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Distribution Patterns of the Morphology, Species, and Sex in the Stingray Species Complex of *Himantura uarnak, Himantura undulata,* and *Himantura leoparda* in Indonesia

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Abstract

The assessment of the morphology and stingray species distribution of the *Himantura uarnak* species complex covers eight sampling sites in western and eastern Indonesia as many as 113 individuals. The observation of the reticulate pattern was accomplished by directly checking the dorsal side of the stingrays, and growth observations also carried out. The distribution patterns were analyzed using the principal component analysis (PCA), while the growth patterns were evaluated using correlation analyses. The reticulation patterns of the *H. uarnak* species complex were highly variable, and the distribution patterns based on the morphological parameters, species, and sex produced four groups. The distribution of the length frequency of *H. undulata* and *H. uarnak* showed a range of different sizes. The body length of the female in the western region is relatively longer than the male, while in the eastern region the female revealed a range in relative size showing that the male was longer than the female. The length-weight relationships of the stingrays were negative allometric, indicating a b-value of less than 3. The b-values of both the male and female *H. undulata* were 1.5860 and 0.4380, respectively; while the male and female *H. uarnak* were 0.2956 and 0.4376, respectively.

Abstrak

Pola Distribusi dari Morfologi, Spesies dan Jenis Kelamin pada Kompleks Spesies Ikan Pari *Himantura uarnak*, *Himantura undulata* and *Himantura leoparda* di Indonesia. Penilaian terhadap morfologi dan distribusi spesies dari ikan pari kompleks spesies *H. uarnak* meliputi delapan lokasi pengambilan sampel di barat dan timur Indonesia sebanyak 113 individu. Identifikasi spesimen mengikuti kunci *family* Dasyatidae, untuk genus *Himantura*. Pengamatan pola retikulasi dilakukan dengan pengecekan langsung pada punggung ikan pari dan observasi pertumbuhan juga dilakukan. Pola distribusi ikan pari dianalisis dengan *principal component analysis* (PCA), sedangkan pola pertumbuhan menggunakan analisis korelasi. Pola retikulasi kompleks spesies *H. uarnak* sangat bervariasi. Pola distribusi berdasarkan parameter morfologi, spesies dan jenis kelamin ikan pari menghasilkan empat kelompok. Distribusi panjang frekuensi *H. undulata* dan *H. uarnak* menunjukkan berbagai ukuran yang berbeda. Panjang tubuh dari betina di wilayah barat relatif lebih panjang dari jenis kelamin jantan, sedangkan wilayah timur menunjukkan bahwa ukuran tubuh dari jenis kelamin jantan relatif lebih panjang dari betina. Hubungan panjang-berat ikan pari menunjukkan alometrik negatif dengan nilai b kurang dari 3. Nilai b pada masing-masing jantan dan betina dari *H. undulata* adalah 1,5860 dan 0,4380, sedangkan masing-masing jantan dan betina *H. uarnak* adalah 0,2956 dan 0,4376.

Keywords: distribution pattern, Himantura uarnak species complex, morphology, reticulation, stingray

1. Introduction

Species complex represent a group of closely related species, where the boundaries between the species are often unclear or vague, and they usually exhibit incomplete reproductive isolation [1-2]. The *Himantura uarnak* species complex includes the *Himantura* genus of the family Dasyatidae, which is the most economical

stingrays in the waters of Indonesia. These stingrays are the target species, and are widely used for consumer and trading products, such as handbags, wallets, belts, and other accessories [3]. Members of this stingray species complex, namely *Himantura uarnak*, *H. undulata*, and *H. leoparda*, are still subject to much debate, with common errors in identification. The identification of stingrays is generally done through a morphological approach, using the reticulation pattern and body shape. However, the results are still debatable, as species revisions of *H. undulata* become *H. leoparda* have been done by Manjaji-Matsumoto and Last [4].

The reticulation patterns of *H. uarnak*, *H. undulata*, and *H. leoparda* differ only in adulthood, with observed differences in body shape during juvenile and adult stages. *Himantura uarnak* has characteristics of the dorsal surface of the body being dominated by a reticulation pattern motif of winding, like a beehive. *Himantura undulata* is characterized by a pattern resembling a leopard in the dorsal area. Similarities between *H. uarnak* and *H. undulata* include the shape of their bodies that plate when juveniles rectangularly widen, while in adulthood this rectangular plate is largely quadrangular. *Himantura leoparda* possesses characteristics similar to *H. undulata*, but there is a little difference in the characteristic ring from a leopard pattern on the dorsal surface of the body. This species also has a subtle difference in squamation and body shape, as well as the development of a color which is unique and complex. Aside from this, the body shape of *H. leoparda* is rhomboidal, not quadrangular as in *H. uarnak* and *H. undulata* [4-6]. To the naked eye, individuals in species complexes look identical one to another, so that the people often incorrectly identify them. Additionally, individuals of this species complex have been a target of exploitation because of their high economic value [7]. This study is very important to verify the number of individuals in each species that have been caught, and to estimate their population sizes in the wild.

