) AND NEW INSIGHTS ON THEIR ZOOGEOGRAPHY

Laurent Pouyaud <sup>(1)</sup>, Rudhy Gustiano <sup>(2)</sup> and Marc Legendre <sup>(1)</sup>

(1) IRD (ex ORSTOM), Catfish Asia Project, Instalasi Penelitian Perikanan Air Tawar, Jalan Ragunan, Pasar Minggu, P.O. Box 7220/jkspm, Jakarta 12540, Indonesia  
and GAMET, B.P. 5095, 34033 Montpellier Cedex 1, France

(2) RIFF, Instalasi Penelitian Perikanan Air Tawar, Jalan Ragunan, Pasar Minggu, P.O. Box 7220/jkspm, Jakarta 12540, Indonesia

### Abstract

With the aim of detecting phylogenetic relationships among Pangasiidae catfishes, 23 enzyme loci were studied on 18 nominal species of the genera *Pangasius* and *Helicophagus* (Roberts & Vidthayanon, 1991). In order to assess the taxonomic position of *Laides* genus in the Schilbeidae or in the Pangasiidae, 2 species belonging to the genus *Laides* and 1 species of the genus *Pseudeutropius* (Siluriformes, Schilbeidae) were added in the study.

The results indicate that the species *Laides hexanema* and *Laides sinensis* appear to be genetically related with the Pangasiidae. The phylogenetic tree obtained shows a clustering of species which validate the genus *Helicophagus* but indicates that the genus *Pangasius* is polyphyletic.

The genus *Pangasius* is composed of 3 genetic differentiated groups. Group 1 is composed by 3 species belonging to two different morphological entities validated by Roberts and Vidthayanon (1991) as possible subgenus. *Pangasius hypophthalmus* and *P. gigas* which share common genetic characters validate the subgenus *Pangasianodon*. The possession of many private alleles by *P. pleurotaenia* confirms the morphological originality of this species which was therefore considered to belong to the monotypic subgenus *Pteropangasius*. The second group consists of *P. micronema*, *P. macronema*, *P. lithostoma* and *P. polyuranodon* and the last group represents all the other species of the genus *Pangasius*. By contrast *Neopangasius* considered as a possible subgenus by Roberts and Vidthayanon (1991) is polyphyletic. Although relative genetic similarities were found between *P. humeralis* and *P. nieuwenhuisii* which are located in group 3, the species *P. lithostoma* appears to be more closely related to species belonging to the group 2. By reference to the *Helicophagus* genus, the genetic distances estimated between these genetic entities suggest that *Pangasianodon* and *Pteropangasius* could be elevated to the genus level. Low genetic distance between groups 2 and 3 lead us to maintain species of both groups in the genus *Pangasius*. In the same way the genus *Laides* could be placed in Pangasiidae as proposed by Roberts (1989). The results also confirm the nominal species revision proposed by Roberts and Vidthayanon (1991).

New insights are given in this paper, like the presence of *Pangasius djambal* in all major basins of Sumatra and the fact that, contrarily to previous statements, this species was never utilised for aquaculture in Indonesia so far. *Helicophagus typus* is not extinct, three specimens were caught in the Batang Hari river (Sumatra) in February 1997 and two specimens in the Kapuas river (West Kalimantan) in June 1997. Two possible new species were discovered, the first one (ref. sp1) occurring in Sumatra (Musi, Batang Hari and Indragiri rivers), in Kalimantan (Mahakam River) and in Vietnam (Mekong delta), the second one (ref. sp2) was observed in East Kalimantan (Berau River). The taxon sp1 shares several characteristics with *Pangasius pangasius*. However this latter species is not supposed to be represented in the Mekong River nor in Indonesian waters.

### INTRODUCTION

The tropical Asian catfish family Pangasiidae is characterised by a noticeable ecological and

morphological diversity. In term of distribution, some species are endemic like *P. gigas* in the Mekong river, other have disjunctive distribution like *P. macronema* which occurs in mainland Asia

only in the Mekong and Chao Phraya basins, while in the Indonesian archipelago, it is known only from southern Kalimantan and Java. Finally many species have a wide distribution like *P. micronema* and *P. polyuranodon* which are reported from most of the basins of south-east Asia. The Pangasiidae are freshwater fishes, with the exceptions of *P. pangasius*, *P. polyuranodon* and *P. krempfi* which can enter in saline waters. Concerning maximum size and growth rate, the situation is also contrasted. Some species, as *P. macronema* never grow longer than 200 mm SL while *P. gigas* can reach over 3 m for more than 300 kg body weight. These species exhibit a wide range of feeding behaviours.

With these considerations, it is noteworthy that phylogenetic relationships among populations of Pangasiidae remain problematic. Large distribution which leads in many cases to significant population differentiation and difficulty to access to comparative material in foreigner museums were responsible of many misidentifications. Before the systematic revision of Roberts & Vidthayanon (1991), a particular confusion prevailed. These authors studied type specimens and other material of Pangasiidae on 39 nominal species or subspecies, resulting to the recognition of 18 previously described species as valid. Three new species were also described bringing to 21 the total number of species recognised in Pangasiidae. They subdivided Pangasiidae in two genera, *Pangasius* Valenciennes, 1840 and *Helicophagus* Bleeker, 1858. *Neopangasius* Popta, 1904, *Pangasianodon* Chevey, 1930 and *Pteropangasius* Fowler, 1937 were recognised by Roberts & Vidthayanon (1991) as possible subgenus of *Pangasius*. Two species have been placed in the genus *Laides* Jordan, 1919 and their position in Schilbeidae or in Pangasiidae is subject to controversy.

On the basis of the systematic revision of Roberts & Vidthayanon (1991) and in order to assess the phylogenetic relationships among Pangasiid catfish species, 23 enzyme loci were studied on 18 species of the genera *Pangasius* and *Helicophagus*. One species of the genus *Pseudeutropius* (Siluriformes, Schilbeidae) and two species of the genus *Laides* were also characterised at 19 enzyme loci in order to precise the taxonomic position of the genus *Laides*.

