Research Article

Population Genetic Structure and Genetic Diversity in Twisted-Jaw Fish, *Belodontichthys truncatus* Kottelat & Ng, 1999 (Siluriformes: Siluridae), from Mekong Basin

Surapon Yodsiri,^{1,2} Komgrit Wongpakam,^{1,2} Adisak Ardarn,^{1,2}
Chadaporn Senakun,^{1,2} and Sutthira Khumkratok^{1,2}

¹Walai Rukhvej Botanical Research Institute, Mahasarakham University, Kantharawichai District, Maha Sarakham 44150, Thailand

²Biodiversity and Conservation Research Unit, Walai Rukhvej Botanical Research Institute, Mahasarakham University, Kantharawichai District, Maha Sarakham 44150, Thailand

Correspondence should be addressed to Komgrit Wongpakam; komgrit_w@yahoo.com

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The Mekong River and its tributaries possess the second highest diversity in fish species in the world. However, the fish biodiversity in this river is threatened by several human activities, such as hydropower plant construction. Understanding the genetic diversity and genetic structure of the species is important for natural resource management. *Belodontichthys truncatus* Kottelat & Ng is endemic to the Mekong River basin and is an important food source for people in this area. In this study, the genetic diversity, genetic structure, and demographic history of the twisted-jaw fish, *B. truncatus*, were investigated using mitochondrial *cytochrome b* gene sequences. A total of 124 fish specimens were collected from 10 locations in the Mekong and its tributaries. Relatively high genetic diversity was found in populations of *B. truncatus* compared to other catfish species in the Mekong River. The genetic structure analysis revealed that a population from the Chi River in Thailand was genetically significantly different from other populations, which is possibly due to the effect of genetic drift. Demographic history analysis indicated that *B. truncatus* has undergone recent demographic expansion dating back to the end of the Pleistocene glaciation.

1. Introduction

The Mekong is the second most biodiverse river for fish species. It has been estimated that more than 877 fish species can be recorded in the Mekong and its tributaries [1]. However, many species are under threat due to human-mediated environmental change, such as hydropower dam construction [1, 2].

The twisted-jaw catfish (*Belodontichthys truncatus* Kottelat & Ng) is endemic to the Mekong basin [3]. Two species of the genus *Belodontichthys* are found in the Mekong and its tributaries, including *B. dinema* Bleeker, 1851, and *B. truncatus*. The former species occur in central and southern Thailand, Malaysia, Sumatra, and Borneo, while the latter species is found in northeast Thailand, Lao PDR, Cambodia, and Vietnam [3]. *Belodontichthys truncatus* is a very important species for fisheries in Lao PDR and Cambodia where the

fish is caught and exported to Thailand [4]. However, there is no information on the genetic diversity and genetic structure of this important fish, despite being important for natural resource management [5].

In this study, we used the mitochondrial *cytochrome b* (*cyt b*) sequences to examine the genetic diversity, genetic structure, and demographic history of *B. truncatus* in the Mekong and two of its tributaries, the Chi and Mun Rivers in northeastern Thailand. Previous studies indicated that *cyt b* sequences can be successfully used to infer genetic structure and demographic history of freshwater fishes [6–8]. The information presented in this study will be useful for the management of *B. truncatus*. In addition, because this species is widely distributed in the Mekong and its tributaries, understanding its genetic structure and demographic history would shed some light on the effect of historical change

FIGURE 1: Sampling locations for twisted-jaw fish, *Belodontichthys truncatus*, are used in this study. Details of sampling sites are provided in Table 1.

(e.g., Pleistocene climatic and environmental change) on fish biodiversity.

2. Materials and Methods

2.1. Specimen Collection and Identification. A total of 124 fish specimens were collected from seven locations in Thailand and Cambodia (Table 1 and Figure 1). Among these locations, one was from the Chi River and one from the Mun River; both of these are in northeastern Thailand. Three sites were from the Mekong River along the Thailand-Lao PDR border, one from the Mekong River in Cambodia and one from Tonle Sap

Lake in Cambodia. Specimens were identified following the description of *Belodontichthys truncatus* by Kottelat & Ng [3].