The purpose of this study was to determine the distribution pattern, reticulation, and growth of the *H. uarnak* species complex in Indonesian waters, based on morphometric measurements (length-weight relationship), species, and sex.

2. Methods

Sampling sites. Data collection was conducted from August 2006 until October 2011, covering eight sampling sites in western and eastern Indonesia (Figure 1).

Specimen collection. Sampling was conducted at the fish landing sites (production centers) or booked directly through stingray fishing collectors. Each sample was documented, and all relevant information was recorded, including date of sampling, specimen labels, museum codes, catchment population, location of catchment, and local name. Specimens were collected only from individuals that were small and medium size, while the larger sizes were documented only, from all directions and special parts. Handling of the samples followed the procedures for the preparation of specimens for the National Museum of Zoology Bogoriense (MZB), Cibinong. Each specimen was immersed in a 4-7% formalin solution for 2-3 weeks,

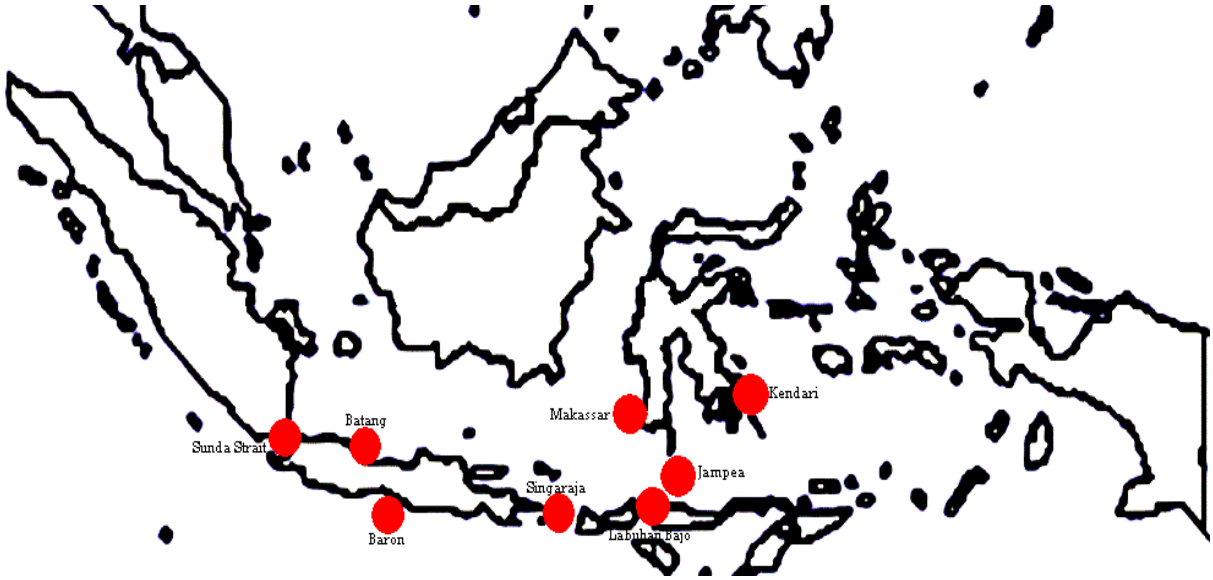

Figure 1. Sampling Locations of *Himantura uarnak* Species Complex

and then re-immersed in a solution of 70% alcohol before being deposited into the MZB. The specimen collection will be used for further research on the identification of the species based on the stingray's DNA barcoding.

Identification of specimens. To identify the specimens, the identification key was followed for the Dasyatidae family, *Himantura* genus [4,8-12]. The morphological data was recorded by measuring body width (W) and body length (L) using a ruler, while the body weight (BW) was measured by using scales.

Sex determination was done by separating each individual by clasper or non-clasper. A genital clasper is found only on male stingrays (Figure 2 and Table 1). Interviews of fishermen were also conducted to obtain additional information as supporting data (location of catchment, fishing ground, and amount of the catchment).

Identification of the spot pattern motif was done by observing these patterns directly on the top of the stingray body. *Himantura uarnak* complex species generally have a pattern motif where the spots are small, while they tend to be large in juveniles of the same species (Figure 3a). As adults, these spots form a distinct reticulation (Figure 3b), honeycomb (Figure 5a), leopard pattern (Figure 5b), and leopard rings on the dorsal surface (Figure 5b type 2) [4,13].

Himantura uarnak complex species also have special features that distinguish between *H. uarnak* and *H. undulata* or *H. leoparda*. The features on *H. uarnak* include two prominent spiny pearls lying in the heart and center of the disk-shaped crescent, followed by smaller spines (Figure 4a). However, in *H. undulata* or *H. leoparda* the two pearls are both heart-shaped and thorny, followed by heart-shaped small thorns (Figure 4b).

Data analysis. The fish length to weight relationship is expressed in the form: $W = aL^b$ [14-16], where W = weight, L = length, and a = the coefficient of fish growth. To test the value of b = 3 or b ≠ 3, a t test (partial testing) is used, with the hypothesis:

H0: b = 3, length-weight relationship is isometric.

