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Intra-species variations of *Photopectoralis bindus* (Family: Leiognathidae) collected from two geographical areas in East Java, Indonesia

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Abstract

Ponyfishes (Family: Leiognathidae) contributed $\pm 22\%$ of all demersal catch in East Java, and also Indonesia. *Photopectoralis bindus* (Valenciennes, 1835), orangefin ponyfish, was the most dominant species in the catch. This study intended to test the hypothesis on whether separate stock can be identified from two geographical areas of about 400 km apart, with two geographical barriers of narrow traits. Samples of *P. bindus* from Banyuwangi and Tuban were collected for isometric growth dimension, morphometric, and genetic analysis (mt DNA region COI). The results showed that there was no single parameter indicating the presence of separate subpopulation between the areas. Isometric growth dimension closely resembled to the same species found in Channai Coast, India. Morphometric analysis showed overlap in shape of samples from both areas. Alignment of DNA sequence showed 100% similarity one to the other, with 99.2% similarity with the same species found in the Philippines. Geographical distribution of the species was far longer distance than was thought before, from the analogy of Mantis Shrimp at Java Sea.

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Introduction

Fishery management is simply regulating fishing effort in such way to avoid over-fishing, and hence, fish stock is maintained at sustainable level (Mous *et al.*, 2005). Thus, fish stock is the basis and considered central to fishery science and fishery management (Cadrin, 2000; Cadrin *et al.*, 2004). Fish stock is defined as sub-group of species inhabiting certain (discrete) geographical area (Sparre & Venema, 1989; Cadima, 2003). The group (stock) is characterized by having the same parameters such as growth, mortality, body shape, or shared a common gene pool.

Growth is commonly defined by a measure of increase body dimension, particularly length and weight, which provides the best estimates of organism's growth status in certain area and time (Mazlan & Seah, 2006; Kishakudan & Reddy, 2012). Length-weight relationship in fishes usually follows a cube law: $W = a * L^3$; where W stands for weight, L stands for length; "a" is a constant and "3" is considered as isometric growth dimension. In the field, the exponent, 3, may not always the case as surrounding environment influences the growth. The formula can best be expressed as: $W = a * L^b$. A species, separated in two groups, and inhabiting different geographical areas, may have different value of isometric growth dimension, "b". If this occurs, each group is treated as a stock and considered different from the adjacent stock.

Stock identification methods have developed in parallel with the advancement of morphometric techniques (Pollar *et al.*, 2004; Bagherian & Rahmani, 2009; Cronin-Fine *et al.*, 2013). Body shape can always explain species. But within species differences in body shape may considered informative to separate fish stock. Body shape can be expressed as a ratio between two related morphometry, such as body depth ratio to standard length. Sajina *et al.* (2011) applied body shape morphometrics (truss box method) and distinguished two different stock of *Megalaspis cordyla* between that in Bay of Bengal

dan Arabian Sea subpopulation. Difference in morphometric characters does not always indicate genetic variations unless populations have had isolated each other in long period of time. Vasconcellos *et al.* (2008) distinguished body shape difference within Brazilian population of yellowtail snapper. However, analysis of mitochondrial DNA sequences (633bp of control region) does not support the conclusion. On the contrary, genetic study on mantis shrimp in Java Sea (Barber *et al.*, 2000) showed genetic variation as close as 300 km apart. So, both genetic and morphometric can be used in combination to better understand the stock.

Based on the latest finding, there at least 15 leiognathid's species morphologically described from catches of artisanal fisheries of East Java (Wiadnya *et al.*, 2014). The most dominant species, *Photopectoralis bindus* (Valenciennes, 1835), was observed to be site specific. The catches from the north area (extension of Java Sea) usually having bigger body size (standard length) than the same species collected from south-eastern part of the region, Banyuwangi. This study is intended to test whether both groups belong to different stock species. The results may help Indonesian fishery managers to identify the presence of separate stock in two adjacent geographical areas. Before any detail fishery management measures are in place, this information on stock and its geographical dispersal may crucial to support management.