## MATERIALS AND METHODS

216 individuals belonging to 62 populations of four genera (*Pangasius*, *Helicophagus*, *Laides*, *Pseudeutropius*) were analysed. The geographic origin (river system and location) and size of the samples are indicated in Figure 1 and table 1.

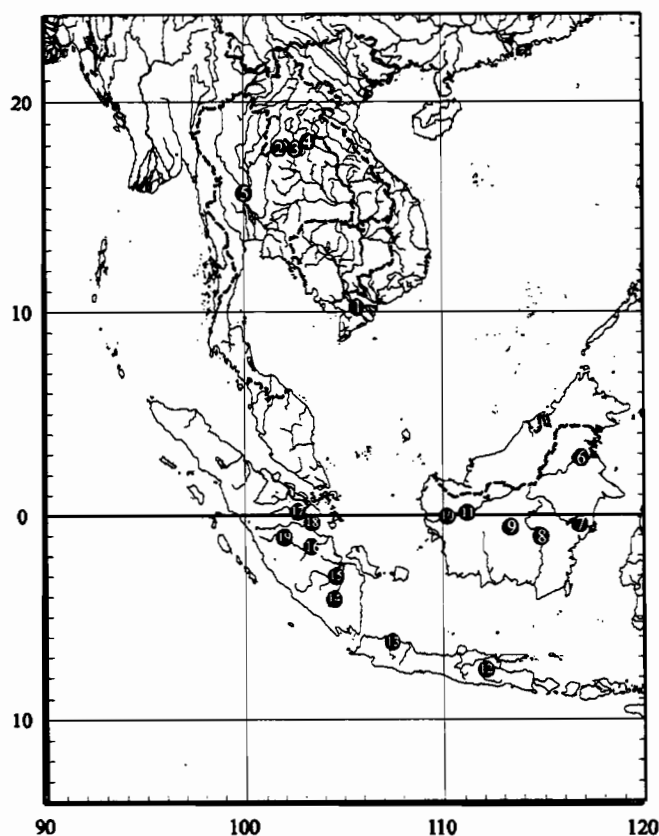


Figure 1: Sampling locations of studied specimens.

Captured specimens were dissected and the tissue samples were stored in liquid nitrogen for transfer to the laboratory. They were then stored at  $-20^{\circ}\text{C}$  for several months pending analysis. Two eyes and  $2\text{ cm}^3$  of muscle and liver were removed from each individual. To obtain optimal results, the samples were homogenised several hours before analysis. Each specimen was identified with key available in Roberts & Vidthayanon (1991) and stored in formalin for further examination.

The methods of starch gel electrophoresis were adapted from Pouyaud & Agnès (1995). Table 2 shows the enzyme systems and the buffers used as well as the organs in which the different loci were expressed. The nomenclature is that proposed by Shaklee *et al.* (1990).