H1: b ≠ 3, length-weight relationship is allometric, wherein:

Positive allometric if b > 3 (gain weight faster than increase length), and

Negative allometric if b < 3 (increase length faster than gain weight).

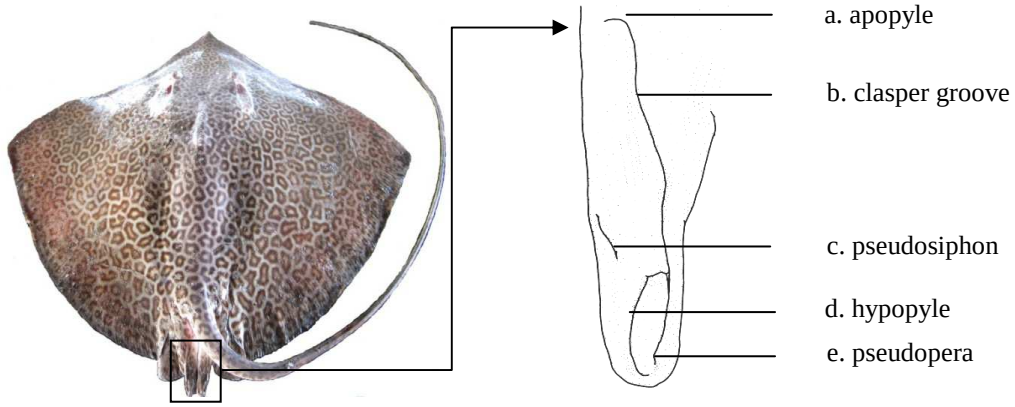

Figure 2. Clasper, Male Genitalia on Stingrays

Table 1. The Number of Individuals in the *H. uarnak* Species Complex based on Reticulation Patterns and Sex

Locations	Species					
	H. uarnak		*H. undulata*		*H. leoparda*	
	♂	♀	♂	♀	♂	♀
Batang, Central Java	0	0	33	51	1	0
Sunda Strait, Banten	0	0	1	2	0	0
Makassar, South of Sulawesi	6	5	0	0	0	0
Jampea, Selayar islands	2	5	0	0	0	0
Kendari, Southest of Sulawesi	1	2	0	0	0	0
Labuan Bajo, East Nusa Tenggara	2	0	0	0	0	0
Singaraja-Buleleng, Bali	0	0	0	1	0	0
Baron, Yogyakarta	0	0	0	1	0	0
Total per sex	11	12	34	55	1	0

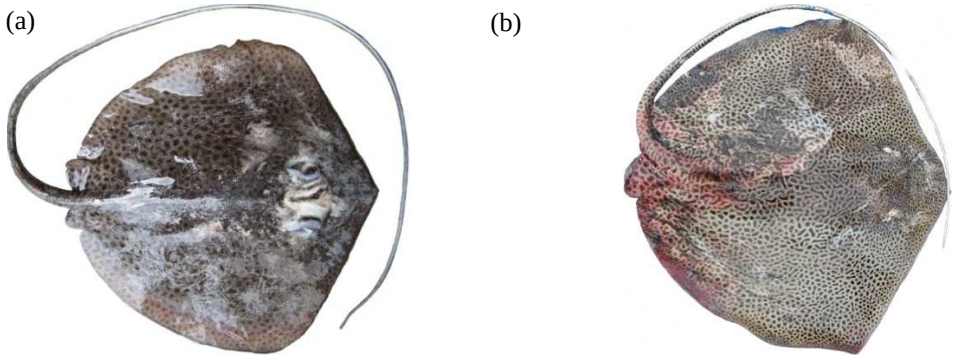

Figure 3. (a) *H. uarnak* Juvenile, (b) *H. uarnak* Adult

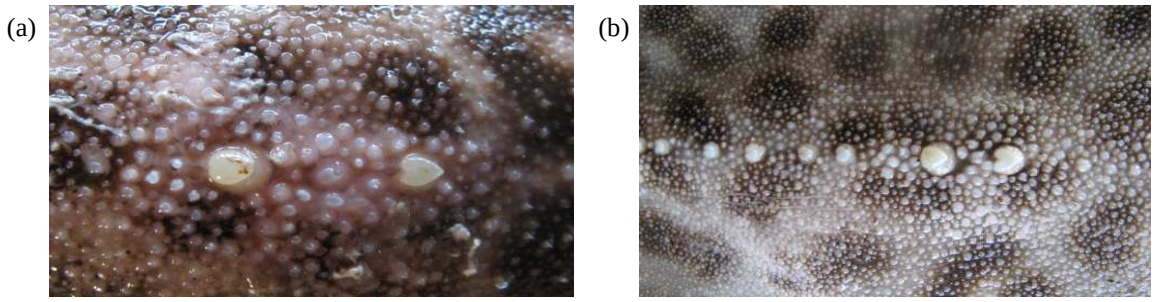

Figure 4. (a) The Pearl of *H. Uarnak*, (b) The Pearl of *H. undulata* or *H. Leoparda*

The geographical distribution patterns of the *H. uarnak* species complex were created by combining the morphological data and species from each region to capture the data. The data were analyzed using the principal component analysis (PCA) and cluster analysis program, MINITAB version 14.12 [17-18]. The data on the growth of *H. leoparda* was combined with *H. undulata* because the sample size was very small (n = 1).

