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Morphological Comparative of *Osteochilus spilurus* (Cyprinidae) from Three Sundaland Island in Indonesia Using Geometric Morphometric

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ABSTRACT

Morphological comparisons of freshwater fish from the geographical disconnection region can serve more effectively using geometric morphometric. One of the least affected species with a wide distribution on the Sundaland river is *Osteochilus spilurus* (Bleeker, 1851). A limited human-caused distribution was created by the lack of attention to this species and provided the potential for accurate morphological variations. Body shape and morphometric analysis were done using a photograph of a live or fresh fish sample from four rivers in Sumatera and Bangka – Belitung island. Body shape pattern constructs by SAGE 1.04 for landmark-based measurements, TPSdig 2.1 was used, and comparative analysis using Kruskal-Wallis. Morphometric and body shape differences happen on each island. Morphological plasticity as an outcome of adaptation reaction to water streams and predators is thought to contribute to this distinction. Fish harvesting and ancient geographical isolation possibly causing genetic variation but required further.

INTRODUCTION

Osteochilus spilurus (Bleeker, 1851) as the accepted name has several synonyms: *Dangila spilurus* (Bleeker, 1851), *Rohita oligolepis* (Bleeker, 1853), *Osteochilus oligolepis* (Bleeker, 1853), *Osteochilus oligolepsis* (Bleeker, 1853), and *Osteochilus spilurus* (Bleeker, 1851). They have a body characteristic of the silvery color, several small pores at the cone-snout, the dorsal height gradually increases, forged shape caudal fin, a pair of barbels above and below the mouth, and a large black dot at the caudal peduncle. The lateral line consists of 28-30 stripped scales with 4½ scale above and below it. Dorsal fins formulation is D.12 -13 and A.6 -7 for anal one (Kottelat *et al.*, 1993).

These species' distribution is widely on a river in Sumatera, Bangka Belitung Archipelago, Malay Peninsular, dan Kalimantan (Roberts, 1989; Hui and Kottelat, 2009; Esa *et al.*, 2012; Fahmi *et al.*, 2015; Kurniawan *et al.*, 2020). They do not have a common name and trade name but have various Indonesian names such as *Cempedik, Kepaet, Bantak Batu, Aro, Buruk Perut,* and *Seluang*. Each region has its naming, with some of them intersecting in other fish species. There is no utilization report of these species outside Belitung Island. At least its utilization caused low threats to this species, so that IUCN (International Union for Conservation of Nature and Natural Resources) was assessed as Least Concern species (Huckstorf, 2012).

Lack of attention to this species can make the minimum probably human-caused distribution. It allows us to study the distribution and morphological variations of fish that have been connected thousands of years ago. Morphological comparisons can serve more accurately differences due to geographical disconnection. A comparative study can be done on body shape and morphometric size. Analysis and interpretation of body shape can be more effective using geometric morphometric. However, the traditional method can prove the differences in form statically (Parsons et al., 2003). For this reason, we use geometric morphometrics to analyze body shapes and measurements of *O. spilurus* from various geographic areas. The results can be information about species, a reference for cultivation development and conservation.

MATERIALS AND METHODS

Sampling and Sample Processing

O. spilurus were collected from field sampling on four locations: Lenggang River at Belitung Island (-2.919301 S, 108.109824 E) in May 2019, Lebak River at Bangka Island (-2.072623 S, 105.870000 E) in March 2019, Lelabi River at Bangka Island (-1.8454722 S, 105.7493332 E) at March 2019, and Way Merah River at North Lampung, Sumatera Island (-4.7309944 S, 104.841025 E) at February 2020 (Figure 1). Lebak River and Lelabi River at Bangka Island are not connected and have an estuary to the different sea.