Species	CL	Sampling origin	Abbrev.	N	(Hobs)	(P <sup>95</sup> )
<i>Pangasius Hypophthalmus</i>	5	Tchao Praya Nakhom Sawan Thailand	HYP THA 2	3	0.0725	0.1739
<i>Pangasius Hypophthalmus</i>	1	Mekong Can Tho Viet Nam	HYP VIE 1	6	0.0870	0.3478
<i>Pangasius Hypophthalmus</i>	2	Mekong Thabo Thailand	HYP THA 1	6	0.0290	0.1304
<i>Pangasius Hypophthalmus</i>	13	CRIFFI Strain Sukamandi Indonesia	HYP JAV 2	3	0.0725	0.1739
<i>Pangasius macronema</i>	5	Tchao Praya Nakhom Sawan Thailand	MAC THA 2	5	0.1826	0.3478
<i>Pangasius macronema</i>	3	Mekong Nong Khai Thailand	MAC THA 1	4	0.1196	0.2174
<i>Pangasius macronema</i>	1	Mekong Can Tho Viet Nam	MAC VIE 1	5	0.0696	0.1739
<i>Pangasius pleurotaenia</i>	5	Tchao Praya Nakhom Sawan Thailand	PLE THA 2	5	0.0348	0.1304
<i>Pangasius pleurotaenia</i>	4	Mekong Bung Kan Thailand	PLE THA 1	1	0.0435	0.0435
<i>Pangasius pleurotaenia</i>	1	Mekong Can Tho Viet Nam	PLE VIE 1	1	0.0000	0.0000
<i>Pangasius larnaudii</i>	5	Tchao Praya Nakhom Sawan Thailand	LAR THA 2	4	0.0978	0.2174
<i>Pangasius larnaudii</i>	3	Mekong Nong Khai Thailand	LAR THA 1	1	0.0000	0.0000
<i>Pangasius larnaudii</i>	1	Mekong Can Tho Viet Nam	LAR VIE 1	1	0.0000	0.0000
<i>Pangasius conchophilus</i>	1	Mekong Can Tho Viet Nam	CON VIE 1	5	0.0609	0.1304
<i>Pangasius conchophilus</i>	2	Mekong Thabo Thailand	CON THA 1	3	0.0580	0.0870
<i>Pangasius conchophilus</i>	5	Tchao Praya Nakhom Sawan Thailand	CON THA 2	4	0.0326	0.0435
<i>Pangasius sanitwongsei</i>	4	Mekong Bung Kan Thailand	SAN THA 1	3	0.0000	0.0000
<i>Pangasius krempfi</i>	1	Mekong Can Tho Viet Nam	KRE VIE 1	3	0.0290	0.0870
<i>Pangasius gigas</i>	3	Mekong Nong Khai Thailand	GIG THA 1	2	0.0652	0.1304
<i>Helicophagus waandersii</i>	4	Mekong Bung Kan Thailand	WAN THA 1	4	0.0326	0.1739
<i>Helicophagus waandersii</i>	16	Batang Hari Jambi Sumatra Indonesia	WAN SUM 3	2	0.0000	0.0000
<i>Helicophagus waandersii</i>	5	Tchao Praya Nakhom Sawan Thailand	WAN THA 2	3	0.0000	0.0000
<i>Helicophagus typus</i>	16	Batang Hari Jambi Sumatra Indonesia	TYP SUM 3	3	0.0290	0.0870
<i>Pangasius djambal</i>	16	Batang Hari Jambi Sumatra Indonesia	DJA SUM 3	7	0.0311	0.1739
<i>Pangasius djambal</i>	15	Musi Palembang Sumatra Indonesia	DJA SUM 2	4	0.0435	0.0870
<i>Pangasius djambal</i>	12	Brantas Jombang Java Indonesia	DJA JAV 1	10	0.0739	0.2609
<i>Pangasius djambal</i>	8	Barito Muara Tewe Kalimantan Indonesia	DJA KAL 3	1	0.0000	0.0000
<i>Pangasius djambal</i>	17	Indragiri Rengat Sumatra Indonesia	DJA SUM 4	7	0.0435	0.1304
<i>Pangasius bocourti</i>	1	Mekong Can Tho Viet Nam	BOC VIE 1	7	0.0807	0.3043
<i>Pangasius bocourti</i>	4	Mekong Bung Kan Thailand	BOC THA 1	4	0.0471	0.1739
<i>Pangasius sp1</i>	15	Musi Palembang Sumatra Indonesia	SP1 SUM 2	5	0.0435	0.0435
<i>Pangasius sp1</i>	7	Mahakam Samarinda Kalimantan Indonesia	SP1 KAL 2	4	0.0543	0.2174
<i>Pangasius sp1</i>	1	Mekong Can Tho Viet Nam	SP1 VIE 1	3	0.0870	0.1304
<i>Pangasius micronema</i>	17	Indragiri Rengat Sumatra Indonesia	MIC SUM 4	5	0.0783	0.2174
<i>Pangasius micronema</i>	10	Kapuas Sanggau Kalimantan Indonesia	MIC KAL 5	4	0.1196	0.2174
<i>Pangasius micronema</i>	12	Brantas Dam Karet Java Indonesia	MIC JAV 1	5	0.0783	0.2609
<i>Pangasius micronema</i>	18	Indragiri Teluk Kuantan Sumatra Indonesia	MIC SUM 5	3	0.1014	0.2174
<i>Pangasius micronema</i>	14	Tulang Bawang Kotabumi Sumatra Indonesia	MIC SUM 1	3	0.0725	0.1739
<i>Pangasius micronema</i>	8	Barito Muara Tewe Kalimantan Indonesia	MIC KAL 3	2	0.1522	0.3043
<i>Pangasius sp2</i>	6	Berau Tanjung Redeb Kalimantan Indonesia	SP2 KAL 1	3	0.0145	0.0435
<i>Pangasius nasutus</i>	9	Kayanan Palangkaraya Kalimantan Indonesia	NAS KAL 4	3	0.0290	0.0870
<i>Pangasius nasutus</i>	10	Kapuas Sanggau Kalimantan Indonesia	NAS KAL 5	2	0.0435	0.0435
<i>Pangasius nasutus</i>	8	Barito Muara Tewe Kalimantan Indonesia	NAS KAL 3	1	0.0435	0.0435
<i>Pangasius nasutus</i>	16	Batang Hari Jambi Sumatra Indonesia	NAS SUM 3	3	0.0000	0.0435
<i>Pangasius nasutus</i>	15	Musi Palembang Sumatra Indonesia	NAS SUM 2	3	0.0000	0.0435
<i>Pangasius nasutus</i>	17	Indragiri Rengat Sumatra Indonesia	NAS SUM 4	1	0.0435	0.0435
<i>Pangasius nieurwenhuisii</i>	7	Mahakam Samarinda Kalimantan Indonesia	NIE KAL 2	4	0.0761	0.1739
<i>Pangasius lithostoma</i>	11	Kapuas Sintang Kalimantan Indonesia	LIT KAL 6	1	0.0000	0.0000
<i>Pangasius humeralis</i>	11	Kapuas Sintang Kalimantan Indonesia	HUM KAL 6	1	0.0000	0.0000
<i>Pangasius polyuranodon</i>	17	Indragiri Rengat Sumatra Indonesia	POL SUM 4	8	0.1087	0.2609
<i>Pangasius polyuranodon</i>	8	Barito Muara Tewe Kalimantan Indonesia	POL KAL 3	4	0.0435	0.1304
<i>Pangasius polyuranodon</i>	1	Mekong Can Tho Viet Nam	POL VIE 1	6	0.0870	0.2174
<i>Pangasius polyuranodon</i>	5	Tchao Praya Ayuttaya Thailand	POL THA 2	1	0.1304	0.1304
<i>Pangasius polyuranodon</i>	10	Kapuas Sanggau Kalimantan Indonesia	POL KAL 5	1	0.0870	0.0870
<i>Pangasius polyuranodon</i>	15	Musi Palembang Sumatra Indonesia	POL SUM 2	3	0.1739	0.2609
<i>Pangasius polyuranodon</i>	16	Batang Hari Jambi Sumatra Indonesia	POL SUM 3	3	0.1159	0.1739
<i>Pangasius polyuranodon</i>	14	Tulang Bawang Kotabumi Sumatra Indonesia	POL SUM 1	3	0.0725	0.1739
<i>Laides hexanema</i>	5	Tchao Praya Nakhom Sawan Thailand	HEX THA 2	2	0.0263	0.0526
<i>Laides hexanema</i>	19	Batang Hari Muara Bungo Sumatra Indonesia	HEX SUM 6	3	0.0175	0.0530
<i>Laides hexanema</i>	4	Mekong Bung Kan Thailand	HEX THA 1	2	0.0263	0.0526
<i>Laides sinensis</i>	4	Mekong Bung Kan Thailand	SIN THA 1	2	0.0000	0.0000
<i>Pseudeutropius brachyoptes</i>	19	Batang Hari Muara Bungo Sumatra Indonesia	BRA SUM 6	3	0.0000	0.0000

**Table 1:** Species identification, code of sampling origin (CL, cf. Fig. 1), sampling origin, species abbreviations (Abbrev.), size of samples (N), observed heterozygosity ( $H_{obs}$ ) and polymorphic loci indices ( $P^{95}$ ).