To determine the size distribution of the length and weight of the stingrays in each fishing area, the observations were made based on the division of the territories, by western and eastern regions. The western region included: (1) Sunda Strait (Banten), (2) Batang (Central Java), and (3) Baron (southern coast of Yogyakarta); while the eastern region included: (4) Singaraja (Bali), (5) Labuan Bajo (eastern Nusa Tenggara), (6) Jampea (Selayar Island, southern Sulawesi), (7) Makassar Strait (southern Sulawesi), and (8) Kendari (southeastern Sulawesi).

3. Results and Discussion

The reticulation patterns of the *H. uarnak* species complex were evaluated. Material samples of the stingrays were successfully collected from 8 sampling sites in seven fishing area in Indonesian waters. The total sample size numbered 113 individuals, consisting of 70 females and 43 males (Table 2).

According to Arlyza *et al*. 2013 [19], the *H. uarnak* species complex has four different species, consisting of *H. uarnak*, *H. undulata*, *H. leoparda*, and *H. tutul*, a new species in this complex [20]. The morphological identification result proved to be the opposite of molecular study between *H. undulata* and *H. leoparda*; wherein, *H. leoparda* apparently was *H. undulata* based on mitochondrial and nuclear DNA, or otherwise [21].

Length-weight relationship of stingrays. The length-weight relationship analysis using the data on body length and wet weight of the stingray samples was used to evaluate the individual growth patterns of *H. undulata* and *H. uarnak*. The length-weight relationship model *H. undulata* and *H. uarnak*, male and female, are presented in Table 3 and Figure 6.

The results of testing the correlation of the length-weight relationships of *H. undulata* were values of correlation (r) of 80.4% for stingray males and 90.3% for female stingrays (Figure 6a). *Himantura uarnak* exhibited values of correlation (r) of 90.6% for male stingrays and 96.7% for female stingrays (Figure 6b). From the b values obtained from the t test ($\alpha = 0.05$) compared to the value of b, it is known that the growth patterns of *H. undulata* males and females are negatively allometric. Additionally, *H. uarnak* males and females are also negatively allometric.

Table 2. The Number of Individuals in the *H. uarnak* Species Complex, based on Patterns of Reticulation and Sex

Species	Motif type						Total
	Type1		Type2		Type3		
	♂	♀	♂	♀	♂	♀	
H. uarnak	4	7	4	2	3	3	23
H. undulata (89) + *H. leoparda* (1)	2	3	25	31	5	24	90
Total	6	10	29	33	8	27	113

Note: 29 individuals without photos and 17 juvenile individuals were not included in identifying the types of motif patterns. The motif patterns of the *H. uarnak* species complex were divided into three types for *H. uarnak* and *H. undulata*. The motif patterns of *H. leoparda* only have one type, and tend to be similar to *H. undulata* type 2 (Figure 5).

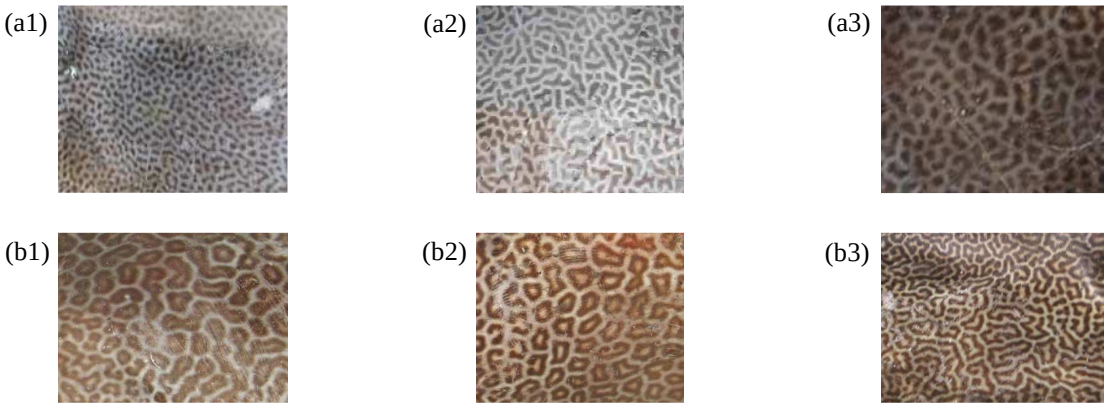

Figure 5. The Patterns of Motifs on *H. uarnak* Species Complex based on Reticulation: (a) *H. uarnak;* 1. Type1R; 2. Type2R; 3. Type3R. (b) *H. undulate*; 1. Type1; 2. Type2; 3. Type3