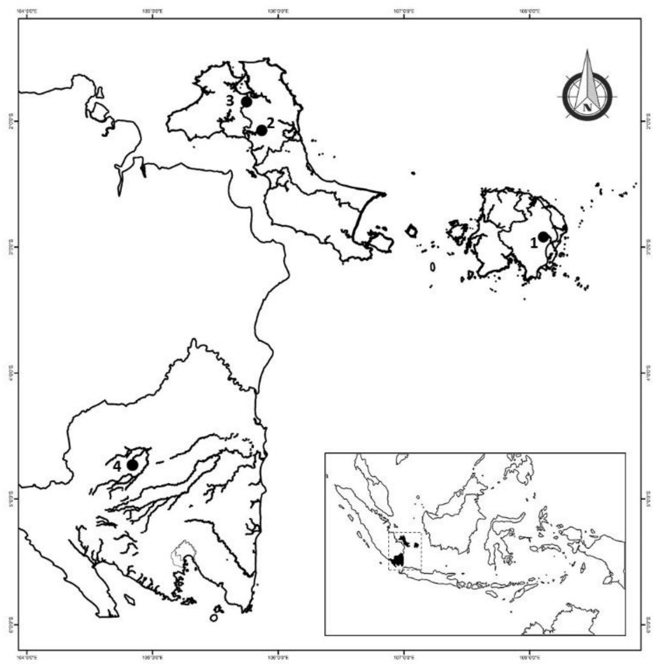

Figure 1. Sampling location of *O. spilurus.*
1). Lenggang river, Belitung. 2). Lebak river, Bangka. 3). Lelabi river, Bangka, 4). Way Merah river, Lampung.

Fish samples were obtained from fishers according to the fishing gear they used. Samples from the Way Merah River, Lampung were caught using a lift net, while other sampling locations used a trapped net. All samples verified their morphological character as *Osteochilus spilurus* based on Weber and de Beaufort (1916) and Kottelat et al. (1993) description. Fish samples were photographed alive or fresh, then immersed in 5% formalin solution as preservation techniques in laboratory storage.

Body Shape Analysis

Individually of sixty-three samples from all sampling locations were photographed three times (tri-replicated) using a 13 MP camera on the left lateral side equipped with a ruler. Shape analysis was done digitally with fourteen morphological landmarks, as in Figure 2. The photographs of the fish samples are converted to TPS files using TPSutil version 32. Determination of landmarks using Thin-plate spline digitalized (TPSdig) software version 2.1 and transformed to Symmetry and Asymmetry Geometric Data Analysis (SAGE) program version 1.04 to get the principal component (Astuti *et al*., 2020).

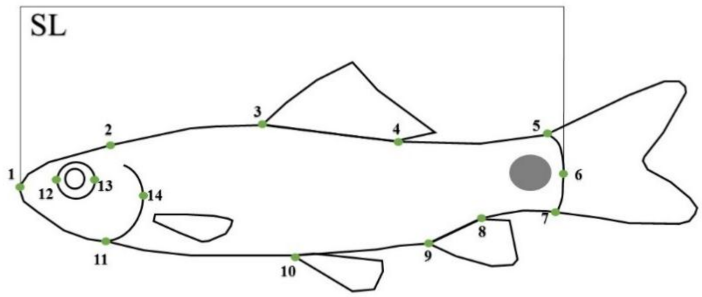

Figure 2. Position of landmark points used on morphometric analysis

Morphometric analysis

Morphometric measurements have also used the landmarks on the TPS file in TPSdig version 2.1. They are taken following the standard length (SL: 1-6), head length (HL: 1-14), head depth (HD: 2-11), eye diameter (ED:12-13), snout length (SnL: 1-12), pre-dorsal length (PreDL: 1-3), pre-ventral length (PreVL: 1-10), pre-anal length (PreAL: 1-9), dorsal base length (DbL:3-4), anal base length (AbL: 8-9), and caudal depth (CD: 5-7). The outcome data were presented as a ratio by comparing them to the head length for ED and SnL. The other measurement ratio is obtained from comparison with the Standard length. Non-parametric Kruskall-Wallis analysis using SPSS version 22 is applied to identify the morphometric variations among populations. Principal component analysis (PCA) to determine the pattern of spread from the population of *O. spilurus*, which was created with the MVSP 3.1 program.