Mean observed heterozygosities ( $H_{obs.}$ ) and polymorphic ( $P^{95}$ ) indices were computed using the GENETIX package (Belkhir *et al.*, 1996). Standard genetic distances were estimated using the Nei's formula (1978). Phenograms were generated from distance matrix by Fitch cluster analysis using the PHYLIP package (Felsenstein, 1989). The robustness of the data set was tested by a resampling method such as bootstrapping (SEQBOOT in PHYLIP). This method is well developed by Felsenstein (1985) and involves the creation of new data set by sampling  $N$  characters randomly with replacement, so that the resulting data set has the same size as the original, but some characters have been left out and others are duplicated. The random variation of the results from analysing these bootstrapped data sets can be shown statistically to be typical of the variation that we would get from collecting new data sets. Bootstrap values were computed over 500 replications of resampled distances matrix.

A multivariate analysis (factor analysis of correspondence) was performed using the BIOMEKO program (Lebreton *et al.*, 1990) from a matrix of alleles coded in presence or absence for each individual and each locus from raw electrophoresis data. This statistical analysis method was used with the goal to assess the

genetic relatedness of species belonging to genus *Laides*.

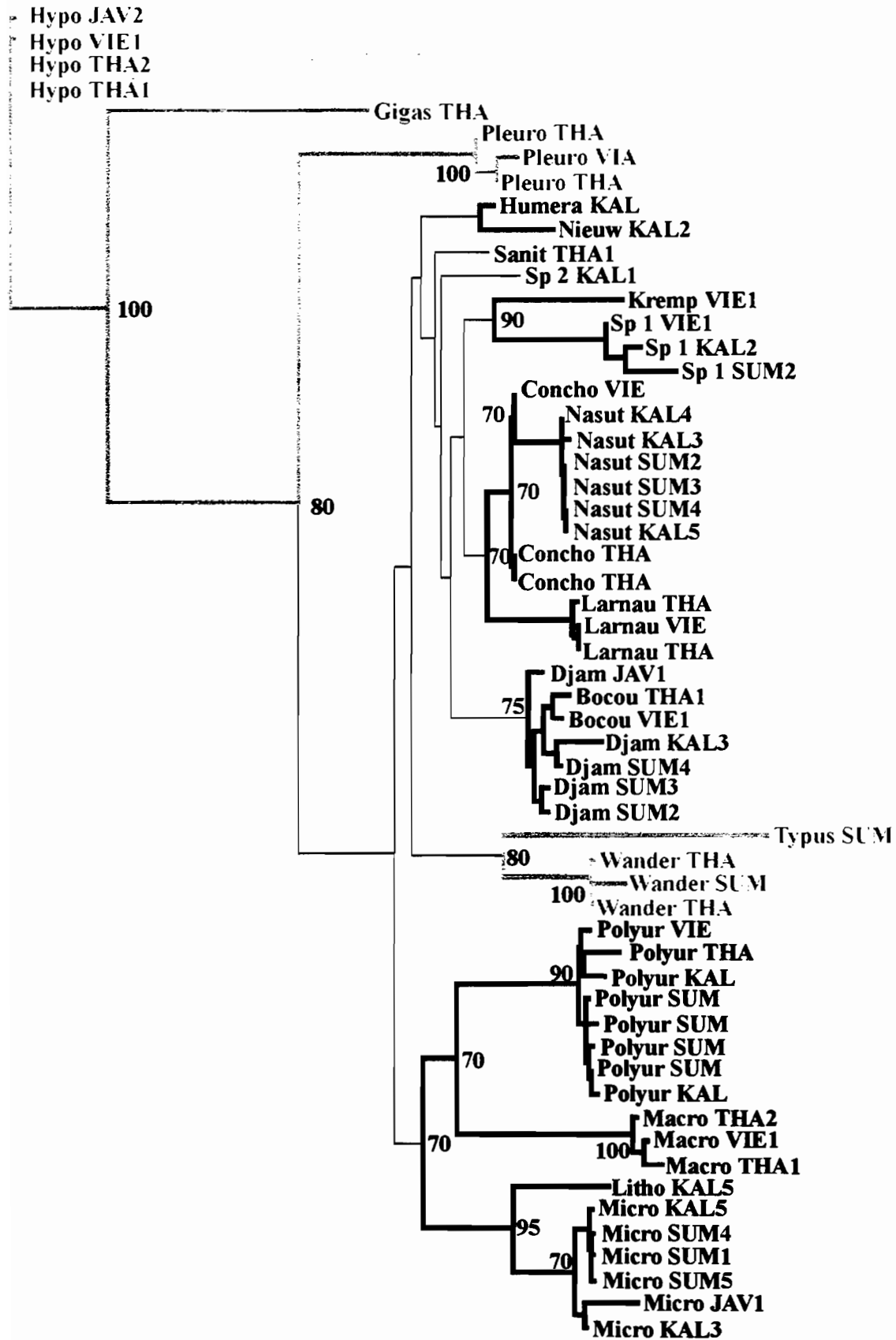
## RESULTS

The study concerned 23 loci (Table 2) excepting for species belonging to *Laides* and *Pseudeutropius* genera for which loci Sod-3, Sod-4, Adh and Sdh were not considered due to difficulty of interpretation. Analysis of the zymograms showed considerable polymorphism at the loci studied with a total of 199 alleles evidenced for all species. Only the locus Ldh-1 was monomorphic for the same allele in all the samples. The average rate of observed heterozygosity ( $H_{obs.}$ ) for all loci per population was between 0.000 and 0.1826 and the average rate of polymorphism ( $P^{95\%}$ ) between 0.000 and 0.3478.