Table 3. Model of the Length-weight Relationships of *H. uarnak* and *H. undulata*

The model of length-weight relationship of *H. undulata*	r value	The model of length-weight relationship of *H. uarnak*	r value
$W = 49.780\ L^{1.586}$ male	80.4%	$W = 44.83\ L^{0.2956}$ male	90.6%
$W = 9.8340\ L^{0.438}$ female	90.3%	$W = 8.592\ L^{0.4376}$ female	96.7%

According Syahailatua [22], variations in the value of b can be determined by differences in sex, level of maturity of the gonads, the number of samples used in the analysis, and the condition of the fish caught and sampled at the time. Further, Biring [23] states that fish growth is influenced by heredity, sex, age, disease, food, and water temperature. By comparing the results of previous studies of the growth patterns of stingrays (as obtained [15] in the waters of the Java Sea from the *Dasyatis kuhlii* family Dasyatidae) similarities exist in the length-weight relationships: W = 0.2279 L23686, r = 0.7028 with a value of b = 2.3686 for female fish, and W = 0.1085 L26086, r = 0.872 with a value of b = 2.6086 for male fish.

Referring to the results of this study, and the other studies of stingray growth patterns, it can be stated that most stingrays have a negative allometric growth pattern. This suggests that the length of the fish increases faster than the body weight [23-24]. However, according to Devadoss [25], the stingray *Dasyatis imbricatus* has a very high b value of 3.6907. This means that *D. imbricatus* has a positive allometric growth pattern [26]. This was unusual, and Devadoss did not mention the sex of the stingrays. Generally, there is a marked difference between the b values of male and female stingrays.

Length and weight distribution of stingrays. *Himantura undulata* and *H. uarnak* have distributions varying in length and weight in the fishing areas that were examined: Batang in Central Java, the Sunda Strait, Yogyakarta, Kendari, Labuan Bajo, Jampea, and Makassar. The minimum body length of the male (*H. undulata* and *H. uarnak*) is 26 cm, while the longest males are 120 cm. Most individuals have a long interval, with the interval being 92-120 cm of 20 heads, and the lowest interval being from 38.5 to 86 cm. A total of 18 individual heads had the highest weights, and the interval of 26-36 kg had the lowest number of

individuals with 4 heads. The interval weight with the highest number of individuals was about 25-79 kg with 18 heads, and in the interval from 0.8 to 20 kg there were 18 heads (Figure 7).

The minimum body length of the female (*H. undulata* and *H. uarnak*) is 22 cm, while the longest are 152.5 cm. Most individuals had a long interval, from 102 to 152.5 cm (38 heads), while the 54-98 cm interval had the lowest number of 20 individuals, and 22-33 kg had 5 heads. The weight of the interval with the highest number of individuals was about 30-84 kg (38 heads) and the range of 5-24 kg had the lowest number of individuals (13 heads) (Figure 8). Thus, there were indicators that the male and female stingrays caught were still above average size.