Materials and methods

Samples and species identification

Samples for the study were collected from two fish landing sites (**Fig. 1**), Banyuwangi (Lat. -8.218389; Lon. 114.386250), and Tuban (Lat. -6.809667; Lon. 111.885408), from April to September 2014. Morphological characters used to determine species was based on Fowler (1904) as *Leiognathus virgatus*; Weber & de Beaufort (1964), James (1975), Jones (1985), as *L. bindus*; Sparks *et al.* (2005), Chakrabarty *et al.* (2008), Abraham *et al.* (2011), Larson *et al.* (2013) that currently valid as

Photopectoralis bindus (Valenciennes, 1835). A 53 morphological characters was compiled to assure the species, and type specimen used in this morphological identification was deposited at Museum Zoologicum Bogoriense (MZB), Bogor (catalog: MZB.FISH. 22125).

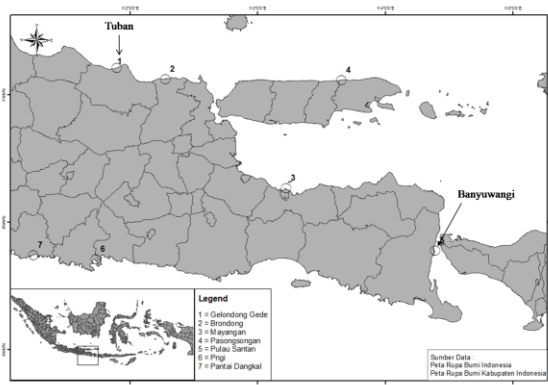


Fig. 1. Map of sampling sites – Banyuwangi represents area connected to Indian Ocean and Tuban is typical for Java Sea. Two narrow straits may act as geographical barriers in avoidance of sock mixing.

Isometric growth dimension and morphometric

Isometric growth dimension was estimated using length-weight relationship. Samples of *P. bindus* from Banyuwangi (n = 123), and Tuban (n = 97) for measurement were obtained from fishermen’s catch using Beach Seine and Mini-Trawl. Individual body weight was measured using portable and battery power weighing scale of max. 500 g (0.1 g). The standard length was measured with dial caliper (0.1 mm). Cube law of length-weight relationship was transferred into log-liner following the equation:

$$\text{Log}_e(W) = \text{Log}_e(a) + b * \text{Log}_e(L)$$

Where ‘W’ stands for weight (g), ‘L’ stands for length (mm), ‘a’ is constant, and ‘b’ is isometric growth dimension (Mazlan & Seah, 2006; Kishakudan, 2012). The constant ‘a’ and ‘b’ were derived by the method of linier least squares. The growth dimensions, ‘b’, from both sites (Banyuwangi and Tuban) were compared through t-distribution, $\alpha = 0.05$ following procedure in SPSS v.16.

Difference samples were obtained for morphometric approach. Prior to measurement, all samples were preserved in formaldehyde 4% for 48 hours, diluted with running water for another 48 hours, and permanently stored in saturated alcohol (96%). Morphometric measurements (a straight distance between two anatomical landmarks) were based on Lagler *et al.* (1977), recorded to the nearest 0.1 mm using dial caliper (**Fig. 2**; see also Wiadnya *et al.*, 2014).

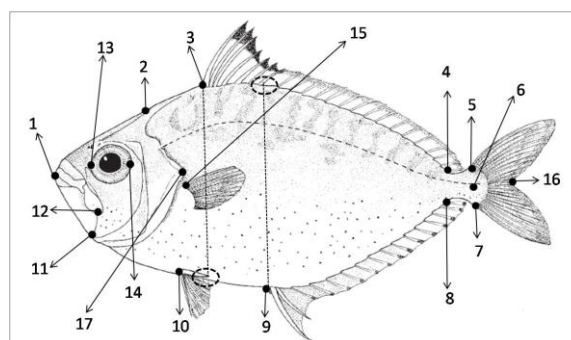


Fig. 2. Point of anatomical landmarks used as bases for morphometric measurements (figure was redrawn from Carpenter & Niem, 2001); SL = straight distance between 1-6; FL = 1-16; DBD = 3 to abdomen; ABD = 9 to dorsal; MBD = usually lays between DBD and ABD; PDL = 1-3; PAL = 1-9; PVL = 1-10; PPL = 1-15; UpCL=4-5; LoCL = 7-8; DFB = 3-4; AFB = 8-9; HL = 1-17; NL = 1-2; SNL = 1-13; OBD = 13-14; UpML = 1-12; LoML = 1-11; POL = 14-17