A phylogenetic tree (Fig. 2) was obtained from the matrix of pairwise Nei's genetic distances between all pair of taxa. The results confirm all nominal species proposed by Roberts and Vidthayanon (1991). Bootstrap tests validated four genetic differentiated groups. Within these groups, 7 significant clusters were observed, in which occurrence probability was above 0.70. However internal topology of the genetic network was not

Enzyme system	Abbreviation	Locus	Tissue source	Electrode buffer*
Aspartate aminotransferase	AAT	<i>Aat</i>	Liver	TEB
Alcohol dehydrogenase	ADH	<i>Adh</i>	Liver	POULIK ½
Creatine kinase	CK	<i>Ck-1</i>	Eyes	MC 2
		<i>Ck-2</i>	Eyes	MC 2
Fructose biphosphatase	FBP	<i>Fbp</i>	Liver	MC 2
Glucosephosphate isomerase	GPI	<i>Gpi-1</i>	Muscle Eyes	RW
		<i>Gpi-2</i>	Muscle Eyes	RW
Isocitrate dehydrogenase	IDHP	<i>Idhp-1</i>	Muscle	MC 2
		<i>Idhp-2</i>	Liver	MC 2
Lactate dehydrogenase	LDH	<i>Ldh-1</i>	Eyes	MC 2
		<i>Ldh-2</i>	Eyes	MC 2
Malate dehydrogenase	MDH	<i>Mdh-1</i>	Eyes	MC 2
		<i>Mdh-2</i>	Eyes	MC 2
Mannose phosphate isomerase	MPI	<i>Mpi</i>	Liver	POULIK ½ TEB
Phosphoglucomutase	PGM	<i>Pgm</i>	Muscle	RW
6-Phosphogluconate dehydrogenase	6PGD	<i>6Pgd</i>	Liver	MC 2
		<i>Prot-1</i>	Muscle	MC 2
Protein Total	PT	<i>Prot-2</i>	Muscle	MC 2
Superoxide dismutase	SOD	<i>Sod-1</i>	Liver	POULIK ½
		<i>Sod-2</i>	Liver	POULIK ½
		<i>Sod-3</i>	Liver	MC 2
		<i>Sod-4</i>	Liver	MC 2
Sorbitol dehydrogenase	SDH	<i>Sdh</i>	Liver	POULIK ½

**Table 2:** Enzymes systems, buffers, locus, tissue specificity and electrode buffer investigated in the study. MC 2, Morpholine citrate, pH 6.2; RW, Ridgeway, Lithium hydroxide-borate, pH 8.3; POULIK ½, boric acid-sodium hydroxide, pH 8.2; TEB, Tris-borate-EDTA, pH 8.6.



**Figure 2:** Phenogram produced by Phylip on 20 species of Pangasiidae. This tree was generated from distance matrix by Fitch Cluster Analysis. Bootstrap values were computed over 500 replications of resampled distance matrix.

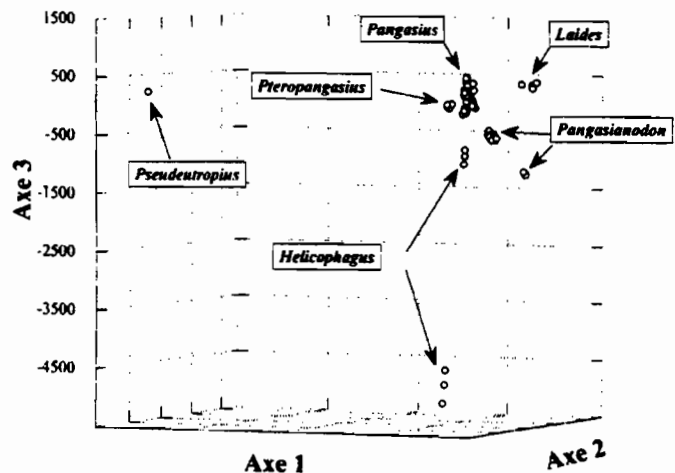
significant. The cluster 1 is composed of *P. hypophthalmus*, *P. gigas* and *P. pleurotaenia*, the cluster 2 is defined by *H. waandersii* and *H. typus*, the cluster 3 by *P. conchophilus*, *P. nasutus* and *P. larnaudii*, the cluster 4 by *Pangasius* sp1 and *P. krempfi*, the cluster 5 by *P. djambal* and *P. bocourti*, the cluster 6 by *P. nieuwenhuisii* and *P. humeralis* and finally the cluster 7 comprises *P. micronema* and *P. lithostoma*, *P. polyuranodon* and *P. macronema*. *Pangasius sanitwongsei* and *Pangasius* sp2 constitute a particular situation because they are characterised by the possession of many alleles shared with species belonging to clusters 3, 4 and 5. *Pangasius* sp1 and *Pangasius* sp2 mentioned above are probably new species because they were not identified with the specific keys proposed by Roberts & Vidthayanon (1991). *Pangasius* sp1 was caught both in Indonesia (Kalimantan at Samarinda on Mahakam River; Sumatra at Palembang on Musi River, Jambi on Batang Hari River and Rengat on Indragiri River) and in Vietnam (Binh Dai and Can Tho in Mekong delta). *Pangasius* sp1 is genetically related to *P. krempfi*, nevertheless they are reproductively isolated because no intermediate genotype was observed in sympatric condition as in the Mekong Delta. *Pangasius* sp2 was observed only in East Kalimantan at Tanjung Redeb on Berau River.

The multivariate analysis projections of individuals referring to their genotype multilocus relatedness is presented on Fig. 3. On the first projection (plane formed by axis 1 and 2) the axis 1 which is the most informative clearly shows a huge genetic divergence between Schilbeidae and Pangasiidae families. Axis 1 also reveals a genetic differentiation between *P. pleurotaenia* and all other pangasiid species. On the second projection (plane formed by axis 2 and 3), the species belonging to genus *Lalides*, *P. hypophthalmus* and *P. gigas* form three genetically independent entities, formally separated on axis 2 from the group constituted by other pangasiids. *Helicophagus waandersii* and *H. typus* are differentiated from the other species on axis 3.