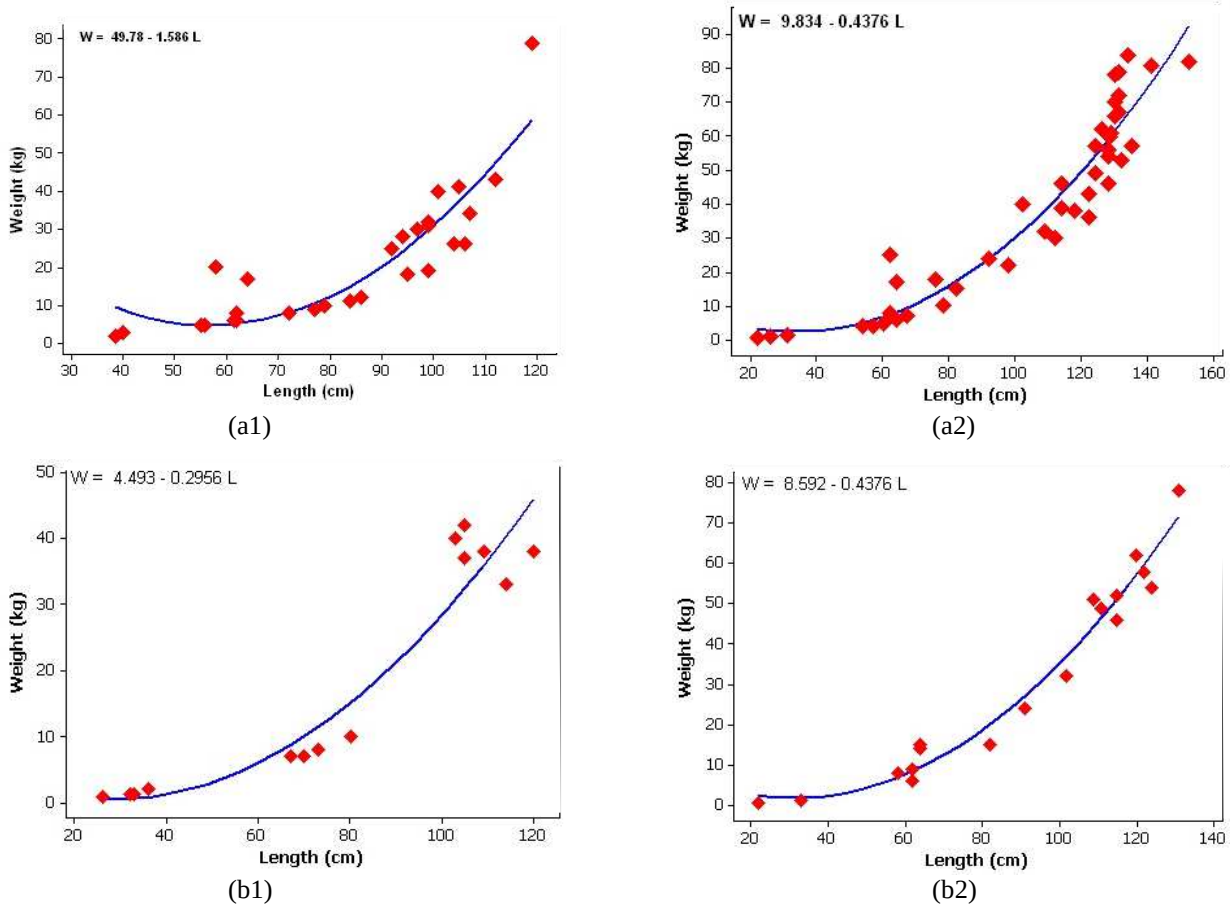

Figure 6. The Weight-length Relationship of *H. undulata* (Figure a1 and a2) and *H. uarnak* (Figure b1 and b2), Both for Male and Female

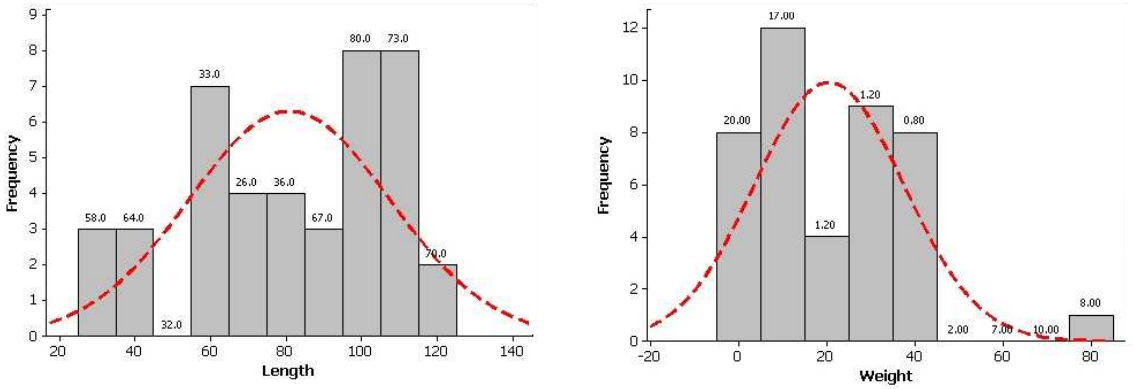

Figure 7. Length and Weight of Frequency Distribution of *H. undulata* and *H. uarnak*, According to Observations of the Male Sex

The maximum body lengths of *H. undulata* and *H. uarnak* individuals caught during the study were 120 cm to 142 cm (male and female), in the Batang, Central Java location. According to Last and Stevens [12] and White *et al*. 2006 [13], the total size of the stingrays can reach a maximum width of 410 cm by a length of 140 cm. Furthermore, according to Manjaji-Matsumoto and Last [4], they can reach a minimum total size of 446.5 mm and 413.7 mm, however, whether male or female was not recorded.

The total length differences could be due to several possibilities, such as differences in the locations of the fish samples, representativeness of the samples taken, and high fishing pressure on the stingrays. The same fish species living in different aquatic locations will experience different growth rates due to factors within, as well as outside factors that affect the possible size of the fish [23].

According to Effendi [23], the factors that are difficult to control include heredity, sexing, age, parasites, and disease. External factors affect the fish's growth and food temperature. Assuming that the stingray samples taken are representative of the population with regard to the maximum body length, in the Kendari area, Labuan Bajo, Jampea, and Makassar the stingrays are relatively small, compared to Batang in Central Java, the Sunda Strait, and Yogyakarta. This indicates that there are high pressure fishing activities, particularly in the areas of Kendari, Labuan Bajo, Jampea, and Makassar. This is influenced by the frequency of public consumption of stingrays, as it is known that people in the four regions are likely to consume stingrays as a source of animal protein from the sea, especially the Bajo and Bugis ethnic groups. This information was obtained through discussions with several fishermen in each sampling location.

The results of the principal component analysis used to determine the distribution pattern from the entire parameter of stingrays produced the following results: root traits for the three main components, called F1, F2, and F3, describe the distribution patterns of the morphological parameters, species, and sex, respectively, and are presented in Table 4.