2.2. DNA Extraction, Polymerase Chain Reaction, and Sequencing. Genomic DNA was extracted from the tissue using the Genomic DNA Extraction mini kit (RBC Bio-Science, Xindian City, Taiwan). A fragment of the *cytochrome b* (*cyt b*) gene was amplified using the primers Glu31 (5'GTG-ACTTGAAAAACCCACCGTT3') and Cat.Thr29 (5'ACC-TTCGATCTCCTGATTACAAGAC3') [9]. The amplification reaction with a total volume of 50 μ l contained

TABLE 1: Details of sampling locations for *Belodontichthys truncatus* used in this study and haplotype and nucleotide diversity.

Location (code)	River	Geographic region	N	Collection date	Haplotype diversity	Nucleotide diversity
Yasothon Province, Thailand (CHYT)	Chi	15°47'24.82''N 104°08'34.04''E	2	10/11/2014	0	0
Mueang District, Maha Sarakham Province (CHMK)	Chi	16°13'18.29''N 103°20'3.13''E	2	14/08/2014	0	0
At Samat District, Roi Et Province (CHRO1)	Chi	15°40'38.17''N 104°06'48.87''E	4	6/11/2014	0.8333 ± 0.2224	0.001958 ± 0.001641
Phanom Phrai District, Roi Et Province (CHRO2)	Chi	15°41'41.50''N 103°07'38.64''E	14	10/11/2014	0.5055 ± 0.1581	0.003413 ± 0.002077
Ubon Ratchathani Province, Thailand (MUUB)	Mun	15°14'29.71''N 104°57'20.64''E	10	11/11/2014	0.8667 ± 0.0850	0.00205 ± 0.00141
Nong Khai Province Thailand (MKNK)	Mekong	18°01'28.29''N 101°52'54.34''E	13	8/11/2014	0.9487 ± 0.0506	0.00405 ± 0.00242
Khong Chiam District, Ubon Ratchathani Province, Thailand (MKUB)	Mekong	15°23'26.82''N 105°29'39.14''E	13	17/10/2014	0.9359 ± 0.0507	0.00401 ± 0.00240
Khemarat District, Ubon Ratchathani Province, Thailand (MKKR)	Mekong	16°02'32.16''N 105°13'32.39''E	28	14/10/2014	0.8810 ± 0.0367	0.00271 ± 0.00165
Phnom Penh Province, Cambodia (MKCDPP)	Mekong	11°33'28.18''N 104°56'14.13''E	12	18/11/2014	0.8788 ± 0.0751	0.00242 ± 0.00158
Tonle Sap Lake, Pursat Province, Cambodia (MKCDPO)	Tonle Sap Lake	12°02'19.24''N 104°43'43.54''E	26	15/11/2014	0.8062 ± 0.0594	0.00365 ± 0.00212
<i>Total</i>			124		0.9765 ± 0.0051	0.003179 ± 0.001826

2 μ l MgCl₂ (50 mM), 5 μ l of 10x PCR buffer, 1.6 μ l of mixed dNTPs (10 μ M), 2 μ l of each primer (10 μ M), 0.4 μ l of Taq DNA polymerase (5 u/ μ l), and 2 μ l DNA template. The temperature profile was as follows: 94°C for 3 min, followed by 35 cycles of 94°C for 30 seconds, 48°C for 1 min, and 72°C for 1.30 min with a final extension at 72°C for 7 min [10]. The PCR products were checked by 1% agarose gel electrophoresis and purified using a High Yield Gel/PCR DNA fragment extraction kit (RBC BioScience, Taiwan). Sequencing was performed at the Macrogen DNA sequencing service (Seoul, Korea) using the same primers as in the PCR.