## DISCUSSION

### Speciation and evolutionary processes

The tropical Asian catfish family Pangasiidae displays a strong genetic differentiation with 199



**Figure 3:** Multivariate analysis projections of individuals referring to their multilocus relatedness.

alleles evidenced on 23 loci. For comparison the phylogenetic relationships assessed by Pouyaud & Agnès (1995) among species belonging to genera *Oreochromis*, *Sarotherodon*, *Tilapia*, *Chilochromis*, *Pelmatochromis*, *Tylochromis*, *Hemichromis* and *Chromidotilapia* (Cichlidae) revealed 95 alleles on 24 loci. Fossil record of the family Pangasiidae (Sanders, 1934 in Roberts & Vidthayanon, 1991) from Tertiary deposits (during period between 65 and 1 million years before present) in central Sumatra indicate that occurrence of this family in south-east Asia is ancient. Under these conditions, cumulative fluctuations of sea water levels during this period have undoubtedly fashioned the pangasiid group leading to the notable ecological and morphological diversity observed actually. Large river basins like Mekong, Tchao Phraya and Kapuas have probably played a major role in terms of refuge zone during the past. These rivers possess *P. nasutus* (Malay peninsula, Indonesia); *P. bocourti* (Vietnam and Thailand) and *P. djambal* (Indonesia). The genetic distances between both couples of species ( $d = 0.109$  between samples of *P. nasutus* from Muara Tewe in Kalimantan and *P. conchophilus* from Mekong in Vietnam;  $d = 0.158$  between samples of *P. bocourti* from Thailand and *P. djambal* from Java in Indonesia) are comparable with average distances occurring within the species *P. polyuranodon* ( $d = 0.106$  between population of Muara Tewe in Kalimantan and population caught in Tchao Phraya) or *P. micronema* ( $d = 0.145$  between population of Teluk Kuantan in Sumatra and population of Solo in Java).

### Systematic implications

The multivariate analysis clearly indicate that *Laides hexanema* and *Laides sinensis* are genetically related with the pangasiid species. Nei's genetic distances (Table 3) confirm that *Laides* species are genetically closer to pangasiid species than to *Pseudeutropius* species. Therefore the genus *Laides* should be placed in Pangasiidae as firstly proposed by Roberts (1989).

	<i>Pseudeutropius</i> (Schilbeidae)	<i>Laides</i>
<i>Laides</i>	1.19	
Pangasiidae	1.05	0.52

**Table 3:** Nei's genetic distances between Pangasiidae, *Laides* and Schilbeidae.

The phylogenetic tree obtained shows a clustering of species which validate the genus *Helicophagus* but cannot agree the actual composition of the genus *Pangasius* which is polyphyletic. *Neopangasius*, *Pangasianodon* and *Pteropangasius* are the three subgenus recognised by Roberts & Vidthayanon (1991).

Our study confirms the species relatedness described within these three groups except in the subgenus *Neopangasius* in which *P. lithostoma* could not be inserted. This species is genetically closer to *P. micronema*. Considering the genus *Helicophagus* as a reference, average genetic distances between each group (Table 4) suggest that *Pangasianodon* and *Pteropangasius* should be elevated to the genus level. The genus *Pangasianodon* would then be composed by *P. hypophthalmus* and *P. gigas* and the genus *Pteropangasius* by only one species *P. pleurotaenia*. *Neopangasius* may remain valid with *P. nieuwenhuisii* and *P. humeralis* as a subgenus of *Pangasius*. *Pangasius lithostoma*, *P. micronema*, *P. macronema*, *P. polyuranodon*, *P. lithostoma*, *P. sanitwongsei*, *P. krempfi*, *P. larnaudii*, *P. nasutus*, *P. conchophilus*, *P. djambal*, *P. bocourti* could be maintained in the genus *Pangasius*. Additional genetic analyses using other markers like ribosomal sequences may

provide evidence of other subgenus in the *Pangasius* genus.

### Possible finding of two new species

*Pangasius* sp1 was caught in Sumatra (Palembang, Musi River; Rengat, Indragiri River), in Kalimantan (Samarinda, Mahakam River) and in Vietnam (Mekong River). In these localities this species was misidentified as *P. djambal*. This species shares many characteristics with *Pangasius pangasius* like gill raker counts, its occurrence in saline waters (reported by fishermen both in Indonesia and Vietnam) and a relatively similar palatal dentition. As in *P. pangasius*, the palatal dentition is curved and constituted by related close-sets. Referring to the dentition picture available in Roberts & Vidthayanon (1991) for *P. pangasius*, the *Pangasius* sp1 dentition presents some differences. The two vomerine tooth plates are always linked like an horizontal number 8. On each side the palatine tooth plates are small, round and separated. As no samples of *Pangasius pangasius* could be obtained at this stage from India or Bangladesh for comparison, the status of *Pangasius* sp1 remains unclear. A detail morphological examination of the specimens collected during this study will be carried out in a near future. They may represent a new pangasiid species, but at the present state the possibility that these specimens belong to the species *P. pangasius* cannot be discarded. In this latter hypothesis, *P. pangasius* would become the pangasiid species with the largest geographic distribution, from India to Vietnam and Indonesian archipelago.

*Pangasius* sp2 was caught in East Kalimantan (Tanjung Redeb, Berau river). In this location the species was misidentified as *P. polyuranodon*. This species share genetic characteristics with species such as *P. djambal* and *P. bocourti*. The internal position of this fish in the phylogeny and its isolated biogeographic area (basin regarding to the Macassar strait along the Wallace line) may indicate that this species has colonised this area since a very ancient period. Further analysis will permit to precise if this species has conserved

	<i>Pangasianodon</i>	<i>Pteropangasius</i>	<i>Helicophagus</i>	<i>Pangasius 1</i>
<i>Pteropangasius</i>	0.656			
<i>Helicophagus</i>	0.701	0.675		
<i>Pangasius 1</i>	0.568	0.538	0.230	
<i>Pangasius 2</i>	0.607	0.530	0.180	0.080

**Table 4 :** Nei's genetic distances between each possible genus and subgenus in pangasiids.

primitive characters. The two vomerine form a single large median tooth plate like in *P. polyuranodon*, but the palatines tooth plates are very elongate and are parallel with the lateral sides of the vomerine plate. Up to now this shape of palatal dentition was never reported in the pangasiids.

