The Use of tpsDig in Re-Describing Morphometry of Barbodes binotatus Caught in Java Island, Indonesia

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Abstract. Barbodes binotatus is a group of freshwater fish and this species is an endemic and most dominant category of freshwater species in Indonesia. Morphological examinations at the level of intraspecific populations by involving extensive sampling localities have never been conducted before. Morphological characteristics through truss morphometry analysis of body shape can be used to analyze shape changes during growth, discriminate genera or species as well as identify good quality of population for fish broodstock. This aims of research were to determine the relationship between fluctuating asymmetry and morphological character of B. binotatus across different regions from Java. A total of 650 samples were analyzed and collected from a total of 24 sampling sites around Java Island. Individual specimens for each population were analyzed using thin plate spline (tpsDig) method with a total of 14 anatomical landmarks. The result of this research inform that PCA analysis showed highly significant different of each population. Research with genetic approach is needed to determine population groupings across distribution area.

1. Introduction
Barbodes binotatus is categorized as a freshwater fish that easily found in freshwater ecosystem. B. binotatus has special characteristics which distinguish it from other freshwater fish species with the form of black spots on the tail and under the dorsal fin. B. binotatus has not been developed optimally in the field of aquaculture, so this species still has a great opportunity to be developed [1]. B. binotatus is generally distributed in natural environments and this species used as a biological indicator in aquatic environment [2]. Morphological studies on this species were carried out to reveal differences in phenotypic features that may differ from other locations. Morphological and morphometric studies are still currently carried out using manual procedures and the percentage of calculation errors tend to higher than other (±0,1 cm), and tpsDig approach attempt to minimize measurement errors to analyze morphometric characters of the species [3][4]. This approach provides landmark and morphometric geometric information that used as the basis for calculating the percentage of morphometric differences in the body shape of the species [5][6]. Fish morphometric variations are formed due to ontogeny products in species that give an impact growth differences and maturation rates [7]. The tpsDig approach
is also used to obtain morphometric geometric data and make it easier to measure the biological shape, variation, and shape covariance of the organisms [8]. The results of the calculations are represented in the coordinates of the truss morphometry data [9][10]. Truss morphometry visualization represents with plot and shape coordinate [11]. Analysis of the distribution of species and tracing morphological characters provide basic information regarding differences in individual phenotypic characters influenced by their environment. A comparison between morphometric characters and morphological analysis will provide data on the percentage differences in individual characters that can be traced as the percentage of species variation in population.

2. Methodology

2.1 Sampling Activity
A total of 650 fishes were collected from 24 different locations. Sampling was conducted with a total of 24 locations covering East Java (Tuban, Pamekasan, Ngawi, Kediri, Blitar, Malang, Sumber Maron, Sumber Taman, Pasuruan, Banyuwangi, Bondowoso), Central Java (Wonosobo, Jogjakarta, Magelang, Sleman, Surakarta, Tegal, Demak), and West Java (Sukabumi, Bogor, Bandung, Cirebon, Tasikmalaya) (Fig.1). Samples were taken in natural habitats with several types of environmentally friendly fishing gear such as nets, fishing rods, traps, and scoops. All fresh samples were stored in 96% alcohol to keep them in good condition.

2.2 Digitization Specimens
Digitizing is a term to describe the process of transferring media to digital form. Digitization is carried out to create digital document archives or digital library collections. Digitization on this research is carried out using tpsUtil to collect all digital data and transferred it into tpsDig document [12]. Landmark digitizations are selected according to the body shape of the organism compared to several other previously marked species (Fig.2). The results of this landmark-marking are the basis data for calculating
truss morphometry coordinate points to identify morphological variations in the body shape of organisms (Table 2).

![Figure 2. Landmark-marking of *Barbodes binotatus*](image)

The morphometric characters were used to digitize the landmark point (Fig.2). A total of 15 landmark points were used in this step to calculate the variation in body shape analysis.