2.3. Data Analysis. A fragment of 1,024 bp of the *cyt b* gene was obtained from 124 specimens. Sequences were deposited in GenBank under the accession numbers KY607016–KY607139. Genealogical relationships between haplotypes were estimated using a median joining (MJ) network (Bandelt et al., 1999) calculated using Network v 4.6.1.0 (<http://www.fluxus-engineering.com>). Haplotype diversity and nucleotide diversity were calculated using Arlequin ver. 3.5 [11]. The population pairwise F_{ST} calculated in Arlequin was used to infer the genetic structure. The significance test statistic was obtained from 1,023 permutations. To avoid bias, due to a small sample size, populations with less than five specimens were omitted from the genetic structure analysis. A Mantel test [12] was used to determine the relationship between the genetic distance (F_{ST} from Arlequin) and the geographical distance (km) for an isolation-by-distance (IBD) model. The Mantel test was implemented in IBD ver. 1.52 [13] using 1,000 randomizations. The mismatch distribution was used to test the signature of the population expansion. Populations that have undergone a recent past demographic expansion show a unimodal mismatch distribution [14]. The sum-of-squares deviation and Harpending's raggedness index [15] were used to test the deviation from the sudden expansion model. A mismatch distribution was estimated using Arlequin. Fu's F_S [16] and Tajima's D [17] statistical tests were used to test the population equilibrium. A large negative value from these tests was expected for the demographic expansion.

3. Results

3.1. Genetic Diversity. A total of 124 sequences from seven sampling locations were obtained in this study, and 40 haplotypes were identified. Haplotype diversity in each location ranged between 0 in two populations (CHYT and CHMK) of the Chi River in northeastern Thailand and 0.9487 in Nong Khai Province (MKNK) for the Mekong River with an overall average of 0.9765 (Table 1). The nucleotide diversity in each population ranged from 0 in two populations (CHYT and CHMK) of the Chi River to 0.0041 in Nong Khai Province (MKNK) for the Mekong River with an overall average of 0.0032 (Table 1).

3.2. Mitochondrial DNA Genealogy. The median joining network (Figure 2) revealed no major phylogeographic breaks among the 124 sequences included in the analysis. There is no

FIGURE 2: Median joining (MJ) network of 124 mitochondrial cytochrome b (*cyt b*) sequences of *Belodontichthys truncatus*. Each haplotype is represented by a circle and sizes of circles are relative to number of individuals sharing a specific haplotype. Haplotypes are labelled according to the river.

FIGURE 3: Mismatch distribution of 124 mitochondrial cytochrome b (*cyt b*) sequences of *Belodontichthys truncatus* representing observed and expected pairwise differences under sudden population expansion model. Mismatch distribution for *B. truncatus* is consistent with predictions of sudden population expansion model (SSD = 0.0047, $P = 0.4750$; Harpending's raggedness index = 0.0349, $P = 0.5270$).

evidence of geographic association between the haplotypes. The core haplotype that has the highest frequency is shared by 35 specimens from all three major rivers (Mekong, Chi and Mun) of the Mekong Basin. Overall, the haplotype is a star-like shape, which is the characteristic of a recent expansion in the population.

3.3. Genetic Structure. The population pairwise F_{ST} revealed an overall low level of genetic structuring between the populations of *Belodontichthys truncatus*. No significant F_{ST} values were observed except for comparisons between a population (CHRO2) from the Chi River and the other populations, where most were significantly different (Table 2). A Mantel test revealed no significant relationships between the genetic and geographic distances ($r = -0.0528$, $P = 0.3590$).

3.4. Demographic History. A mismatch distribution analysis revealed a unimodal mismatch graph (Figure 3), which is a characteristic of a recently expanding population that is

TABLE 2: Pairwise F_{ST} (below diagonal) and associated P value (above diagonal) between populations of *Belodontichthys truncatus* collected during August–November 2014 in Thailand and Cambodia. Population codes are according to Table 1.