It consisted of 20 measurements for each individual sample: standard length (SL), fork length (FL), dorsal body depth (DBD), anal body depth (ABD), maximum body depth (MBD), pre-dorsal length (PDL), pre-anal length (PAL), pre-pelvic length (PVL), pre-pectoral length (PPL), upper caudal peduncle length (UpCL), lower caudal peduncle length (LoCL), dorsal fin base (DFB), anal fin base (AFB), head length (HL), nuchal length (NL), snout length (SNL), orbit diameter (OBD), Upper maxilla length (UpML), lower maxilla length (LoML), and post-orbital length (POL). Truss-morphometries were constructed (Sparks and Chakrabarty, 2007; Chakrabarty *et al.*, 2010) based on SL, except for NL, SNL, OBD, UpML, LoML, and POL that used HL throughout. For geometric

morphometric analysis, Principal Component Analysis (PCA) was performed to show the axes on which species groups are best distinguished by shape. The calculation was supported with computer-based software of SPSS ver.16.0, and graph construction based on Excel program.

Genetic (DNA sequence)

Four individuals, two from each site were sampled for genetic study. Total DNA genome were collected from dorso-lateral tissue of the fish, preserved in acetone, and stored at -50°C prior to laboratory procedure. DNA extraction (Asahida *et al.*, 1996) was performed in 1.5 ml volume containing 400 μl TNESU 8 buffer extract (TNES-Urea: 8 M urea; 10 mM Tris-HCl, pH 7.5; 125 mM NaCl; 10 mM EDTA; 1% SDS), and 0.8 mg Proteinase K. The mix solution was incubated at 36°C for 8 h. The DNA was extracted with Phenol-Chloroform (1:1), 2 ethanol, 0.3 M NaCl, and TE Buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). Partial mt DNA región COI was amplified using PCR with primer (Ward *et al.*, 2005):

FishF1-5' TCAACCAACCACAAAGACATTGGCAC3'
and,

FishR1-5' TAGACTTCTGGGTGGCCAAAGAATCA3'

PCR was performed at 10 μl volume containing dd H_2O 5.65 μl , 10X Fast Buffer 1 μl , dNTP mix 1 μl , each primer of 0.5 μl , SpeedSTAR *taq* polymerase 0.05 μl , and DNA template 0.5 μl based on protocol in Takara Inc., adjusted for Taq Enzyme SpeedSTAR HS DNA polymerase. PCR were carried out over 30 cycles with program setting: denaturation at 98°C for 5s, annealing at 55°C for 15s, and extension at 72°C for 20s. PCR product, after visualized in 1.2% agarose gel, was purified follows Kit protocol of GE ExoSAP-IT. Sequencing was done based on BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystem 3500). The sequence was aligned (reverse complement, pairwise alignment, and consensus) using BioEdit (Hall, 1999). Phylogenetic reconstruction was based on maximum-likelihood method, bootstrap method with 1000 replicates and all parameters were set at default.

Results and discussions

Based on both approaches, the species used in this study was morphologically and genetically confirmed as *Photopectoralis bindus* (Valenciennes, 1835). The most distinguishing characters are: prominent orange color of membrane of spinous dorsal fin at half-distal part, that ventrally separated with a line of dark or black color; dorso-lateral part of the body was ornamented with dark irregular vermiculate or semi-circular marking in a zigzag pattern, starting from behind the head and ending closely to the end of soft dorsal ray (laterally extending down to middle half of the body, through lateral line); a black curved band following posterior angle of the lower maxilla; and a golden yellow color at the upper part of the eye (**Fig. 3**).

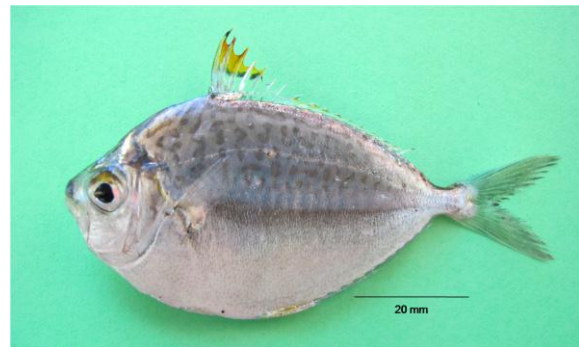


Fig. 3. Photograph of species *Photopectoralis bindus* (Valenciennes, 1835) collected from Banyuwangi (Lat. -8.218389 ; Lon. 114.386250) that used for species identification.