#### *Some new data on zoogeography*

*Helicophagus typus* was considered as probably extinct in Sumatra because the last specimen was collected in Palembang in 1908 (Roberts & Vidthayanon, 1991). We can assume that *H. typus* still occurs in the Batang Hari River (Sumatra, Jambi) where 3 specimens (around 600 mm) were collected the 27 February 1997 and two supplementary specimens (621 and 342 mm SL) were found in the Kapuas River on the 27 June 1997 (Sanggau and Sintang, West Kalimantan).

Until now, *Pangasius djambal* was reported only from Java and Kalimantan, we can assume however that this species occurs in all major basins of Sumatra as Musi, Batang Hari and Indragiri Rivers. The species is abundant on the markets in these locations. Nevertheless, this species has a restricted distribution in Java where it seems to be present only in Solo and Brantas Rivers. Contrarily to the situation stated by Roberts and Vidthayanon (1991), *Pangasius djambal* was never utilised in aquaculture in Java until now, as anywhere else in Indonesia. The only pangasiid species cultured in Indonesia is *Pangasius hypophthalmus* which was introduced from Thailand in 1972.

Because of human activities located on river banks, organic and chemical pollution, dams construction, over-fishing, many endemic species are threatened with extinction as is the case of *Pangasius gigas* in the Mekong. In Indonesia, such a situation is observed in Java where *P. djambal* and *P. micronema* could be found only in the Brantas and Solo Rivers in central Java. Local information strongly suggests that these species have now disappeared from anywhere else on the island. By contrast, *Pangasius hypophthalmus* which has been found in the Citarum River (West Java) and Batang Hari River tributaries (Sumatra) could now be considered as part of the Indonesian ichthyofauna.

#### ACKNOWLEDGMENT

The authors are grateful to Sophie Lenormand, Jacques Slembrouck and Antoine Pariselle (IRD), Sudarto, Wartono Hadie, Didi Sadili, Anang Hari Kristanto and Oman Komarudin (CRIFI/RIFF, Indonesia), Maskur (Loka BAT, Jambi), Nguyen Van Thuong (Can Tho Univ., Vietnam), Le Thanh Hung and Philippe Cacot (CIRAD) and Chawalit Vidthayanon (DOF, Thailand) for their help in collecting fish samples.

#### REFERENCES

- Belkhir K., Borsa P., Goudet J., Chikhi L. & Bonhomme F. (1996) GENETIX, logiciel pour la génétique des populations. Version 3.0. Université Montpellier II, Montpellier, France.
- Felsenstein J. (1989) PHYLIP—phylogeny inference package (Version 3.2). *Cladistics*, 5: 164-166. Lebreton J. D., Roux M., Banco G., Bacou A. M. (1990) BIOMECO (Biometry-Ecology), version 3.9 Statistical ecology software for PC and compatibles. Montpellier: CEFE-CNRS.
- Pouyaud L. & Agnès J.F. (1995) Phylogenetic relationships between 21 species of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *Journal of Fish Biology*, 47, 26-38.
- Roberts T.R. (1989) *The freshwater fishes of western Borneo (Kalimantan barat, Indonesia)*. Memoirs of the California Academy of Sciences 14, xii+210pp.
- Roberts T.R. & Vidthayanon C. (1991) Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 143, 97-144.
- Shaklee J.B., Allendorf F.W., Morizot D.C. & Whitt G.S. (1990) Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society*, 119, 2-15.



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



Proceedings of the mid-term workshop of the  
“Catfish Asia Project”  
Cantho, Vietnam, 11-15 May 1998



Edited by :  
Marc LEGENDRE  
Antoine PARISELLE



## CONTENTS

	Page
<b>FOREWORD</b>	1
<b>CONTENTS</b>	3
<b>CONTEXTS AND RESEARCH GOALS</b>	
<b>Legendre M.</b> The Catfish Asia project: backgrounds, aims and prospects.-----	7
<b>Lazard J.</b> Interest of basic and applied research on <i>Pangasius</i> spp. for aquaculture in the Mekong Delta: situation and prospects.-----	15
<b>Sadili D.</b> Marketing of pangasiid catfishes in Java and Sumatra, Indonesia.-----	21
<b>BIOLOGICAL DIVERSITY</b>	
<i>CHARACTERISATION OF SPECIES, POPULATIONS AND STRAINS</i>	
<b>Teugels G.G., Legendre M. &amp; Hung L.T.</b> Preliminary results on the morphological characterisation of natural populations and cultured strains of <i>Clarias</i> species (Siluriformes, Clariidae) from Vietnam.-----	27
<b>Teugels G.G., Gustiano R., Diego R., Legendre M. &amp; Sudarto.</b> Preliminary results on the morphological characterisation of natural populations and cultured strains of <i>Clarias</i> species (Siluriformes, Clariidae) from Indonesia.-----	31
<b>Pariselle A. &amp; Komarudin O.</b> First results on the diversity of gill parasites of some catfishes host species in South East Asia.-----	37
<b>Pouyaud L., Hadie W. &amp; Sudarto.</b> Genetic diversity among <i>Clarias batrachus</i> (Siluriformes, Clariidae) populations from the Indochina Peninsula and Indonesia Archipelago.-----	43
<b>Pouyaud L., Gustiano R. &amp; Legendre M.</b> Phylogenetic relationships among pangasiid catfish species (Siluriformes, Pangasiidae).-----	49
<b>Volckaert F., Hellemans B. &amp; Pouyaud L.</b> Preliminary data on genetic variation in the genus <i>Clarias</i> and <i>Pangasius</i> on the basis of DNA microsatellite loci.-----	57
<i>BIO-ECOLOGY</i>	
<b>Thuong N.V., Hung H.P., Dung D.T. &amp; Kha L.A.</b> Preliminary data on species composition and distribution of pangasiid catfishes (Siluriformes, Pangasiidae) in the lower Mekong River basin.-----	61
<b>DIVERSIFICATION AND OPTIMISATION IN AQUACULTURE PRODUCTION</b>	
<i>REPRODUCTION</i>	
<b>Cacot P.</b> Description of the sexual cycle related to the environment and set up of the artificial propagation in <i>Pangasius bocourti</i> (Sauvage, 1880) and <i>Pangasius hypophthalmus</i> (Sauvage, 1878), reared in floating cages and in ponds in the Mekong delta.-----	71
<b>Legendre M., Subadgja J. &amp; Slembrouck J.</b> Absence of marked seasonal variations in sexual maturity of <i>Pangasius hypophthalmus</i> brooders held in ponds at the Sukamandi station (Java, Indonesia).-----	91
<b>Legendre M., Slembrouck J. &amp; Subadgja J.</b> First results on growth and artificial propagation of <i>Pangasius djambal</i> in Indonesia.-----	97

