FISHERIES SCIENCE 2006; **72:** 469–476

Genetic diversity and population history of the migratory catfishes *Pangasianodon hypophthalmus* and *Pangasius bocourti* in the Cambodian Mekong River

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ABSTRACT: Polymerase chain reaction (PCR) restriction fragment length polymorphism analysis of mitochondrial DNA was applied to the genetic structure and evolutionary history of the more ancestral *Pangasianodon hypophthalmus* ($n = 82$), and the recently speciated catfish *Pangasius bocourti* ($n = 90$) from the Cambodian Mekong River. Both pangasiids were characterized by a lack of genetic population structure that may result from high levels of contemporary gene flow. Genetic diversity was lower in *P. hypophthalmus* than in *P. bocourti*. However, a different evolutionary history was inferred for both species based on genealogical and demographic analyses (mismatch analysis, Tajima's *D*- and Fu's F_S-tests). The genetic profile of the more ancestral *P. hypophthalmus* shows indications of a recent population bottleneck, whereas the recently speciated *P. bocourti* shows signatures of historical population expansion. This study stresses the importance of preserving the migration routes in the Cambodian Mekong basin in order to maintain the genetic diversity and long-term integrity of both species.

KEY WORDS: **bottleneck, fisheries, mitochondrial DNA, Pangasiidae, population genetics, restriction fragment length polymorphism.**

INTRODUCTION

The importance of historical biogeography in shaping intraspecific genetic structure and the ability of mitochondrial DNA (mtDNA) to retain a history of past isolation have been well demonstrated.[1–5] Demographic historical events such as growth or range expansion following a bottleneck leave signatures in both genetic diversity and phylogenetic structure,[6–8] while vicariance events and climatic cycles may be linked to deeper phylogenetic structure.[2,9,10] Just two studies combining phylogenetic, population genetic and distributional approaches have provided insight into intraspecific patterns and processes for South-East Asian freshwater fishes (catfish *Hemibagrus nemurus*[1] and cyprinid *Barbodes gonionotus*[11]).

Fish ecology and production within the Mekong system is driven by the annual flood cycle, which is under the influence of the south-west monsoon (June–October). Thousands of square kilometers of floodplain throughout the Mekong system and the Tonle Sap Lake in Cambodia are inundated to a surface 4–6 times larger than in the dry season. Its extremely diverse fish community, with over 1200 species recorded (Cambodia in itself counts over 500 fish species), reflects past climatic and geologic processes that have brought together the fauna of several river systems.[12] Although human activities, such as habitat modification, deforestation, deterioration of water quality, water capture for hydropower and irrigation, and overfishing, currently threaten all fisheries of the Mekong basin, no group is more at risk than both Pangasiid species.[13,14]

Pangasianodon hypophthalmus Sauvage 1878 and *Pangasius bocourti* Sauvage 1880 belong to the South-East Asian catfish family of the Pangasiidae.[15] *Pangasianodon hypophthalmus* occurs in large rivers of the Mekong and Chao Phraya basins and is very common in the Lower Mekong River.[12] *Pangasius bocourti* is apparently a Mekong endemic, known from large rivers in the Lower

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Received 12 January 2005. Accepted 11 November 2005.

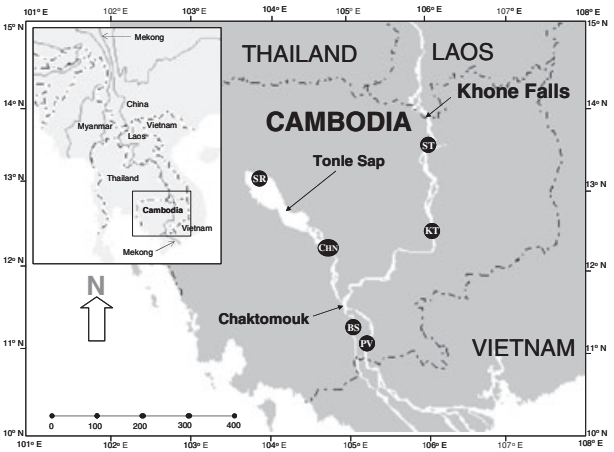

Fig. 1 Sampling locations of *Pangasianodon hypophthalmus* and *Pangasius bocourti* in the Cambodian Mekong River basin. Tonle Sap River: Siem Reap (SR, 13°13′N, 103°52′E) and Kompong Chnang (CHN, 12°16′N, 104°41′E). Upstream Mekong River: Stung Treng (ST, 13°32′N, 105°57′E) and Kratie (KT, 12°37′N, 106°01′E). Downstream Mekong River: Prey Veng (PV, 11°08′N, 105°14′E). Bassac River: Bassac (BS, 11°24′N, 105°00′E).