More general characters include: body deep and compress; antero-ventral profile more convex than antero-dorsal region in front of dorsal fin; occipital region shows a slight concavity; short but sharp nose with black melanophore at the tip; mouth terminal and pointed forward or slightly downward when protracted; commencement of mouth when close was above the lower level of the eye; lower maxilla profile almost straight; lateral line was initially clear but became obsolete from the end of dorsal fin ray; and caudal fin was deeply forked. Some additional characters that can be observed from the fresh samples are scattered melanophore along the ventral part of the body; and concentrated (dots)

melanophore at the middle of the body, forming a longitudinal line from behind pectoral fin to the end of soft dorsal ray (**Fig. 3**); anterior part of the third and fourth dorsal fin spine were serrated, anterior

part of the third anal fin serrated, and as well as the lower part of opercle. Morphometric characters of the species are compiled in **Table 1**.

Table 1. Morphometric measurement used to identify the species of *Photopectoralis bindus* (Valenciennes, 1835) in East Java (n = 10).

	Means	Max.	Min.
Standard length, SL (mm)	69.5	77.8	65.3
As percentage of SL:			
Head length, HL	28.9	29.9	27.6
Maximum body depth	59.6	61.0	56.2
Anal body depth	57.0	59.0	53.8
Dorsal body depth	57.4	58.7	54.3
Pre-dorsal length	44.0	46.0	40.9
Pre-pelvic length	42.1	43.1	40.0
Pre-anal length	57.7	58.3	56.7
Dorsal-fin base	65.6	67.7	64.2
Anal-fin base	52.6	55.9	50.4
As percentage of HL			
Snout length	32.6	35.2	30.8
Orbit diameter	35.8	37.4	34.5
Post-orbital length	33.4	34.6	31.6
Pre-pectoral length	107.2	110.8	103.9

Isometric growth dimension

Sample size of individuals from Tuban used in the calculation was about 30 mm larger than that of Banyuwangi (**Fig. 4A** and **4B**). Growth dimension (b) of sample from Banyuwangi was statistically higher ($p < 0.05$) than 3, indicating allometric growth dimension, with confidence interval for 'b' was $3,071 \leq b \leq 3,219$ (**Fig. 4C**). The growth dimension of *P. bindus* from Tuban was found to be isometric, with confidence interval of b was ranging between $2,952 \leq b \leq 3,080$ (**Fig. 4D**). However, using t-test of statistical analysis indicated that the difference was not significant at $\alpha = 0.05$. Length-weight relationship of *P. bindus* from both sites are:

$$W = 0.0000142 * L^{3.145} \text{ (n = 123; r = 0.9915) for Banyuwangi, and}$$

$$W = 0.0000556 * L^{3.016} \text{ (n = 97; r = 0.9945) for Tuban}$$

Result of isometric growth dimension of the same species measured in Chennai Coast (Kishakudan & Reddy, 2012) was quiet similar with this result, as follows:

$$\text{Males} \quad : W = 0.00001318 * L^{3.0240} \text{ (r = 0.9857)}$$

$$\text{Females} \quad : W = 0.00001342 * L^{3.0238} \text{ (r = 0.9869)}$$

$$\text{Indeterminate} \quad : W = 0.00000919 * L^{3.0348} \text{ (r = 0.9602)}$$

Considering there was no significant difference between sex, Kishakudan & Reddy (2012) pooled all the data to derive singular equation, and it resulted in a new equation of:

$$W = 0.000011989 * L^{3.0515} \text{ (r = 0.9788)}$$

This growth dimension lays in between Banyuwangi and Tuban. The growth dimension of *P. bindus* was found to the highest compared to *Secutor (Deveximentum)* and *Gazza* collected from the same fishing ground (Kishakudan & Reddy, 2012).