	CHRO2	MKKR	MKNK	MKUB	MKCDPO	MKCDPP	MUUB
CHRO2		<0.0001	<0.0001	0.0090	<0.0001	0.0090	0.0631
MKKR	0.1107		0.0450	0.1982	0.4594	0.5405	0.0450
MKNK	0.0970	0.0605		0.1441	0.1892	0.0631	0.3063
MKUB	0.0517	0.0148	0.0128		0.5676	0.6036	0.2883
MKCDPO	0.0963	−0.0029	0.0162	−0.0065		0.5946	0.1531
MKCDPP	0.0577	−0.0085	0.0435	−0.0106	−0.0079		0.0631
MUUB	0.0397	0.0756	0.0108	0.0098	0.0267	0.0618	

Bold characters indicate statistically significant differences at P values adjusted by Bonferroni's correction.

consistent with the star-like shape of the mtDNA genealogy. Harpending's raggedness index (0.0349, $P = 0.5270$) and the sum-of-squares deviation (SSD = 0.0047, $P = 0.4750$) were not significantly different from the simulated data under the sudden population expansion model. Population expansion was also supported by highly significant negative values for both Tajima's D (−2.5364, $P < 0.001$) and Fu's F_s (−26.2649, $P < 0.001$). The expansion time, estimated based on the fish *cyt b* evolutionary rate of 1% Myr [18] and assuming a generation time of two years for *B. truncatus*, was 20,458 years ago.

4. Discussion

The levels of genetic diversity observed in *B. truncatus* ($h = 0.9765$, $\pi = 0.003179$) based on the *cyt b* mtDNA sequences are similar to other fish species of the family Siluridae. For example, butter catfish, *Ompok bimaculatus*, in India ($h = 0.8270$, $\pi = 0.00391$) [19] and *Trichomycterus areolatus* from Chile [8]. However, the genetic diversity found in *B. truncatus* is higher than other catfish species from the Mekong River [20].

Mismatch distribution analysis indicated a recent demographic expansion in *B. truncatus* that dated back to 20,458 years ago, which is at the end of the last glaciation. Previous studies found a significant influence from the Pleistocene glaciation on genetic diversity, genetic structure, and demographic history in many Southeast Asian floras and faunas [e.g., [21–23]]. During the Pleistocene glaciations, climatic and environmental conditions in Southeast Asia were cooler and drier [24]. Evidence indicated that the water level and water flow in large rivers in Southeast Asia, including the Mekong, reduced during the Pleistocene glaciation [25]. After the climatic conditions recovered at the end of the Pleistocene, the water level and water flow increased, which could trigger the population expansion in *B. truncatus*.

The population genetic structure analysis based on the F_{ST} values revealed that a population from the Chi River was genetically significantly different from almost all other populations from the Mekong but not from the Mun River. Mitochondrial genealogy indicated no evidence of divergent lineages for *B. truncatus* from different rivers, thus indicating that the genetic differentiation between the Chi and Mekong River populations is likely to be a recent event. The sharing

of the mtDNA haplotypes between fish from these rivers suggests that there is some gene flow between these populations. There are two possible explanations for the significant genetic differentiations between the populations from the Chi River and the Mekong River. The Chi River is part of the Mekong, and it originates from a mountainous area in the upper part of northeastern Thailand. The Chi River joins the Mun River in Ubon Ratchathani Province and then flows into the Mekong. Near the location (approximately 5.5 km) of the Mun and Mekong confluence, there is a dam (Pak Mun dam) that was constructed in 1994. This dam could be a barrier to gene flow between *B. truncatus* populations in the Chi and Mun Rivers and the Mekong. The effects of dams on gene flow have been investigated in other fish species [26–28]. However, given that this dam is only 23 years old, it is unlikely to have had an impact on the genetic structure. The more likely explanation for the genetic differentiation of the Chi River populations from the Mekong River is the effect of genetic drift. It has been suggested that the random sampling of the alleles from the source population of the colonizer could lead to the genetic differentiation of the population by the effect of genetic drift [29]. Although populations from the Chi River possess considerable genetic diversity, the relatively low haplotype diversity in this population (0.5055), compared to the others that contributed to the significant pairwise F_{ST} values, supports genetic drift playing a role in the genetic differentiation.