Based on the characteristic roots, the distribution pattern of the morphological parameters (length, weight, width, and reticulation), species, and sex of the stingrays suggests that a variety of the main components are adequately represented with only two axes (F1 and F2 > 75%) (Table 4). This implies two main components (F1 and F2 = 87.9%) were able to explain most of the information contained in the parameters. The parameter greatly affecting component F1 was length (0.476), whereas for F2 it was sex (-0.753).

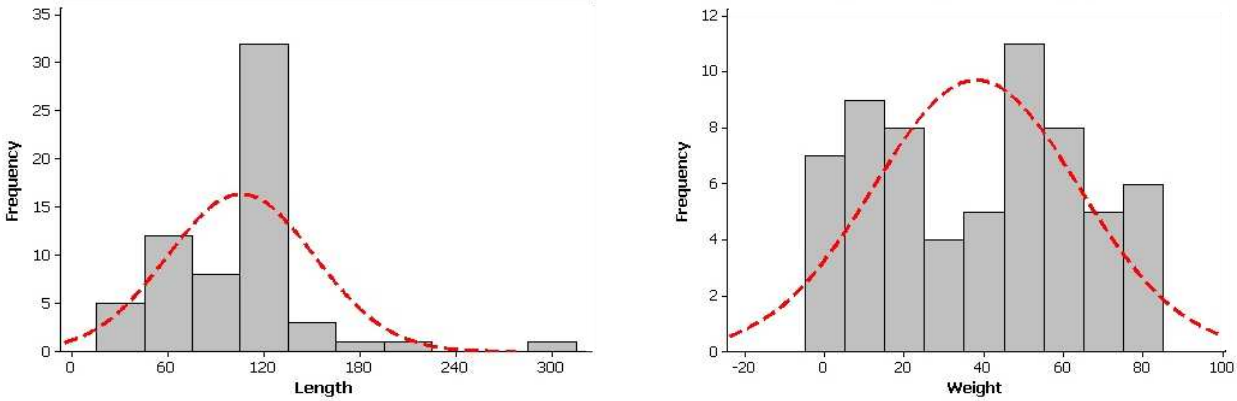

Figure 8. Length and Weight of Frequency Distribution of *H. undulata* and *H. uarnak*, According to Observations of the Female Sex

Table 4. Eigenvalues for the Major Components of the Stingray Distribution Patterns

Parameter	Main component		
	F1	F2	F3
Eigenvalue	4.1698	1.1068	0.4075
Proportion	0.695	0.184	0.068
Cumulative %	0.695	0.879	0.947
Length	**0.476**	0.172	-0.160
Weight	0.462	0.261	0.264
Width	0.452	0.175	-0.333
Motive reticulation	0.425	0.182	0.447
Sex	0.238	**-0.753**	0.495
Species	0.344	-0.522	-0.590

The results of the morphological analysis of the distribution pattern parameters can be seen in Figure 9. The analysis of the results explains that there were four groups in the distribution of the morphological parameters, species, and sex of the stingrays. The first group was dispersed in Bali, Yogyakarta, and Labuan Bajo, the second group included the locations of Batang in Central Java and Kendari, the third group was spread throughout Sunda and the Makassar straits, while the fourth group centered on Jampea (Selayar Island, Southern Sulawesi).

The group formations in Figure 9 are based on the degree of similarity in the morphological data, species, and sex of the stingrays. The groupings were different, presumably because the environmental factors of the water in each region are likely to be different. Ridho [27], in a principal component analysis (PCA) on the index of diversity and uniformity with environmental factors and depth, showed that high diversity and uniformity of demersal fish are affected by low salinity, dissolved oxygen levels, and high temperatures [27].

The composition and type of water are attributed to differences in the condition of different water environments (habitats), where about 15% of elasmobranches are endemic to a particular habitat [28]. The results of this study indicate that the patterns of species distribution, morphology, and sex of stingrays are not spread evenly. Sometimes, 3 or 4 regions have only one species, allegedly because several species of stingrays have a specific distribution [21]. The western region (Batang, Central Java) of stingrays have lengths and weights that are higher than in other locations, for both males and females. In the eastern regions, the length and weight vary with the location of the stingrays. The samples of stingrays taken in Makassar have the longest body size for males, while the longest female stingray samples were found in Kendari. Based on the morphological parameters, species, and sex of the stingrays, the observation location was grouped by similarity. The results obtained show that, in general, there are four groups based on the location of observations in each region (Figure 10).