RESULTS

1. *Body Shape*

All samples confirmed as *Osteochilus spilurus* on their morphological character follow Weber and de Beaufort (1916) and Kottelat et al. (1993) description. Landmarks on body parts related to fins and heads were successfully used to compare body shape between populations. Results of SAGE indicate the body shape variation of *O. spilurus* in Lenggang River (Belitung Island), Lelabi and Lebak River (Bangka Island), and Way Merah River (Sumatera Island) very significant in individuals parameters and sides identification per individual analysis ($P < 0.05$). The pattern of major variations in the shape and CP scores summary histogram of *O. spilurus* is shown in Figure 3. The Lenggang River samples have a lower PC1 (major principal component) score than other sampling locations. The score is 30.5%, while other populations have scored 53.43%, 46.87%, 49.58% for Lebak, Lelabi, and Way Merah Rivers.

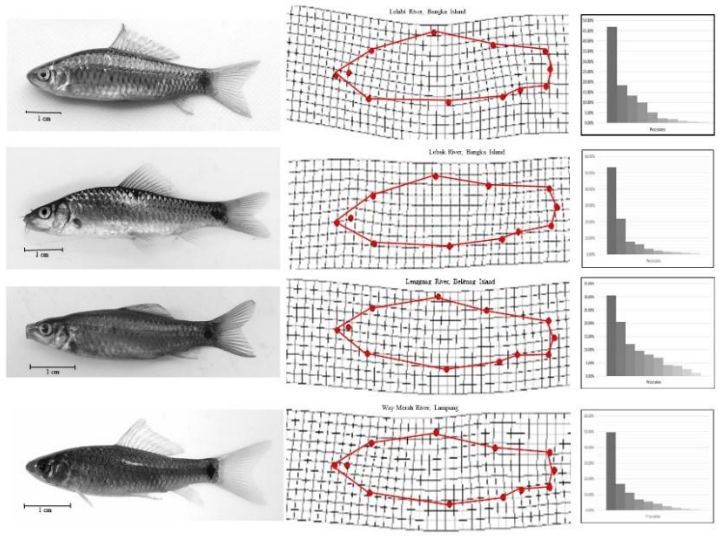

Figure 3. Summary of geometric morphometric analysis of body shape among *O. spilurus*

2. Morphometric

Morphometric character measurements using a photograph in TPSdig improve measurement accuracy and facilitate the sampling process. Photos that can be digitally enlarged make it easier to pinpoint landmarks. Sizing for a live fish is also possible in a short time. The standard length of the lowest and maximum *O.spilurus* was found in samples from the Lelabi River, Bangka Island, respectively 33.44 mm and 61.04 mm. Morphometric measurements based on landmarks are presented in ratio and analyzed using Kruskal-Wallis as in Table 1. A pattern of morphometric spread from *O. spilurus* population is shown in Figure 4.

Table 1. Morphometric Ratio of *O. spilurus* and The Kruskal-Wallis Analysis

Ratio	Average + SD				Kruskal-Wallis H
	Lenggang	Lebak	Lelabi	Way Merah	
SL	41.98±2.31	53.67±1.58	49.58±8.33	45.99±3.23	23.793**
HL_SL	20.83±1.22	21.62±1.35	22.74±0.91	23.35±1.21	26.338**
HD_SL	17.70±0.62	17.61±0.77	19.38±0.60	18.53± 0.79	28.759**
ED_HL	36.01±2.04	27.26±0.96	31.03±2.35	29.45±2.03	37.418**
DL_SL	23.13±1.81	24.49±1.78	27.96±2.10	27.36±1.62	31.802**
HC_SL	13.09±0.71	13.62±0.60	13.83±0.49	12.61±0,61	23.302**
AL_SL	8.80±0.98	8.51±1.21	9.23±1.23	8.77±0.74	4.554^{ns}
PDL_SL	48.32±1.00	48.27±1.30	48.69±1.28	47.75±1.08	5.076^{ns}
PAL_SL	75.65±1.27	75.73±0.70	78.33±1.33	77.16±1.45	24.998**
PVL_SL	52.77±1.23	52.13±1.71	53.91±1.05	53.96±1.53	12.076**