and the Middle Mekong basin.[12] Remarkable in both species is their migratory behavior. Adult *P. hypophthalmus* and *P. bocourti* leave the Tonle Sap Lake and Mekong floodplain to migrate upstream to the spawning grounds, located downstream between the Khone Falls and the town of Kratie (KT) in Cambodia (Fig. 1) during October–March.[16] After spawning, they migrate downstream to the feeding grounds in Siem Reap (SR), Kompong Chnang (CHN), Prey Veng (PV) and Bassac (BS) (Fig. 1). Within the Mekong region, *P. hypophthalmus* and *P. bocourti* are dominant food fish, a common basis of local wild-capture fisheries and flagship catfish species.[17]

Little information is available on the phylogenetic characteristics of the family Pangasiidae. Two studies based on allozyme and mitochondrial markers reported a deep phylogenetic structure in pangasiid catfishes.[18,19] *Pangasianodon hypophthalmus* diverged from its closest living relative (*P. gigas*) approximately 8.0–6.0 Ma BP, while *P. bocourti* and its sister species *P. djambal* shared a common ancestor 1.0–0.3 Ma BP.[18,19]

However, despite the commercial importance of both Mekong catfish species their intraspecific diversity has never been studied. It is not known whether both species contain multiple gene pools that resulted from historical isolation across their wide natural distribution range. The latter information is important to guide the conservation management of both catfish species and ascertain their long-term survival.

The current paper has historical and contemporary components. Firstly, the evolutionary history of the more anciently diverged *P. hypophthalmus* and the recently speciated *P. bocourti* were studied to gain information about historical population subdivision and demographic history. Secondly, the population genetic structure and diversity of both Mekong catfish species were investigated. The implications of our genetic results, combined with ecologic and fisheries data, are discussed for the management of both catfishes in the Mekong basin.

MATERIALS AND METHODS

Fish sampling

Five locations in the Cambodian Mekong River were sampled in 2001 to obtain 82 specimens of *Pangasianodon hypophthalmus* and 90 specimens of *Pangasius bocourti* (Fig. 1).

Fish tissues (tail fin) were collected and stored in a salt-saturated DMSO solution (20% dimethylsulfoxide, 250 mM ethylenediaminetetracetic acid [EDTA] and 5 M NaCl) and kept at room temperature for transport to the laboratory for DNA analysis. Samples were transferred to pure ethanol and stored at room temperature upon arrival.

DNA extraction and PCR amplification

Small pieces of fin (50–100 mg) were digested with proteinase K (10 mg/mL) in CTAB buffer (2% cetyltrimethyl-ammonium bromide, 1.4 M NaCl, 20 mM EDTA at pH 8.0, and 100 mM Tris[hydroxymethyl] aminomethane at pH 8.0) and incubated at 55°C overnight. Total genomic DNA was isolated with a standard phenol–chloroform extraction protocol.[20] A fragment of 2.1 kb, that contained the cytochrome *b* gene and the control region, was amplified with two universal primers: (i) a forward primer complimentary of ND5/6, 5′-TGA YAT GAA AAA CCA TGG TTG TAA-3′;[21] and (ii) reverse primer HN20, 5′-GTG TTA TGC TTT AGT TAA GC-3′.[22] The polymerase chain reaction (PCR) was performed in a volume of 25 μL containing 1 × reaction buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8 at 25°C, and 0.01% Tween-20), 2 mM $MgCl_2$, 0.5 U of SilverStar *Taq* polymerase (Eurogentec, Seraing, Belgium), 200 μM dNTPs, 0.8 μM each of forward and reverse primer, and 1 μL (10–100 ng) of total genomic DNA. The amplification

procedure was as follows: initial denaturation at 95°C for 3 min followed by a cycle of denaturation at 95°C for 30 s, annealing at 46°C for 30 s, and final elongation at 72°C for 2 min 30 s in a thermocycler (Trioblock, Biometra, Göttingen, Germany). This cycle was repeated 35 times, after which an additional elongation step was done at 72°C for 7 min.

Restriction fragment length polymorphism analysis

The amplified fragments were subsequently screened for polymorphism using 10 restriction endonucleases: *Hae* III, *Hinf* I, *Mbo* I, *Taq* I, *Alu* I, *Rsa* I, *Hpa* II, *Tas* I, *Fnu* DII and *Tru*1 I, all recognizing tetranucleotide palindromic sequences. Restriction digestion was carried out overnight in a 10-μL volume using 5 μL of each PCR amplified mtDNA product and 10 U of restriction enzyme. Resulting mtDNA fragments were separated by electrophoresis on 1.2–2% agarose gels alongside a 100 bp DNA ladder used for fragment sizing.

