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Short Communication

Morphological differences in *Neolissochilus soroides* (Pisces: Cyprinidae) populations in national parks in eastern Thailand

Jenjit Khudamrongsawat[1*], Jes Kettratad[2], Natchar Sukkhee[2], Ponchai Wanusrut[3], Pituk Intasorn[4], Nipon Pinyo[5], and Sornpasok Tapcheewin[6]

[1] *Department of Biology, Faculty of Science, Mahidol University, Ratchathewi, Bangkok, 10400 Thailand*

[2] *Department of Marine Science, Faculty of Science, Chulalongkorn University, Pathum Wan, Bangkok, 10330 Thailand*

[3] *Khao Chamao-Khao Wong National Park, Klang, Rayong, 21110 Thailand*

[4] *Namtok Phlio National Park, Lamsing, Chanthaburi, 22190 Thailand*

[5] *Khao Khitchakut National Park, Khao Khitchakut, Chanthaburi, 22210 Thailand*

[6] *Namtok Khlong Kaeo National Park, Bo Rai, Trat, 23140 Thailand*

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Abstract

Phlio waterfall (PL) in Namtok Phlio National Park in eastern Thailand is well known for the abundance of a stream fish, *Neolissochilus soroides* (Cyprinidae) for many decades. It had been observed that morphology of *N. soroides* population at PL appeared to be much thinner than those in nearby populations. To clarify this issue, we sampled individuals of *N. soroides* from four populations associated with four headwater streams and examined their body profiles using 11 morphological characteristics. Results revealed that some individuals of PL population were significantly thinner than others ($p < 0.005$). This difference was not influenced by the environment nor genetics, and may be related to health of the fish, which will require further investigation.

Keywords: body landmark, mahseer, morphology, Namtok Phlio, *Neolissochilus soroides*

1. Introduction

Neolissochilus soroides (Cyprinidae) is a freshwater mahseer commonly found in clear headwater streams located in protected areas in eastern Thailand. This species had served as popular tourist attraction for decades, especially at Phlio waterfall (PL) in Namtok Phlio National Park in Chantaburi Province, Thailand. Here, tourists could swim, watch, and even feed the fish as seen in the number of local stores at the front gate of the national park selling vegetables, fish food, and bread until the Department of National Parks, Wildlife, and Plant Conservation (DNP) announcement in 2015 that forbade food feeding to the fish (Royal Thai Government Gazette No. 132/ section 103/ page 18). Later, it was noticed that some individuals of *N. soroides* at Phlio waterfall showed shallow body profile or appeared to be thin compared to those inhabiting other waterfalls in nearby national parks of the same region. Since morphology of the fish may be influenced

*Corresponding author
Email address: khudamrong@gmail.com

by either environmental conditions (Cheng *et al.*, 2018) or genetics (Park, Powell, Gillings, Gaston, & Williamson, 2020) or both (Rohlf, 1990), it was possible that the observation of difference in body shape of *N. soroides* in PL actually represented intraspecific variation.

We proposed to examine variations in body shape of *N. soroides* populations in eastern Thailand and to identify characters that distinguished individuals at PL using important landmarks on the body. Morphological variation of a single species reflects their ecological adaptation or genetic differences of populations and could be quantified or described using body landmark data and many multivariate models (Cadrin, 2000; Fitzgerald, Nanson, Todd, & Davis, 2002; Strauss & Bookstein, 1982; To & Cl, 2015). Such analyses tremendously benefit fisheries management and conservation.

2. Materials and Methods

2.1 Sampling sites

We conducted sampling in northwestern end of the Cardamom Mountains during summer of 2018 and 2019. We collected samples from Khao Chamao waterfall (CM, 12.9129 N, 101.7246 E) (N = 35), Klong Kaeo waterfall (KK, 12.6183 N, 102.5771 E) (N = 52), Krating waterfall (KT, 12.8392 N, 102.1208 E) (N = 39), and Phlio waterfall (PL, 12.5295 N, 102.1842 E) (N = 29). All sampling areas are national parks under the supervision of Department of National Park, Wildlife, and Plant Conservation (DNP) and serve for ecotourism and education. Fish were collected using dipnets and cast nets. Then, samples were euthanized on ice (Wilson, Bunte, & Carty, 2009), preserved in 10% formalin, and stored in 70% ethanol. Voucher specimens were kept at Department of Biology, Faculty of Science, Mahidol University, in Bangkok.

Water quality data from the sampling sites were obtained from national park reports in 2018. Evaluation of surface water quality was based on National Environmental Quality Promotion and Conservation Act (2535 B.E.), section 32.