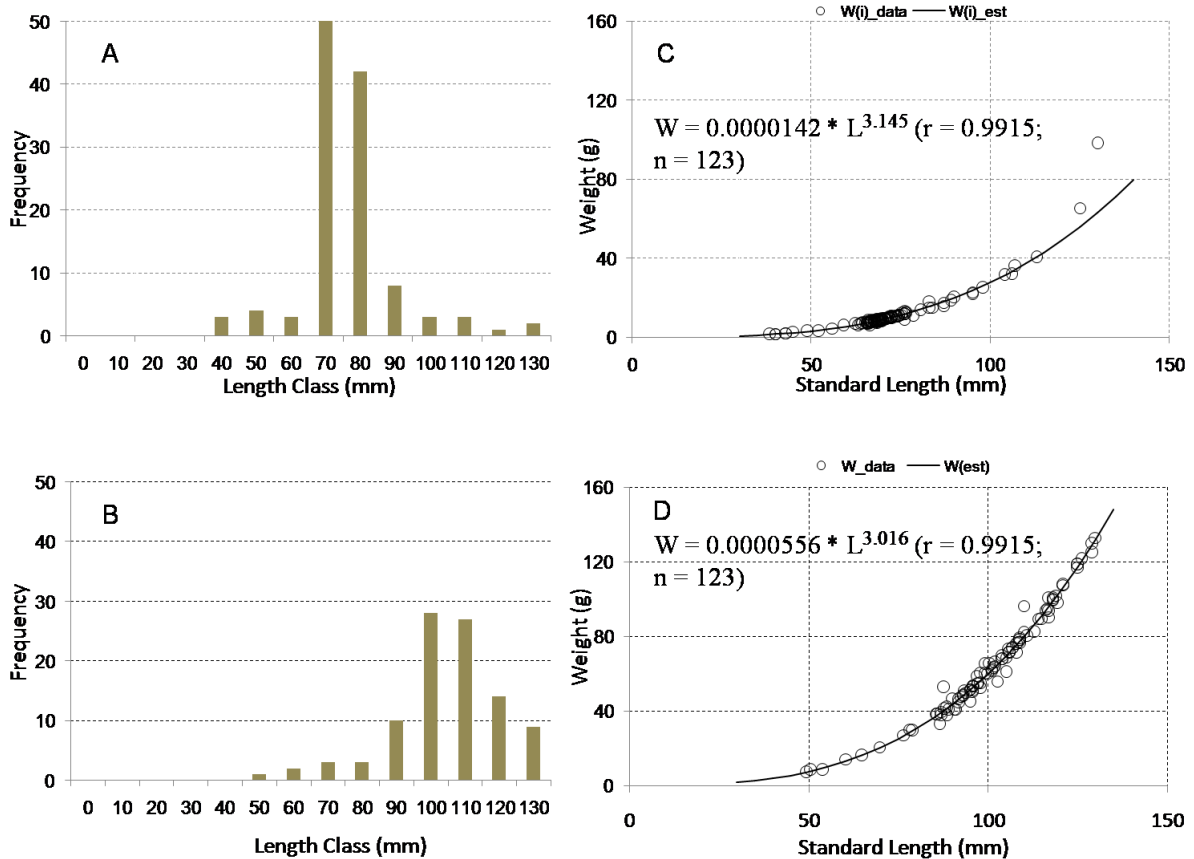


Fig. 4. (A) Length frequency of samples from Banyuwangi used for growth dimension analysis; (B) samples from Tuban; (C) isometric growth dimension derived from length-weight equation (Banyuwangi); (D) isometric growth dimension from Tuban.

Morphometric characters

Based on analysis of variance, there was no single geometric morphometric difference ($p > 0.05$) among 20 characters of *P. bindus* between two sites. Cross correlation matrix (r) among the 20 characters also indicated that there was no strong relationship. Principal Component Analysis (PCA) can reduced the parameters into six main components. PC1 explained around 20% of the variation due to ratio between body depth and standard length, and between anal fin base with standard length. However, both characters were not significant to show the difference in body shape due to difference geographical areas, Banyuwangi and Tuban.

Plot of Principal Component (PC1) with PC2 of *P. bindus* from both sites, Banyuwangi and Tuban, was indicated in Fig.5. It shows that geometric

morphometric body shape of both samples are overlap one to the other.