Data analysis

Variation in mtDNA haplotype frequencies,[23] haplotype (*h*, standard genetic diversity) and nucleotide (π, average gene diversity over all loci) diversities,[24] overall and pairwise F_{ST} values,[25] and minimum spanning (genealogical) networks[26] among *P. bocourti* and *P. hypophthalmus* samples were assessed using ARLEQUIN software.[27] Differences in haplotype and nucleotide diversity of both catfishes were statistically assessed by performing a parametric Student's *t*-test on dependent variables using STATISTICA version 6.0 software (StatSoft, Tulsa, OK, USA).

To infer population demographic history of both catfishes, the frequency distribution of pairwise differences between mtDNA haplotypes (i.e. mismatch distribution)[28,29] were examined, and Tajima's *D*-test of neutrality[6,30] and Fu's F_S-test of neutrality[31] were evaluated.

A mismatch analysis was performed using ARLEQUIN to compare the demographic history of both species. Populations which have been stable over time are predicted to have a more balanced phylogeny shape and a bi- or multimodal mismatch distribution.[7] Populations which have gone through growth or expansion are expected to have a star-like phylogenetic shape and unimodal mismatch distribution.[7,8] The fit between observed and expected distributions was tested using the sum of squared deviations (SSD) for the estimated stepwise expansion models.[32]

Tajima's *D*-statistic values for departure from neutrality on total numbers of segregating sites were calculated using ARLEQUIN. Tajima's *D*-statistic is close to zero in populations that have remained stable in size over time.[33] Under the assumption of neutrality, a significant negative value of *D* predicts either a population expansion which has undergone recent increases in size because rare alleles are more abundant than expected,[34,35] or a recent past selective sweep.[6,36] A significant positive value of *D* predicts a long-past population subdivision.[36] The significance of the *D*-statistic was tested by simulating a distribution (1000 replicates) of *D*-values under the null hypothesis of population stability (i.e. neutrality).[27] Like Tajima's *D*-test,[6] Fu's neutrality test[31] is based on the infinite-site model without recombination, and is also used to infer population history. The F_S-statistic is very sensitive to population demographic expansion, which generally leads to large negative F_S values.[31] The Fu's F_S-statistic values were estimated using ARLEQUIN. The significance of the F_S-statistic values were tested by simulating a distribution (1000 replicates) of F_S-values under the null hypothesis of population stability (i.e. neutrality).[27] Significance levels of multiple tests were sequential Bonferroni corrected according to Rice.[37]

RESULTS

Genetic diversity and population differentiation

Of the 10 restriction enzymes used, five (*Hae* III, *Hinf* I, *Alu* I, *Rsa* I and *Tru*l I) in *Pangasianodon hypophthalmus* and seven (*Hinf* I, *Mbo* I, *Tag* I, *Alu* I, *Rsa* I, *Hp* II and *Tru*l I) in *Pangasius bocourti* were polymorphic. Seven mtDNA haplotypes were detected in *P. hypophthalmus* and 17 haplotypes in *P. bocourti* (Table 1). In *P. hypophthalmus*, the mean haplotype diversity was 0.499 (range 0.295–0.674), and the mean nucleotide diversity was 0.022 (range 0.009–0.048). In contrast, for five *P. bocourti* samples, the mean haplotype diversity was 0.728 (range 0.576–0.836), and the mean nucleotide diversity was 0.048 (range 0.036–0.058). Three unique (singleton) haplotypes were found in *P. hypophthalmus* and 10 in *P. bocourti*. On average, haplotype diversity in *P. hypophthalmus* was nearly significantly lower than that in *P. bocourti* ($t = 2.727$, $P = 0.053$), while nucleotide diversity was significantly lower in *P. hypophthalmus* than in *P. bocourti* ($t = 4.113$, $P = 0.014$).

Overall population differentiation (fixation index, F_{ST}) in *P. hypophthalmus* was 0.022 and statistically insignificant ($P = 0.139 > 0.05$). Similarly,

Table 1 Genetic diversity and results of population genetic tests in *Pangasianodon hypophthalmus* and *Pangasius bocourti*