<b>Xuan L.N. &amp; Liem P.T.</b> Preliminary results on the induced spawning of two catfish species, <i>Pangasius conchophilus</i> and <i>Pangasius</i> sp1, in the Mekong delta.-----	103
<b>Kristanto A.H., Subadgja J., Slembrouck J. &amp; Legendre M.</b> Effects of egg incubation techniques on hatching rates, hatching kinetics and survival of larvae in the Asian catfish <i>Pangasius hypophthalmus</i> (Siluriformes, Pangasiidae).-----	107
<b>Campet M., Cacot P., Lazard J., Dan T.Q., Muon D.T. &amp; Liem P.T.</b> Egg quality of an Asian catfish of the Mekong River ( <i>Pangasius hypophthalmus</i> ) during the process of maturation induced by hCG injections.-----	113
<b>Legendre M., Slembrouck J., Subadgja J. &amp; Kristanto A.H.</b> Effects of varying latency period on the <i>in vivo</i> survival of ova after Ovaprim- and hCG-induced ovulation in the Asian catfish <i>Pangasius hypophthalmus</i> (Siluriformes, Pangasiidae).-----	119
 <b>LARVAL BIOLOGY AND REARING</b>	
<b>Hung L.T., Tuan N.A., Hien N. V. &amp; Cacot P.</b> Larval rearing of the Mekong catfish, <i>Pangasius bocourti</i> (Siluriformes, Pangasiidae): <i>Artemia</i> alternative feeding and weaning time.-----	127
<b>Slembrouck J., Hung L.T., Subadgja J. &amp; Legendre M.</b> Effects of prey quality, feeding level, prey accessibility and aeration on growth and survival of <i>Pangasius hypophthalmus</i> larvae (Siluriformes, Pangasiidae).-----	137
<b>Subadgja J., Slembrouck J., Hung L.T. &amp; Legendre M.</b> Analysis of precocious mortality of <i>Pangasius hypophthalmus</i> larvae (Siluriformes, Pangasiidae) during the larval rearing and proposition of appropriate treatments.-----	147
 <b>NUTRITION, FEEDING AND GROWTH</b>	
<b>Hung L.T., Tuan N. A., Phu N.V. &amp; Lazard J.</b> Effects of frequency and period of feeding on growth and feed utilisation on <i>Pangasius bocourti</i> in two Mekong catfishes, <i>Pangasius bocourti</i> (Sauvage, 1880) and <i>Pangasius hypophthalmus</i> (Sauvage, 1878).-----	157
<b>Hung L.T., Lazard J., Tu H.T. &amp; Moreau Y.</b> Protein and energy utilisation in two Mekong catfishes, <i>Pangasius bocourti</i> and <i>Pangasius hypophthalmus</i> .-----	167
<b>Phuong N.T. &amp; Hien T.T.T.</b> Effects of feeding level on the growth and feed conversion efficiency of <i>Pangasius bocourti</i> fingerlings.-----	175
<b>Phuong N.T., Thi M.V. &amp; Hang B.T.B.</b> The use of plant protein (soybean meal) as a replacement of animal protein (fish meal and blood meal) in practical diets for fingerlings of <i>Pangasius bocourti</i> .-----	179
<b>Liem P.T. &amp; Tu H.T.</b> Rearing of <i>Pangasius bocourti</i> fry (Siluriformes, Pangasiidae) fed different diets in concrete tanks.-----	187
 <b>HYBRIDS EVALUATION</b>	
<b>Kiem N.V. &amp; Liem P.T.</b> Some biological characteristics of <i>Clarias batrachus</i> and Preliminary results of the hybridisation between <i>Clarias batrachus</i> x <i>Clarias gariepinus</i> .-----	191
<b>Lenormand S., Slembrouck J., Pouyaud L., Subadgja J. &amp; Legendre M.</b> Evaluation of hybridisation in five <i>Clarias</i> species (Siluriformes, Clariidae) of African ( <i>C. gariepinus</i> ) and Asian origin ( <i>C. batrachus</i> , <i>C. meladerma</i> , <i>C. nieuhofii</i> and <i>C. teijsmanni</i> ).-----	195
<b>Minh L.T.</b> Preliminary results on the relationship between growing stage and body composition in <i>Clarias macrocephalus</i> , <i>Clarias gariepinus</i> and their hybrid ( <i>C. macrocephalus</i> female x <i>C. gariepinus</i> male).-----	211

**PATHOLOGY**

- Komarudin O.** Preliminary observations on the infection of the gills of cultivated *Pangasius hypophthalmus* by *Monogenea*.----- 217
- Supriyadi H., Komarudin O. & Slembrouck J.** Preliminary study of the source of *Aeromonas hydrophila* infection on *Pangasius hypophthalmus* larvae.----- 219
- Dung T.T. & Ngoc N.T.N.** Preliminary results of the study of parasitic and red spot diseases on high economical valuable catfish species in the Mekong Delta.----- 223