2.2 Morphological measurement and statistical analyses

Samples were initially examined based on the description in Rainboth (1996) and all were identified as *N. soroides* based on morphological, meristic, and genetic examinations (Khudamrongsawat *et al*., 2021). Assessment of body shape variations was determined using discrete landmarks on fish body, especially the insertion point of fins, (Strauss & Bookstein, 1982; Bookstein, 1989). Eleven traits (Table 1) were selected and measured to the nearest 0.1 mm using digital calipers. Sex of the samples was pooled as this factor could not be differentiated based on morphological examination.

Because the number of fish samples was limited, exclusion of too large or too small individuals may decrease sample size and statistical power. Therefore, size correction was performed following the method suggested by Pinheiro, Teixeira, Rego, Marques, & Cabral, (2005). Data were preliminary screened for normality and $\log_e$-transformed.

Table 1. Lists of morphological traits used in the analysis.

Traits	Characters of traits
ODOP1	origin of dorsal fin to origin of pectoral fin
ODOP2	origin of dorsal fin to origin of pelvic fin
LOP2	snout to origin of pelvic fin
LOD	snout to origin of dorsal fin
LODOA	origin of dorsal fin to origin of anal fin
LODIA	origin of D-fin to posterior end of A-fin
CCD	caudal depth
CCOD	caudal to origin of dorsal fin
CCOP2	caudal to origin of pelvic fin
CCOA	caudal to origin of anal fin
LOP1-OP2	length of origin of pectoral fin to origin of pelvic fin

Bivariate scatter plots of characters against fish standard length (SL) were constructed. Clear outliers were removed. MANOVA was performed in R v4.0.4 (R Core Team 2021) and RStudio v1.3 (RStudio Team 2020) to check if any characters from the samples showed significant differences. Then, linear discriminant function analysis (LD) was performed to distinguish the populations based on body shapes using R package MASS (Venables & Ripley, 2002) and illustration using packages ggplot2 (Wickham, 2016) and dplyr.

We further calculated morphological distance matrix and compared this information with genetic distance based on 8 polymorphic microsatelliate DNA loci (Khudamrongsawat *et al*., 2021). Pairwise genetic distance of four *N. soroides* populations was tested based on random 1000 permutations using Arlequin version 3.5 (Excoffier, Lischer, & Schneider, 2010). Then, Euclidean distances of morphological variations of each population were correlated with their population genetic distances.

3. Results

Body shape of the samples from four populations were generally similar. The cross-validation test revealed that the LD model on average correctly placed samples in their true origin for only 39%. Many samples were mis-classified, but samples from CM, KK, and KT were less likely to be predicted as PL (Table 2). MANOVA indicated significant difference of the distance from the origin of dorsal fin to origin of pelvic fin (ODOP2) relative to SL among these ($p < 0.005$). Samples from PL had smaller ODOP2 relative to SL than other populations ($p < 0.005$). Correlation between SL and ODOP2 of populations from CM, KK, and KT showed similar slope, while PL population exhibited less steep slope also confirming small ODOP2 relative to SL in this population (Figure 1). The scatterplot of two LD functions showed great overlap among samples from CM, KK, KT, and some of PL (Figure 2). Nevertheless, several samples from PL were distinct from others and showed high variations of measurements. The first two discriminant functions explained high percentage of the overall variation in morphometric analysis (77.90% and 12.89%, respectively). The traits that contributed highest weight separating the groups included LOP2 (distance from snout to origin of pelvic fin), CCOD (distance from caudal peduncle to origin of dorsal fin), ODOP2, and LODIA (the distance from the origin of dorsal fin to the posterior end of anal fin). Water data revealed good

Table 2. Probability of assigning mahseers into the correct capture locations based on LD model.

Probability of correct assignment of samples into the group	Places of capture			
	CM	KK	KT	PL
Predicted CM	40%	13%	8%	11%
Predicted KK	43%	67%	54%	39%
Predicted KT	14%	13%	36%	0%
Predicted PL	3%	6%	3%	50%

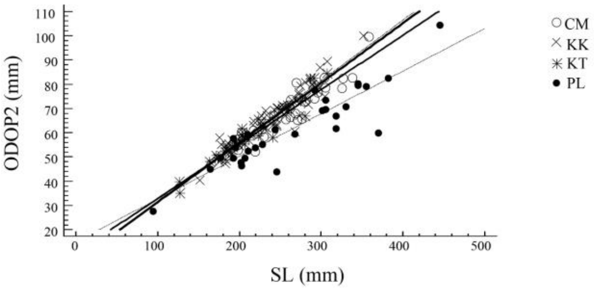

Figure 1. Length from origin of the dorsal fin to the origin of pelvic fin of *N. soroides* from all study locations, black filled circles = samples from PL