Species/HC	Haplotype	ST	KT	SR	CHN	PV	BS	Total
Pangasianodon hypophthalmus								
Ph1	AAAAAAAAAA	8	5	11	–	15	11	50
Ph2	ABAAAAAAAA	3	9	4	–	6	1	23
Ph3	ACAAAAAAAB	–	–	–	–	1	–	1
Ph4	BAAAAAAAAA	–	–	1	–		1	2
Ph5	AAAABAAAAA	–	–	1	–	–	–	1
Ph6	CBAAAAAAAB	–	–	1	–	–		1
Ph7	ACAAAAAAAA	–	2	2	–	–	–	4
h		0.436	0.608	0.674	–	0.481	0.295	0.499
SD		0.133	0.090	0.100	–	0.094	0.156	0.115
π		0.009	0.022	0.048	–	0.019	0.013	0.022
SD		0.008	0.016	0.031	–	0.015	0.012	0.016
Pangasius bocourti								
Pb1	AAAAAAAAAA	10	8	–	9	7	9	43
Pb2	AAABAAAAAA	4	–	–	2	3	1	10
Pb3	AAAABAAAAA	–	–	–	2	3	5	10
Pb4	AABAAAAAAB	2	–	–	1	2	1	6
Pb5	AAAABABAAB	–	–	–	–	1	–	1
Pb6	AABAAAAAAA	–	–	–	3	1	2	6
Pb7	AABABAAAAB	–	–	–	–	1	–	1
Pb8	AADABAAAAA	–	–	–	–	1	–	1
Pb9	AAABBAAAAA	1	–	–	–	–	–	1
Pb10	AAAABABAAA	1	–	–	–	–	–	1
Pb11	AABABAAAAA	2	–	–	1	–	–	3
Pb12	AACAABAAAA	–	–	–	1	–	–	1
Pb13	ABBABAAAAA	–	–	–	1	–	–	1
Pb14	AAAAAABAAB	–	1	–	–	–	1	2
Pb15	AABBBAAAAA	–	1	–	–	–	–	1
Pb16	AABBBAAAAB	–	1	–	–	–	–	1
Pb17	AABBAAAAAA	–	1	–	–	–	–	1
h		0.721	0.576	–	0.784	0.836	0.725	0.728
SD		0.089	0.163	–	0.084	0.065	0.083	0.097
π		0.043	0.052	–	0.051	0.058	0.036	0.048
SD		0.028	0.034	–	0.032	0.036	0.024	0.031

Sampling abbreviations are listed in Figure 1.

HC, haplotype code; *h*, haplotype diversity; π, nucleotide diversity; SD standard deviation; –, not applicable. Capital letters identify fragment patterns.

the F_{ST} value for *P. bocourti* was 0.001 and insignificant ($P = 0.929 > 0.05$). Further, no pairwise F_{ST} value was significant in either catfish after sequential Bonferroni correction (data not shown).

The minimum spanning (genealogical) networks pointed to one very common central haplotype (Ph1) in *P. hypophthalmus* and one (Pb1) in *P. bocourti* (Fig. 2); these haplotypes were observed at all sampling sites. According to predictions from coalescence theory, the centrally positioned haplotypes are most likely ancestral variants.[38] The peripheral mitochondrial variants are connected to the central haplotype with one to just a few mutations, although some derived haplotypes have long branches, particularly in *P. hypophthalmus* (Ph3, Ph5 and Ph6; Fig. 2a). Unique haplotypes were observed in *P. hypophthalmus* at SR (Ph5 and Ph6) and in *P. bocourti* at PV (Pb5, Pb7 and Pb8), ST (Pb9 and Pb10), CHN (Pb12, Pb13) and KT (Pb15, Pb16 and Pb17) (Table 1 and Fig. 2).

Mismatch distribution and neutrality tests

To investigate the hypothesis of population expansion in *P. hypophthalmus* and *P. bocourti*, the distribution of pairwise differences from the segregating sites of the mtDNA haplotypes were computed (Fig. 3). A unimodal mismatch distribution was observed in both catfishes. The mismatch distribution, however, differed substantially between species, as the mean number of differ-

ences was 1.16 in *P. hypophthalmus* and 2.06 in *P. bocourti*. In *P. hypophthalmus* the observed mismatch distribution was significantly different from the expected distribution under the range expansion model, leading to reject the expansion hypothesis. For the mismatch distribution of *P. bocourti*, the sudden expansion hypothesis could not be rejected (Table 2).

Neutrality tests by Tajima's *D*- and Fu's F_S-tests showed that the negative values were significantly different from zero in *P. hypophthalmus*. In *P. bocourti*, Tajima' *D*-statistic was negative and significant from zero, while Fu's F_S-test resulted in a large negative F_S value that was highly significant (Table 2).

DISCUSSION

Historical evolution and demographic history

Along a large section, the Mekong flows downstream of the Khone Falls in the Lower Mekong basin through a very young channel. The river has had major course changes during and following the Pleistocene due to climatic (sea level change) and geologic (tectonic) processes, and only recently assumed its present configuration.[12] The Tonle Sap Lake was formed by the most recent subsidence event of the Cambodian platform just 5720 years ago.[12] This geologic history must have had an important effect on the intraspecific diversity of the aquatic fauna.

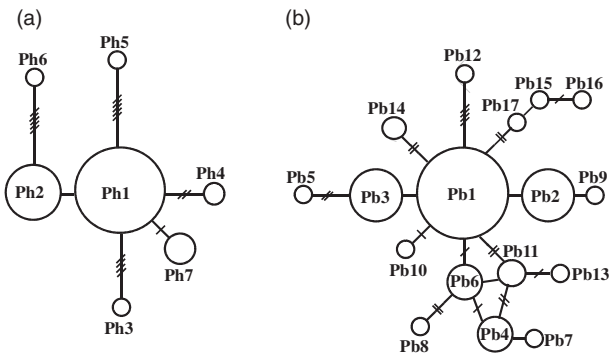

Fig. 2 Minimum spanning networks showing mutational state differences among: (a) *Pangasianodon hypophthalmus* and (b) *Pangasius bocourti* mtDNA haplotypes listed in Table 1 from inferred restriction site differences for 10 restriction enzymes. Dominant (abundant) haplotypes are indicated by larger circles. Slashes denote number of observed mutations.