### Table 1. Morphometric characters

| Landmark Point | Morphometric Location                        |
|----------------|---------------------------------------------|
| 1              | Tip of snout fish                           |
| 2              | Last posterior of nuchal spine              |
| 3              | Dorsal fin - interior insertion             |
| 4              | Dorsal - posterior insertion                |
| 5              | Caudal fin - dorsal insertion               |
| 6              | Midpoint of lateral                         |
| 7              | Caudal fin - ventral insertion              |
| 8              | Anal fin - posterior insertion              |
| 9              | Anal fin - anterior insertion               |
| 10             | Pelvis fin - dorsal base                    |
| 11             | Last of jaw articulation – end of ventral   |
| 12             | Premaxilla - posterior end                  |
| 13             | Midline of orbital eye - anterior margin    |
| 14             | Midline of orbital eye - posterior margin   |
| 15             | Pectoral fin - dorsal base                  |

Morphometric variations among total region were analyzed by comparing the truss morphometry for samples with 6 main comparisons, including the total length and point of standard length, all marked location of eye diameter and the midpoint of standard length, location point of predorsal and standard length, point of pre pectoral length and standard length, location of preanal length and standard length, and the last is body depth and total point of standard length.

### 3. Result and Discussion

All the morphological characters in this research were analyzed by identifying a total of body shape characteristics of *B. binotatus*. Morphological identification was carried out to identify a total landmark point of the species to analyze the determination of all population structure (Table 2).

### Table 2. Morphological Character

| No | Parameter  | East Java       | Central Java     | West Java       |
|----|------------|-----------------|------------------|-----------------|
| 1  | Body of fish| Fusiform/ Normal| Fusiform/Normal  | Fusiform/Normal |
| 3  | Mouth      | Terminal        | Terminal         | Terminal        |
A total of six morphological characters from 650 specimens were analyzed and identified as *Barbodes binotatus* from 24 areas of sampling locations (Table 4).

### Table 3. Morphometric Measurement of *Barbodes binotatus* (cm)

| Characteristic          | Morphometric measurement (cm) |
|-------------------------|-------------------------------|
|                         | East Java (N=450) | Central Java (N=175) | West Java (N=75) |
| SL (standard length)    | 6.871 ± 2.125   | 2.795 ± 1.908   | 4.912 ± 1.088   |
| TL (total length)       | 7.627 ± 2.152   | 5.996 ± 1.852   | 6.182 ± 1.341   |
| BD (body depth)         | 2.531 ± 0.314   | 2.619 ± 0.582   | 1.486 ± 0.579   |
| PDL (pre dorsal length) | 1.918 ± 0.415   | 2.953 ± 1.821   | 2.586 ± 0.722   |
| PAL (pre anal length)   | 2.296 ± 0.814   | 1.581 ± 0.654   | 3.412 ± 0.932   |
| PPL (pre pectoral length)| 3.512 ± 0.557   | 2.862 ± 1.712   | 2.622 ± 0.791   |

The genera of each location are represented by a population of species. A total of 24 sites were represented by each population of the species and scattering result of scattering plot and tpsDig scattering program on the total population was not carried out separately but was collected by placing a data matrix with coordinates. The results show that there are three population groups [Fig. 3].

There are several populations from West Java, and another population that related closely are the population from Central Java that close to each other with population from East Java. This illustrates that some populations from East Java and Central Java have a similar result of morphometric variations and morphological characteristics. The results showed that there were 3 main populations of the total specimens. The difference in morphological variation in a population becomes basic information to reveal the presence and absence of inbreeding activity in a population [13] [14]. Differences in
morphological characters are the impact of individual interactions with the environment, genetic inheritance, and other factors that reveal in a population [15] [16]. In this study, the environmental factor parameters at each location are similar to one another. This illustrates that the similarity in morphology also relevant to similarities in environmental conditions. Morphological characters that reflect the impact of environmental conditions have been proven in previous studies that reveal the existence of morphological variations in species with different environmental conditions [17] [18].

4. Conclusion
The results of the analysis of the population distribution show that some populations are not too far apart, including the sample populations from West Java with Central Java, Central Java with East Java, and East Java with West Java. The highest morphometric variation was obtained from the ratio of body length, dorsal fin length and eye diameter of all populations. Research on genetic approaches is needed to prove the factors that cause some populations related closely with other populations. This can be used to trace inbreeding activity and phylogenetic population from all sampling locations. The addition of a molecular genetic approach also can be used to reveal further findings from the results of this study. These results can be used as initial reference for restocking and aquaculture activities.

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