5. Conclusion

We found that the genetic diversity of *B. truncatus* is considerable compared to other fishes in the Mekong. This implies a large effective population size for this species. The results showed significant genetic differentiation for the population from a Mekong tributary, the Chi River, in Thailand that was genetically significantly different from the other populations due to the effect of genetic drift. We also found that historical environmental change during the Pleistocene has had an effect on the demographic history of this species. Given the recent rapid changes in the Mekong and its tributaries, particularly for the hydropower dam construction, which is predicted to have an effect on the fish productivity and biodiversity [1], further studies should examine the effect of this on genetic structure and diversity on the fish species.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

- [1] G. Ziv, E. Baran, S. Nam, I. Rodríguez-Iturbe, and S. A. Levin, "Trading-off fish biodiversity, food security, and hydropower in the Mekong River Basin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 15, pp. 5609–5614, 2012.
- [2] D. Dudgeon, "Large-scale hydrological changes in tropical Asia: Prospects for riverine biodiversity," *BioScience*, vol. 50, no. 9, pp. 793–806, 2000.
- [3] M. Kottelat and H. H. Ng, "Belodontichthys truncatus, a new species of silurid catfish from Indochina (Teleostei: Siluridae)," *Ichthyological Exploration of Freshwaters*, vol. 10, pp. 387–391, 1999.
- [4] A. Termvidchakorn and K. G. Hortle, "A guide to larvae and juveniles of some common fish species from the Mekong River Basin," MRC Technical Paper 38, Mekong River Commission, Phnom Penh, Cambodia, 2013.
- [5] R. DeSalle and G. Amato, "The expansion of conservation genetics," *Nature Reviews Genetics*, vol. 5, no. 9, pp. 702–712, 2004.
- [6] L. Yang and S. He, "Phylogeography of the freshwater catfish *Hemibagrus guttatus* (Siluriformes, Bagridae): implications for South China biogeography and influence of sea-level changes," *Molecular Phylogenetics and Evolution*, vol. 49, no. 1, pp. 393–398, 2008.
- [7] M. Habib, W. S. Lakra, V. Mohindra et al., "Evaluation of cytochrome b mtDNA sequences in genetic diversity studies of *Channa marulius* (Channidae: Perciformes)," *Molecular Biology Reports*, vol. 38, no. 2, pp. 841–846, 2011.
- [8] E. G. Gonzalez, C. Pedraza-Lara, and I. Doadrio, "Genetic diversity and population history of the endangered killifish *Aphanius baeticus*," *Journal of Heredity*, vol. 105, no. 5, pp. 597–610, 2014.
- [9] P. J. Unmack, A. P. Bennin, E. M. Habit, P. F. Victoriano, and J. B. Johnson, "Impact of ocean barriers, topography, and glaciation on the phylogeography of the catfish *Trichomycterus areolatus* (Teleostei: Trichomycteridae) in Chile," *Biological Journal of the Linnean Society*, vol. 97, no. 4, pp. 876–892, 2009.
- [10] P. J. Unmack, J. P. Barriga, M. A. Battini, E. M. Habit, and J. B. Johnson, "Phylogeography of the catfish *Hatcheria macraei* reveals a negligible role of drainage divides in structuring populations," *Molecular Ecology*, vol. 21, no. 4, pp. 942–959, 2012.
- [11] L. Excoffier and H. E. L. Lischer, "Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows," *Molecular Ecology Resources*, vol. 10, no. 3, pp. 564–567, 2010.
- [12] N. Mantel, "The detection of disease clustering and a generalized regression approach," *Cancer Research*, vol. 27, no. 2, pp. 209–220, 1967.