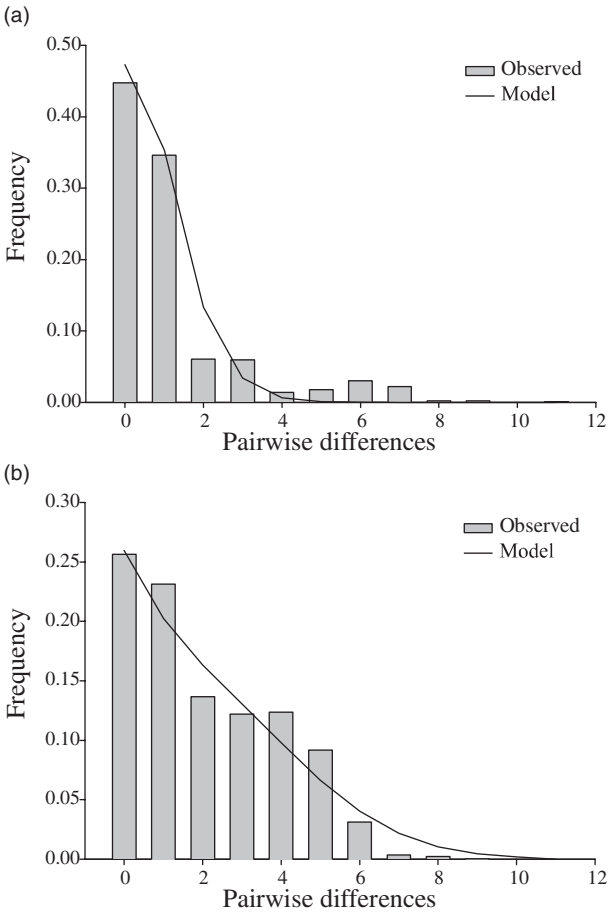

Fig. 3 Mismatch histograms of the number of pairwise differences in haplotype frequency for (a) *Pangasianodon hypophthalmus* and (b) *Pangasius bocourti*. The solid line represents the expected mismatch distribution under the sudden expansion model, and the bars represent the observed pairwise difference from segregating sites of fragment of the cytochrome *b* gene and control region (2100 bp).

Table 2 Results of population genetic tests on *Pangasianodon hypophthalmus* and *Pangasius bocourti*

Species	SSD	*P* (SSD sim. ≥ SSD obs.)	Tajima's *D* statistic	*P* (*D* sim. < *D* obs.)	Fu's F_S statistic	*P* (F_S sim. ≤ F_S obs.)
P. hypophthalmus	0.008	0.027	−1.752	0.016	−13.253	0.000
P. bocourti	0.003	0.867	−1.088	0.031	−27.369	0.000

SSD, Sum of Squared Deviation of the mismatch distribution; *P*, probability; sim., simulated; obs., observed.

Pangasianodon hypophthalmus and *Pangasius bocourti* were characterized by a star-like genealogy and the central haplotype was observed at each sampling location (Table 1). However, there were remarkable differences between both genealogies (Fig. 2). The haplotype network of the recently speciated *P. bocourti* is typical for high gene flow species with populations that have not been sundered by long-term historical biogeographic barriers,[2] whereas the rare divergent haplotypes observed in the more ancestral *P. hypophthalmus* could be the result of different historical processes. According to Grant and Bowen[39] and Avise,[2] this genetic profile can be explained by either: (i) secondary contact between formerly isolated populations or reticulation of isolated lineages associated with low effective population size; or (ii) a strong bottleneck in a formerly large, stable population together with high gene flow. The first scenario is supported by Dodson *et al*.[1] and McConnell,[11] who reported extensive admixture of intraspecific groups of freshwater fish. During the Pleistocene the river mouths of the major Indochinese rivers were interconnected because of low sea levels, whereas during high sea levels, genetic groups evolved allopatrically. However, *P. hypophthalmus* has only been reported from the Chao Phraya basin and it is unclear whether the species is present in neighboring basins. Since there is no evidence that these divergent genetic groups either still exist or have existed in the other basins, this scenario remains speculative. Further, a past subdivision is expected to increase the average number of pairwise differences relative to the total number of mutations, which would lead to a positive Tajima's *D*-statistic.[36] A significant negative value was observed for the latter statistic but the expansion model was rejected. Thus, the star-like genealogy with divergent haplotypes and missing intermediates in *P. hypophthalmus* is more likely to be the result of a bottleneck.[40,41] An excess of distant haplotypes might be the result of a bottleneck event with geographically structured subpopulations. However, the current data do not allow us to exclude a simple bottleneck event in which the contraction leads to a single small population.