** ($p \leq 0.01$) – highly significant, ns – nonsignificant

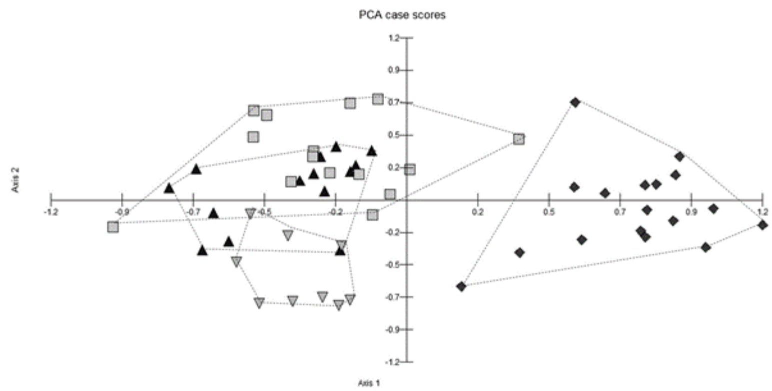

Figure 4. Principal component analysis on the body measurement ratio of *O. spilurus* specimens sampled from four populations: Lenggang River, Belitung Island (◆), Lebak River, Bangka Island (▼), Lelabi River, Bangka Island (■), and Way Merah River, Lampung, Sumatera Island (▲).

DISCUSSION

All specimens from the three islands were described as *O. spilurus* using morphological characters. This method for freshwater fish identifying and distributing is also used to date (Hasan *et al*., 2020; Haryono and Wahyudewantoro, 2020). Various fish species can lead to morphological variations (Burhanuddin and Iwatsuki, 2006). Genetic verification was not conducted at the time because there was only one reference gene of *O. spilurus*, specifically from Sabah, Malaysia, based on mitochondrial cytochrome b (Esa *et al.,* 2012), which was not accompanied by a morphological characteristics report. However, there has not been any difference in meristic characters in the specimens that indicate different species.

Morphometric and body shape differences are an adaptation reaction to their environment in the *O. spilurus* of the three islands. The same case also occurs in the genus Osteochilus from different rivers on the Sumatra island (Roesma and Santoso, 2011; Asiah *et al*., 2019) and is also seen in morphometric *Notopterus notopterus* on different islands populations (Wibowo *et al.,* 2017). Variation of fish body shape can reflect ecological and behavioral differences. Local adaptation leads to similar body shapes of syntopic populations as a response to environmental sharing (Klingenberg *et al.,* 2003). A different phenotype, known as morphological plasticity, is raised in this response (Wimberger, 1992; Pigliucci, 2001; Vences *et al*., 2002). In environments, plasticity also reflects adaptive responses to conditions (Robinson & Parsons, 2002; O'Reilly and Horn, 2004). In response to environmental variables such as water flow (Kelley *et al*., 2017), predation presence (Eklöv and Jonsson, 2007), feeding mode (Olsson and Eklöv, 2005; O'Reilly and Horn, 2004), genetic variation (Svanbäck and Eklöv, 2006), and human harvesting (Avise and Ayala, 2009) of freshwater fishes demonstrate substantial morphological plasticity.

In the three islands, the water flow of the *O. spilurus* ecosystem varies according to river use. On Belitung Island, the Lenggang River has a dam downstream. The 50 m long dam with a 10 meters waterfall height was constructed in 1936-1939 to facilitate tin mining (Intan, 2019). The Lenggang River flows more quickly during the rainy season and when the dam opens. At that moment, *O. spilurus* swim together on the riverbank against the water currents, and local fishers easily capture them using fish traps (Kurniawan *et al.,* 2016). Local community understanding of fish swimming activities contributes to trapping equipment and the direction of installation opposite to water flow (Alos *et al.,* 2012; Fakhrurrozi *et al.,* 2016). During the fishing season, namely in the rainy season, fish specimens from Belitung Island were collected from fishers. So, as they were swimming against the river currents, specimens from Belitung Island were captured.