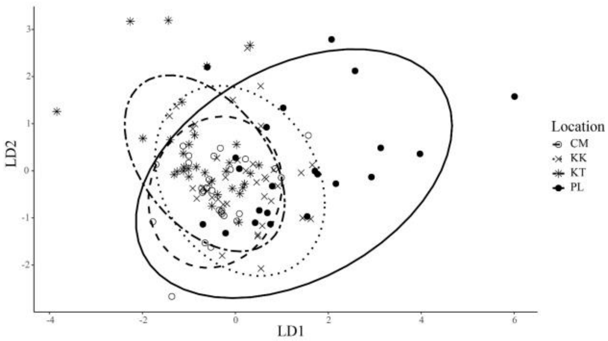

Figure 2. Discriminant analysis of morphological characteristics of *N. soroides* from all study locations, black circles = samples from PL, solid line = data ellipse based on t-distribution at level = 0.95 of PL population.

surface water quality for recreation with clear water, no odor, pH approximately 7.0, DO greater than 6.0 mg/L, and total fecal coliform bacteria less than 2.2 MPN/100 cm^3 (Namtok Phlio National Park, Khao Kitchakood National Park reports). Morphological and genetic distances of studied populations were not related to each other (R^2 = 0.56, p = 0.087). Genetically distant populations such as CM and KK showed similar morphological characters than a closer genetic distant observed in CM and PL (Table 3).

4. Discussion

This study demonstrated morphological differences between *N. soroides* at PL and the comparable populations in nearby waterfalls. Overall morphology of *N. soroides* samples was similar across the region as seen in high overlapping in morphological variations and high mis-classification.

Table 3. Comparison of morphological and genetic distances of *N. soroides* from 4 locations, above diagonal = pairwise genetic distance, below diagonal = morphological distance

Morphological distance	Genetic distance			
	CM	KK	KT	PL
CM	-	0.3864	0.2648	0.1906
KK	0.0042	-	0.3811	0.2873
KT	0.0025	0.0043	-	0.1200
PL	0.0807	0.0800	0.0794	-

However, several samples from PL appeared to be distinct from those in other locations in that they were thinner as indicated by significantly small ratio of ODOP2 and SL compared to others. The discriminant analysis focused on separation of individuals into groups, and so variables that provided high weight were not necessarily related with the group separation as some of them showed high variations within and among groups. The distinct ratio of ODOP2 and SL observed in some PL samples presented an interesting index in addition to other morphometric measurements commonly used as body condition indices to indicate health and perhaps fitness of living organisms (Fitzgerald *et al*., 2002; Labocha, Schutz & Hayes, 2014).

Even though environments and genetics are usually suggested for morphological variations (Rohlf, 1990; Smith & Skúlason, 1996), it may not be the case in this study. The environmental conditions in eastern Thailand are similar across the region as they are located in close proximity with similar elevation and climate. Their habitats are headwater streams with forested covered in protected areas regularly monitored by DNP staff. However, the evaluation of water quality was performed by national park staff, which may not be standardized across all national parks. Intensive and standardized monitoring is recommended for more precise evaluation. In general, pollutions were well controlled and absent from all studied sites.

No correlation between morphological and genetic distances was observed suggesting little or none genetic influence on morphological variations. The genetically distant KK population was morphologically similar to other populations including some individuals from PL. Therefore, genetic differentiation among four populations reflected intraspecific variation of a species and was unlikely a factor affecting morphological distinction found in some samples from PL.

Although factors that were responsible for such variation could not be determined, it was obvious that body shape of several individuals from PL was different, more specifically much thinner than other populations. PL was famous for mahseer abundance and used to be most favorite place for tourists to interact with the fish by feeding them with vegetables and human food. The DNP's decree in 2015 reduced amount of food available for fish at PL, where high density of fish was observed, and may have an impact on the fish. Whether limited amount of food could affect body shape of the fish at PL remains to be investigated. This study provided the idea of morphological variations of *N. soroides* populations and identified distinct body shape of PL population. The fact that fish at PL had thin body shape or shallow body profile may imply their health condition (Fitzgerald *et al*., 2002; Jakob, Marshall & Uetz, 1996), which will require further investigation.

5. Conclusions

Morphological examination of four populations of *N. soroides* revealed thinness of body shape of several individuals from PL populations compared to others. However, causes of such differences could not be identified. We recognized that environmental conditions were not different among four sampling habitats, and variations of genetic characteristics of the studied samples likely presented variations within a species. Therefore, these factors unlikely affected morphological differences among the samples. Whether thin body shape could be associated with health condition remains to be investigated.

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