The geographic distribution of the observed mitochondrial genomes for *P. bocourti* did not suggest any historical subdivision in the Cambodian Mekong basin. Unlike *P. hypophthalmus*, *P. bocourti* clearly showed signatures of a past expansion. Firstly, the genealogical network of *P. bocourti* had a star-like pattern of closely related haplotypes, which is consistent with a population expansion model.[7] Further, the observed unimodal mismatch distribution was well explained by a sudden expansion model and well supported by highly significant negative Tajima's *D*-[34–35] and Fu's F_S-statistics.[31] The more recently speciated *P. bocourti*[18,19] may have experienced population expansion during the Pleistocene (≤1.0 Mya). The growth of *P. bocourti* populations seems to be reflected in its higher mtDNA genetic diversity.[3]

If the genetic profile of *P. bocourti* and *P. hypophthalmus* is interpreted in a historical context, the pattern of *P. bocourti* may be the result of successful colonization of the Mekong basin after its speciation in the late Pleistocene.[18,19] In contrast, the bottleneck signature observed in *P. hypophthalmus* might be the result of dramatic changes in the configuration of the Mekong basin.[12] In the latter case it remains hard to explain why *P. bocourti* did not suffer from these geologic changes.

Intraspecific genetic diversity and population structure

Pangasianodon hypophthalmus and *P. bocourti* showed contrasting levels of genetic diversity, supporting the idea of a different evolutionary demographic history. The diversity values were higher than reported for most freshwater fish species (e.g. North American and European salmon,[42] European catfish,[43] African catfish,[44] northern South American Prochilodontid fishes,[45] and Bolivian Amazon catfishes[46]) with the exception of, amongst others, American shad *Alosa sapidissima*[47] and brown trout *Salmo trutta*.[3] The highest number of haplotypes for *P. hypophthalmus* (six) and *P. bocourti* (eight) was found in the Tonle Sap Lake (SR) and Tonle Sap River (CHN), possibly implying a communal feeding ground. However, this conclusion assumes there are distinct populations in different areas which mix during feeding migration; there is no such evidence in our data set. Overall F_{ST} values suggested little genetic differentiation in the samples of *P. hypophthalmus* and almost no differentiation in *P. bocourti*. Further, all pairwise F_{ST} values in both species were not statistically different, which suggests extensive migrations in the absence of contemporary natural and physical barriers among the four major river branches downstream of the Khone Falls. They reveal that *P. hypophthalmus* and *P. bocourti* each may represent a distinct population in the Lower Mekong River basin. This scenario parallels the evidence based on ecologic surveys along the Mekong River using indigenous fishers' knowledge.[16] However, evidence for unique haplotypes was observed in both catfish species, probably revealing population substructure. Such widely distributed popula-

tions are comparable to several other large river fish species in the Chinese Yangtze River[48] and in major South American rivers.[45,46,49] The mitochondrial data suggests for both species that contemporary migration (gene flow) is sufficiently high to preserve a very weak or absent population structure in the Cambodian Mekong River. This outcome might reflect the unusual life history of both migrants, whose larvae disperse downstream to the nursery grounds after spawning. Both catfish species might have varying degrees of philopatry because they are spatially and temporally relatively predictable in their spawning aggregations.[16] Thorrold *et al*.[50] suggested that even when a substantial proportion of individuals return to their home region, exchange might still be sufficient to observe a panmictic population at the mitochondrial DNA level.

Conservation and management implications

Unlike *P. hypophthalmus*, *P. bocourti* showed a high level of genetic diversity, which might be attributed to different intensities of fishing pressure and exploitation. All life stages of *P. hypophthalmus* are intensively harvested with legal (hooks and lines, trawls, seines, gill nets, set nets, and traps), and illegal and unsustainable (poisons, explosives, electro-shocking and barrages) fishing techniques.[51] Only juvenile *P. bocourti* are commercially collected with multiple hooks and lines in order to meet the demands of aquaculture,[51] while adult fish are rarely observed and not commercially caught because they have a biologically complicated life history (data not shown). Hence, both Mekong catfish species should receive special attention to maintain their genetic diversity for sustainable harvesting and use. Their migration routes between the spawning grounds in the north and feeding grounds in the south must be guaranteed. Dam projects and the concomitant hydrological manipulations built on the migration corridors are a particular threat.[13]

ACKNOWLEDGMENTS

Nam So acknowledges a doctoral scholarship from the Belgian Technical Cooperation (BTC). Research has been funded by the BTC, Katholieke Universiteit Leuven, and the MRC/DANIDA/DoF-FCFMC project of the Mekong River Commission and International Foundation for Sciences. Thanks to the Cambodian National and Provincial Departments of Fisheries for support, the field guides provided by S. Chan, K. Au and colleagues for fish samples from the Bassac River, and B. Hellemans for technical assistance. G. Mair and G. E. Maes provided constructive comments.