- [13] A. J. Bohonak, "IBD (isolation by distance): A program for analyses of isolation by distance," *Journal of Heredity*, vol. 93, no. 2, pp. 153–154, 2002.
- [14] A. R. Rogers and H. Harpending, "Population growth makes waves in the distribution of pairwise genetic differences," *Molecular Biology and Evolution*, vol. 9, no. 3, pp. 552–569, 1992.
- [15] H. C. Harpending, "Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution," *Human Biology*, vol. 66, no. 4, pp. 591–600, 1994.
- [16] Y.-X. Fu, "Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection," *Genetics*, vol. 147, no. 2, pp. 915–925, 1997.
- [17] F. Tajima, "Statistical method for testing the neutral mutation hypothesis by DNA polymorphism," *Genetics*, vol. 123, no. 3, pp. 585–595, 1989.
- [18] E. Bermingham, S. McCafferty, and A. Martin, "Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus," in *Molecular Systematics of Fishes*, pp. 113–126, Academic Press, New York, NY, USA, 1997.
- [19] R. Kumar, B. K. Pandey, U. K. Sarkar et al., "Population genetic structure and geographic differentiation in butter catfish," *Mitochondrial DNA Part A*, vol. 28, no. 3, pp. 442–450, 2017.
- [20] U. Na-Nakorn, S. Sukmanomon, M. Nakajima et al., "MtDNA diversity of the critically endangered Mekong giant catfish (*Pangasianodon gigas* Chevey, 1913) and closely related species: Implications for conservation," *Animal Conservation*, vol. 9, no. 4, pp. 483–494, 2006.
- [21] C. H. Cannon and P. S. Manos, "Phylogeography of the Southeast Asian stone oaks (*Lithocarpus*)," *Journal of Biogeography*, vol. 30, no. 2, pp. 211–226, 2003.
- [22] P. Pramual, C. Kuvangkadilok, V. Baimai, and C. Walton, "Phylogeography of the black fly *Simulium tani* (Diptera: Simuliidae) from Thailand as inferred from mtDNA sequences," *Molecular Ecology*, vol. 14, no. 13, pp. 3989–4001, 2005.
- [23] K. Morgan, Y.-M. Linton, P. Somboon et al., "Inter-specific gene flow dynamics during the Pleistocene-dated speciation of forest-dependent mosquitoes in Southeast Asia," *Molecular Ecology*, vol. 19, no. 11, pp. 2269–2285, 2010.
- [24] D. Penny, "A 40,000 year palynological record from north-east Thailand; implications for biogeography and palaeo-environmental reconstruction," *Palaeogeography, Palaeoclimatology, Palaeoecology*, vol. 171, no. 3–4, pp. 97–128, 2001.
- [25] V. S. Kale, A. Gupta, and A. K. Singhvi, "Late Pleistocene Holocene palaeohydrology of monsoon Asia," in *Palaeohydrology: Understanding Global Change*, pp. 213–232, John Wiley and Sons, New York, NY, USA, 2003.
- [26] H. AnvariFar, H. Farahmand, D. M. Silva, R. P. Bastos, A. Khyabani, and H. AnvariFar, "Fourteen years after the Shahid-Rajaei dam construction: An evaluation of morphometric and genetic differentiation between isolated up- and downstream populations of *Capoeta capoeta gracilis* (Pisces: Cyprinidae) in the Tajan River of Iran," *Genetics and Molecular Research*, vol. 12, no. 3, pp. 3465–3478, 2013.
- [27] M. M. Hansen, M. T. Limborg, A.-L. Ferchaud, and J.-M. Pujolar, "The effects of medieval dams on genetic divergence and demographic history in brown trout populations," *BMC Evolutionary Biology*, vol. 14, no. 1, article 122, 2014.
- [28] L. Zhao, E. L. Chenoweth, J. Liu, and Q. Liu, "Effects of dam structures on genetic diversity of freshwater fish *Sinibrama macrops* in Min River, China," *Biochemical Systematics and Ecology*, vol. 68, pp. 216–222, 2016.

- [29] L. Excoffier and N. Ray, "Surfing during population expansions promotes genetic revolutions and structuration," *Trends in Ecology and Evolution*, vol. 23, no. 7, pp. 347–351, 2008.