The stingrays in the first group have one species, namely *H. undulata*, located in Bali and Labuan Bajo, while the *H. uarnak* contained in Yogyakarta show three types of motifs (type1, type1R, and juvenile), and had a mean size of approximately 49.56 cm long, 59.33 cm wide, and 8.14 kg in weight. This data was obtained from one sex, a female in the territory Yogyakarta and Bali, and a male in the region of Labuan Bajo. The second group had a length of 84.84 cm, width of 96.82 cm, and weight of 30.58 kg, with varying types of motifs (type1, type2, type3, type1R, type2R, type3R, and juvenile), and both sexes from *H. undulata* and *H. uarnak.*

The third group had the same species (*H. undulata* and *H. uarnak*), and a variety of motifs (type1, type2, type1R, type2R, type3R, and juvenile). The mean length of 59.85 cm, width of 71.83 cm, and weight of 7.35 kg was from a female in the Sunda Strait region, while the region of Makassar had both males and females represented. The fourth group consists of *H. undulata* and *H. uarnak*, with motifs of type1 and juvenile, sexing of males and females, and a mean length of about 29.90 cm, width of 34.45 cm, and weight of 1.09 kg. Based on the classification results, we can conclude that the location effect is strong enough in the grouping of the growth parameters at 7 of the stingray fishing areas. The

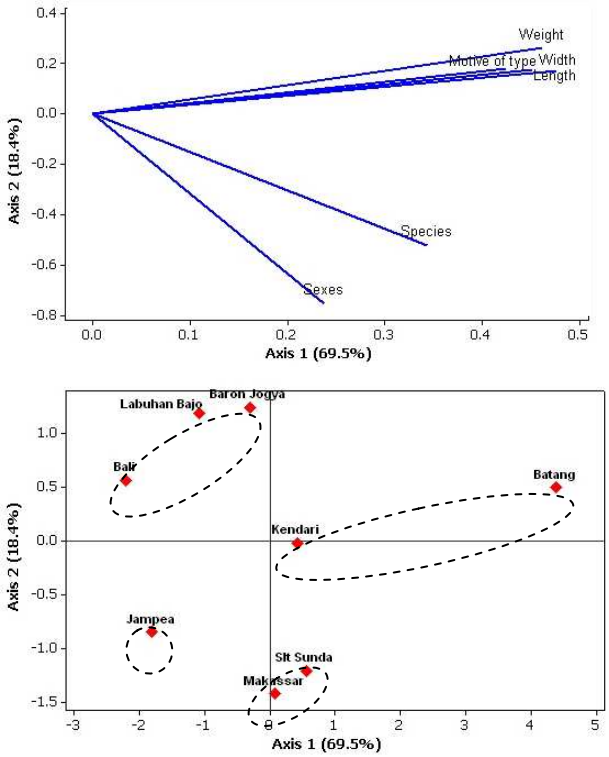

Figure 9. The Correlation Between Morphological Parameters, Species, and Sex of Stingray by Region

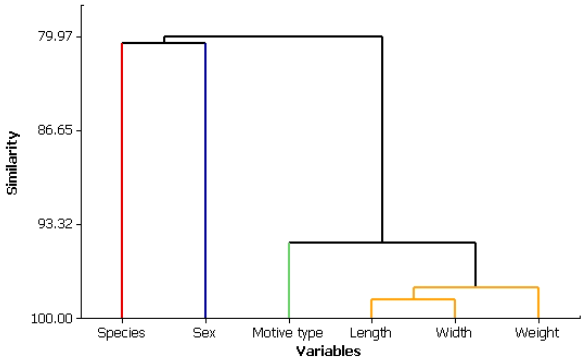

Figure 10. Grouping based on Cluster Analysis of the Similar Characteristics of the Morphological Parameters, Species, and Sex

classification was based on the growth patterns of 7 fishing areas in Indonesia of the *H. uarnak* species complex, grouped into 4 groups per region, with Bali, Labuan Bajo, Baron, and Yogyakarta grouped into one. Batang and Kendari were grouped separately, and the Sunda Strait and Makassar were placed into another group. Jampea and Selayar formed a separate group as well.

Recently, studies based on molecular aspects (mitochondrial and nuclear DNA) show that there has been reproductive isolation in *H. uarnak* in 4 fishing areas: the Makassar Strait, Jampea on the Selayar Island, Kendari, and Labuan Bajo [21].

Based on the comparison between the morphological and molecular approaches (*H. uarnak* species complex divided into four different species, consisting of *H. uarnak*, *H. undulata*, *H. leopard*, and *H. tutul* [19-20]), most of the *H. leoparda* fall into types 1 and 2, while type 3 represents *H. tutul*. The motif patterns of *H. undulata* have only one type (n=1) and tend to be similar to the *H. leoparda* of type 2. This was confirmed from the results of genetic studies using nuclear DNA markers, where type 2 has common alleles with *H. undulata* at three polymorphic intron loci i.e. Cam-3A, Cam-3B, and Cam-3C. However, based on the molecular approach using the mitochondrial DNA gene cytochrome oxidase I (COI) marker, *H. undulata* has a similarity of 92.1% with type 2, and with marker cytochrome b there is a similarity of 90% [21].

4. Conclusions

The reticulation motif patterns of *H. undulata* and *H. uarnak* are different and they were grouped into three types, and the morphology of *H. leoparda* is separate from that of *H. undulata*. The stingrays in the *H. uarnak* species complex have negative allometric growth patterns. The distribution patterns of the *H. uarnak* species complex are based on the morphology, species, and sex from each geographical location, and divided into four groups.

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