REFERENCES

1. Dodson JJ, Colombani F, Ng PKL. Phylogeographic structure in mitochondrial DNA of a South-East Asian freshwater fish, *Hemibagrus nemurus* (Siluroidei, Bagridae) and Pleistocene sea-level changes on the Sunda shelf. *Mol. Ecol.* 1995; **4**: 331–346.
2. Avise JC. *Phylogegraphy. The History and Formation of Species*, 2nd edn. Harvard University Press, Cambridge, Massachusetts. 2000.
3. Bernatchez L. The Evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* 2001; **55**: 351–379.
4. Volckaert FAM, Hänfling B, Hellemans B, Carvalho GR. Timing of the population dynamics of bullhead *Cottus gobio* (Teleostei: Cottidae) during the Pleistocene. *J. Evol. Biol.* 2002; **15**: 930–944.
5. Van Houdt JK, Hellemans B, Volckaert FAM. Phylogenetic relationships among Palearctic and Nearctic burbot (*Lota lota*): Pleistocene extinctions and recolonization. *Mol. Phylogenet Evol.* 2003; **29**: 599–612.
6. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 1989; **123**: 585–595.
7. Slatkin M, Hudson RR. Pairwise comparisons of mitochondrial DNA sequences in stable and exponential growing populations. *Genetics* 1991; **129**: 555–562.
8. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* 1992; **9**: 552–569.
9. Moritz C, Schneider CJ, Wake DB. Evolutionary relationships within the *Ensatina eschotzii* complex confirming the ring species interpretation. *Syst. Biol.* 1992; **41**: 273–291.
10. Brunsfeld SJ, Sullivan J, Soltis DE, Soltis P. Comparative phylogeography of northwestern North America: a synthesis. In: Silvertown J, Antonovics J (eds). *Integrating Ecology and Evolution in a Spatial Context*. Blackwell Science, Oxford. 2001; 319–339.
11. McConnell SKJ. Mapping aquatic faunal exchanges across the Sunda shelf, South-East Asia, using distributional and genetic data sets from the cyprinid fish *Barbodes gonionotus* (Bleeker, 1850). *J. Nat. Hist.* 2004; **38**: 651–670.
12. Rainboth WJ. *Fishes of the Cambodian Mekong*. Food and Agriculture Organization, Rome. 1996.
13. Roberts TR. Mekong mainstream hydropower dams: run-of-the-river or ruin-of-the-river? *Nat. Hist. Bull. Siam Soc.* 1995; **43**: 9–19.
14. Dudgeon D. The ecology of tropical Asian rivers and streams in relation to biodiversity conservation. *Ann. Rev. Ecol. Syst.* 2000; **31**: 239–263.
15. Roberts TR, Vidthayanon C. Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of 3 new species. *Proc. Acad. Natl. Sci. Phil.* 1991; **143**: 97–144.