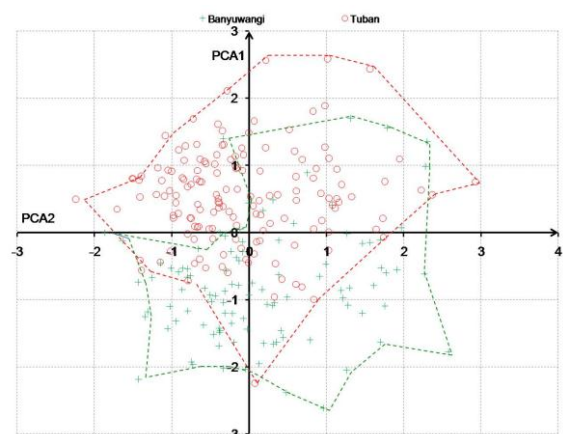


Fig. 5 Plot between Principal Component (PC1) and PC2 for morphometric of samples from Banyuwangi (+) and Tuban (o)

Chakrabarty *et al.* (2010) used morphometric characters (Canonical Variates Analysis) to investigate shape between two groups of type specimen of *Equulites leuciscus*. Graphical plot between Canonical Variates (CV1) and CV2 resulted in really separate groups indicate difference subgroup that finally considered as different species. From the result of PCA plot of this research, *P. bindus* collected from two sites (Banyuwangi and Tuban) was considered as one stock population. Distance of two sites was calculated about 415 km. Barber *et al.* (2000) showed a genetic difference of mantis shrimp for every 300 km apart, in Java Sea. It seems the theory does not match for leioognathid's species.

Phylogenetic analysis

Only three sequences were used in phylogenetic analysis, two sequences from Banyuwangi (WEKS35-15, and WEKS35-16), and one from Tuban (WEKS35-3). One of the sequence was in low quality and did not used in the analysis. Alignment of all three sequences (666 bp) resulted in 100% similarity to each other. These three sequences were closely resemble to sequence of *Leioognathus bindus* collected from the Philippines (Sparks & Dunlap, 2004), with 99.2% similarity (alignment at total 632 bp).

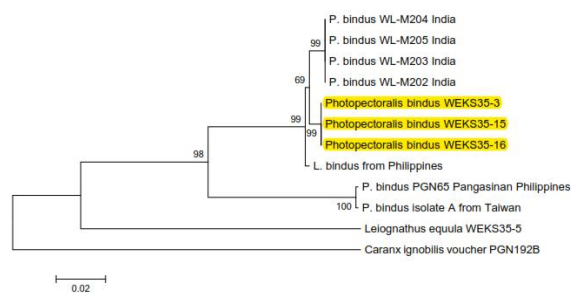


Fig. 6 Phylogenetic reconstruction (maximum likelihood method) of sequences mt DNA region COI of samples from Banyuwangi (WEKS35-15 and WEKS35-16) and Tuban (WEKS35-3) compared with the same species found in Philippines, India, and Taiwan.

Alignment with another four sequences of the same species from another region, India (Lakra *et al.*, 2011) resulted in 99.0% similarity. Finally, it shows 90.5%

similarity with isolate found from Northern Philippines (Pangasinan) and Taiwan (**Fig. 6**).

Individuals found in East Java, India, and together with Philippines can be considered as one species population or one stock of *P. bindus*, being genetically resemble ($\geq 99\%$ similarity to each other). This was also supported with isometric growth dimension, 'b', of samples from East Java that was similar to that collected from India (Kishakudan & Reddy, 2012). On the contrary, the same species found from Pangasinan Northern Philippines (ncbi.nlm.nih.gov), and Taiwan (Chakrabarty *et al.*, 2011) can be treated as another population that is genetically different from East Java, Southern Philippines, and India.

In short, it can be concluded that *P. bindus* found from Banyuwangi and Tuban can be treated as one single population, having similar isometric growth dimension, morphometric characters, and genetic (mt DNA region COI). The stock distribution of this population may reach southern to central Philippines, and India. Separate stock population can be treated with the same species found in Northern Philippines (Pangasinan), and Taiwan (both areas are very close to each other). So, geographical distribution of the species, *P. bindus*, was far longer distance than was thought before, from the analogy of Mantis Shrimp at Java Sea (Barber *et al.*, 2000).

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