Meanwhile, the catching method in Lampung, Sumatera, uses a lift trap with a light attractor. The Way Merah River has an irrigation dam so that the flow of water is slowly regulated and threatens to flood. A lifting net is used to capture fish from the Lelabi and Lebak Rivers on Bangka Island, natural rivers. The difference in water movement between the three rivers allows for variations in fish swimming behavior, which is associated with morphological differences. Fish have a slimmer body shape in existing environments (Collin and Fumagalli, 2011; Kelley *et al.,* 2017). Flowing waters force further swimming by fish (Lupandin, 2005). The swimming activity of the fish allows the body shape to be smaller in depth. If the fish is swimming less, the body is higher. (Ware, 1978; Anderson *et al*., 2005). Food bioenergy is used in swimming activities, thus minimizing mass body formation (Boisclair and Sirois, 1993).

In terms of their swimming ability, predators' presence is known to influence fish's body shape. Several fish species, such as fish from the genus Channa, are predators of *O. spilurus* in the three islands' rivers. The numerous types of predators present in the Lenggang River, Belitung Island have possible that are not present elsewhere, namely the Arowana fish (*Scleropages formosus*). The presence of predators in the artificial pond decreases fish swimming behavior and results in a higher body (Anderson *et al.,* 2005). However, high-density predators in the wild environment impact the smaller anterior body (Walker, 1997; Langerhans, 2009).

The fish feeding mode impacts fish movement. Owing to their attempts to chase prey, fish with mobile feed have shallower bodies (Anderson *et al*., 2005). On Bangka and Belitung islands, the detection of *O. spilurus* stomach content is relatively the same as phytoplankton's dominance (Kartika, 2017; Icas *et al*., 2019). The similarity in types of food eliminates the possibility of morphological plasticity due to their food habits.

At each sampling spot, *O spilurus* has a different perspective. It is positioned as an economically important fish by local people on Belitung, while people on the other two islands pay little to no attention to this fish. In Belitung Island, especially East Belitung, the perception of local individuals who like this fish causes high harvesting to meet market demand for consumption (Kurniawan *et al.,* 2018). Overfishing can trigger smaller individual fishes (Santz *et al.,* 2020). Signs of decline in size and amount of their catch are also perceived by some local people (Kurniawan and Triswiyana, 2019).

The harvesting of fish may impact the mating mechanism (Avise and Ayala, 2009). Most *O. spilurus* are captured in gonad-ripe conditions on Belitung Island (Rizkika, 2017; Kurniawan *et al.*, 2020). Rather than phenotypic plasticity or environmental variation, phenotypic variations in harvested populations are due to evolution (Avise and Ayala, 2009). The geographic isolation of the Bangka, Belitung, and Sumatra Islands from 6000 to 7600 years ago (Voris, 2000; Inger and Voris, 2001) and differences in utilization make it possible to continue the evolutionary process without impacting each other (O'Reilly and Horn, 2004; Turan *et al.*, 2004). Genetic variation research, however, can not be carried out based on morphological changes alone. We believe that further studies on the impact of several environmental variables, including genetic variation, on the plasticity of *O. spilurus* are essential.

CONCLUSION

Differences in body shapes and morphometrics are seen in *O. spilurus* from Way Merah River (Lampung, Sumatera Island), Lebak and Lelabi Rivers (Bangka Island), and Lenggang River (Belitung Island). In these variations, morphological plasticity plays a role in adaptation to water flow and predatory presence. There may be an impact on genetic variations due to fish harvesting and long geographical isolation, but more molecular studies are needed.

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