16. Poulsen AT, Valbo-Jørgensen J. *Fish Migration and Spawning Habits in the Mekong Mainstream: a Survey Using Local Knowledge (Basin-Wide).* MRC Technical Report CD-ROM, Version 1. Mekong River Commission, Phnom Penh. 2001.
17. Hogan Z, Moyle PB, May B, Vander Zanden MJ, Baird IG. The imperiled giants of the Mekong River. *Am. Sci.* 2004; **92**: 228–237.
18. Pouyaud L, Teugels GG, Gustiano R, Legendre M. Contribution to the phylogeny of Pangasiid catfishes based on allozymes and mitochondrial DNA. *J. Fish Biol.* 2000; **56**: 1509–1538.
19. Gustiano R. Taxonomy and phylogeny of Pangasiidae catfishes from Asia (Ostariophysi, Siluriformes). PhD Thesis. Katholieke Universiteit Leuven, Leuven, Belgium. 2003.
20. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning. A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York. 1989.
21. Cronin MA, Spearman WJ, Wilmot RL, Patton JC, Bickham JW. Mitochondrial DNA variation in chinook (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. *Can. J. Fish. Aquat. Sci.* 1993; **50**: 708–715.
22. Bernatchez L, Danzmann RG. Congruence in control region sequence and restriction site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis*, Mitchill). *Mol. Biol. Evol.* 1993; **10**: 1002–1014.
23. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotypes frequencies in a diploid population. *Mol. Biol. Evol.* 1995; **12**: 912–927.
24. Nei M. *Molecular Evolutionary Genetics*. Columbia University Press, New York. 1987.
25. Weir BS, Cockerham CC. Estimating *F*-statistics for the analysis of population structure. *Evolution* 1984; **38**: 1358–1370.
26. Rohlf FJ. Algorithm 76. Hierarchical clustering using the minimum spanning tree. *Comp. J.* 1973; **16**: 93–95.
27. Schneider S, Kueffer JM, Roesseli D, Excoffier L. *Arlequin. Software for Population Genetic Data Analysis*, Version 2.0. Genetic and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva. 2000.
28. Harpending RC. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum. Biol.* 1994; **66**: 591–600.
29. Rogers AR. Genetic evidence for a Pleistocene population expansion. *Evolution* 1995; **49**: 608–615.
30. Tajima F. The amount of DNA polymorphism maintained in a finite population when the neutral mutation varies among sites. *Genetics* 1996; **143**: 1457–1465.
31. Fu Y-X. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 1997; **147**: 915–925.
32. Schnieder S, Excoffier L. Estimation demographic parameters from the distribution of pairwise genetic differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 1999; **152**: 1079–1089.
33. Tajima F. The effect of change in population size on DNA polymorphism. *Genetics* 1989; **123**: 597–601.
34. Bertorelle G, Slatkin M. The number of segregating sites in expanding human-populations, with implications for estimation of demographic parameters. *Mol. Biol. Evol.* 1995; **12**: 887–892.
35. Aris-Brosou S, Excoffier L. The impact of population expansion and mutation rate homogeneity on DNA polymorphism. *Mol. Biol. Evol.* 1996; **13**: 494–504.
36. Simonsen KL, Churchill GA, Aquadro CF. Properties of statistical tests of neutrality for DNA polymorphism data. *Genetics* 1995; **141**: 413–429.
37. Rice WR. Analyzing tables of statistical tests. *Evolution* 1989; **43**: 223–225.
38. Posada D, Crandall KA. Selecting the best-fit model of nucleotide substitution. *Syst. Biol.* 2001; **50**: 580–601.
39. Grant WS, Bowen BW. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *J. Hered.* 1998; **89**: 415–426.
40. Nordborg M, Innan H. Molecular population genetics. *Curr. Opin. Plant. Biol.* 2002; **5**: 69–73.
41. Hwang SY, Lin TP, Ma CS, Lin CL. Postglacial population growth of *Cunninghamia konishii* (Cupressaceae) inferred from phylogeographical and mismatch analysis of chloroplast DNA variation. *Mol. Ecol.* 2003; **12**: 2689–2695.
42. King TL, Spidle AP, Eackles MS, Lubinski BA, Schill WB. Mitochondrial DNA diversity in North American and European Atlantic salmon with emphasis on the Downeast Rivers of Maine. *J. Fish Biol.* 2000; **57**: 614–630.
43. Krieg F, Triantafyllidis A, Guyomard R. Mitochondrial DNA variation in European populations of *Silurus glanis*. *J. Fish Biol.* 2000; **56**: 713–724.
44. Giddelo CS, Arndt AD, Volckaert FAM. Impact of rifting and hydrography on the genetic structure of *Clarias gariepinus* in Eastern Africa. *J. Fish Biol.* 2002; **60**: 1252–1266.
45. Turner TF, McPhee MV, Campbell P, Winemiller KO. Phylogeography and intraspecific genetic variation of prochilodontid fishes endemic to rivers of Northern South America. *J. Fish Biol.* 2004; **64**: 186–201.
46. Coronel JS, Maes GE, Claus S, Van Damme PA, Volckaert FAM. Differential population history in the migratory catfishes *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum* (Pimelodidae) from the Bolivian Amazon assessed with nuclear and mitochondrial DNA markers. *J. Fish Biol.* 2004; **65**: 859–868.
47. Brown BL, Epifanio JM, Smouse PE, Kobak CJ. Temporal stability of mtDNA haplotype frequencies in American shad stocks: to pool or not to pool across years? *Can. J. Fish. Aquat. Sci.* 1996; **53**: 2274–2283.
48. Lu GQ, Li SF, Bernatchez L. Mitochondrial DNA diversity, population structure, and conservation genetics of four native carps within the Yangtze River, China. *Can. J. Fish. Aquat. Sci.* 1997; **54**: 47–58.
49. Sivasundar A, Bermingham E, Orti G. Population structure and biogeography of migratory freshwater fishes (Prochilodus: Characiformes) in major South American rivers. *Mol. Ecol.* 2001; **10**: 407–417.
50. Thorrold SR, Latkoczy C, Swart PK, Jones CM. Natal homing in a marine fish metapopulation. *Science* 2001; **291**: 297–299.
51. Van Zalinge NP, Lieng S, Ngor PB, Heng K, Valbo-Jørgensen J. *Status of the Mekong Pangasianodon Hypophthalmus Recourses, with Special Reference to the Stocks Shared Between Cambodia and Vietnam.* MRC Technical Paper No. 1. Mekong River Commission, Phnom Penh. 2002.