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MORPHOMETRIC ANALYSIS OF *TRIANCHORATUS* PRICE & BERRY, 1966 (MONOGENEA: HETERONCHOCLEIDINAE) FROM *CHANNA* SPP. (OSTEICHTHYES: CHANNIDAE) OF PENINSULAR MALAYSIA

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ABSTRACT. **–** The morphometric data of anchors of 448 specimens belonging to four species of *Trianchoratus* Price & Berry, 1966, from *Channa lucius* (Cuvier) and *C. striata* (Bloch) were statistically analysed using principal components analysis (PCA) and Fisher's linear discriminant (LD) analysis. Both methods were able to differentiate the 448 specimens into four groups which correspond to the four species of *Trianchoratus* viz. *T. malayensis* Lim, 1986, *T. pahangensis* Lim, 1986, *T. longianchoratus* Tan & Lim, 2009, and *T. ophicephali* Lim, 1986, based on the over all size of the three developed anchors and the two main morphometric descriptors are the inner length and outer length of the dorsal anchor. PCA and one-way multivariate analysis of variance (MANOVA) of the individuals within each species indicated the presence of groups or intraspecific morphometric variants within *T. malayensis*, *T. pahangensis* and *T. ophicephali*. There are indications of morphovariants within *T. longianchoratus*, but more specimens are needed to verify the number of the morphometric variants. Preliminary analyses suggest that the intraspecific morphometric variants could be locality-dependent as in *T. malayensis* and *T. ophicephali*, and partially host-dependent as in *T. pahangensis.* The distinguishing morphometric characters for the four *Trianchoratus* species and for each existing morphometric variants of the three species are presented.

KEY WORDS. **–** *Trianchoratus*, Heteronchocleidinae, Intraspecific variation, Morphometric variant, *Channa lucius*, *Channa striata*.

INTRODUCTION

Morphometric data are used extensively in taxonomy for species differentiation. The wide ranges in morphometric data in species descriptions indicate the presence of morphometric variations in nature. Morphologically similar congeneric species that exhibit substantial morphometric variations within their populations may cause difficulties in taxonomy, raising questions as to whether the observed variations are intraspecific or interspecific. Morphometric variations within monogenean populations have been shown by a number of researchers including Lim (1987), Ergens (1991), Jackson & Tinsley (1995), Geets et al. (1999), Davidova et al. (2005), Huyse et al. (2006), and Simkova et al. (2007).

Several statistical methods, for example, principal component analysis (PCA), discriminant analysis (DA), one-way analysis of variance (ANOVA), multivariate analysis of variance (MANOVA) and factor analysis, have been used by various authors to reveal interspecific and intraspecific morphometric variations in monogeneans (Geets et al., 1999; Mariniello et al., 2004; Dmitrieva et al., 2007; Jackson & Tinsley, 2007; Olstad et al., 2007). PCA is a useful exploratory method that reduces the dimension of a data set, allowing important features to be summarized as two-dimensional PCA plots (Jolliffe, 2002).

In a recent taxonomic investigation on *Trianchoratus* Price & Berry, 1966 (Monogenea: Ancyrocephalidae) from *Channa lucius* (Cuvier) and *C. striata* (Bloch), we noted the presence of groups with small but distinct morphometric differences within some *Trianchoratus* species. Briefly, *Trianchoratus* spp. possess three well-developed anchors (two ventral anchors and one dorsal anchor), one comma-shaped vestigial

dorsal anchor and 14 marginal hooks. The presence of these small but distinct morphometric groups within the *Trianchoratus* species gives rise to questions as to whether these observed differences are interspecific or intraspecific variations especially since many of the *Trianchoratus* spp. possess morphologically similar anchors (Lim, 1986; Tan & Lim, 2009). This issue prompted this present study to analyse morphometric data of *Trianchoratus* spp. In this study, PCA, Fisher's linear discriminant (LD) analysis, MANOVA and other related analyses are used first to group the different individuals of *Trianchoratus* from the channids based on their morphometric data with analyses also performed on the individuals within each of the groups formed to determine if morphometric variants are present.

MATERIALS AND METHODS

The morphometric data for this study were from the type and voucher specimens of four species of *Trianchoratus* deposited in the Parasite Collection at the Zoological Museum of University of Malaya. The four species are *Trianchoratus* viz. *T. malayensis* Lim, 1986, *T. pahangensis* Lim, 1986 and *T. longianchoratus* Tan & Lim, 2009, from *C. lucius* and *T. ophicephali* Lim, 1986, from *C. striata*. The two host species were collected from the riverine swamp-lake Tasik Bera, Endau-Rompin and Bukit Merah Reservoirs (Table 1). The monogeneans were collected and prepared as in Lim (1986).

Although the *Trianchoratus* species have morphologically similar copulatory organs which are different in terms of size they are subjected to distortions due to orientations and are hence difficult to measure. They are therefore not included in the analysis. The vestigial anchor and marginal hooks are also not considered in this analysis because they are either obscured by the larger well-developed anchors or are not disposed properly to be measured. In this study five parameters were taken: inner root (IR), outer root (OR), inner length (IL), outer length (OL) and point (pt), for each of the three well-developed anchors (Fig. 1) using Leica image analysis software (QWin Plus) resulting in a total of 15 variables per monogenean (the numbers attached to the parameters denote the position of the anchors, 1 and 2 for the two ventral anchors and 3 for the dorsal anchor). The measurements of the two ventral anchors are treated as separate datasets. All the measurements were taken by the first author. A total of 448 *Trianchoratus* specimens were measured and identified.

***Statistical analyses*. –** All computations were conducted using R (Version 2.8.1; R Core Development Team 2008), and the scripts used are available upon request. The total data set consisting of 448 individuals were analysed first using PCA. Data sets for each of the four species were then analysed separately to detect if there are any morphometric variants. Biplots were used to aid interpretation of the principal component (PC) axes (to reduce the number of Figures in this paper not all biplots are shown but they are available on request). Since the standard errors of the multivariate means are sufficiently small in all four species, the multivariate means were represented simultaneously as Chernoff faces (Chernoff 1973, Johnson and Wichern 2002). The latter provide quick, visual assessment of similarities and differences among the four *Trianchoratus* species. To assess the strength of the current set of morphometric characters in species classification, Fisher's linear discriminant (LD) analysis was performed on the total data set, with equal prior probabilities for all four species. The classification error rate was estimated using Lachenbruch's method (Johnson & Wichern, 2002).

In addition to these standard analyses, we also explored locality and host factors on the distribution patterns of the intraspecific variants, although this study was not designed for this purpose. Except for the *T. longianchoratus* data set, one-way multivariate analysis of variance (MANOVA) on the first two PC scores were done to check if there are any significant differences in the PC means between different morphometric groups in terms of locality. To see the effect of host factors on morphometric variations, MANOVA was done using 102 *T. pahangensis* from two *C. lucius* from the same locality, Bukit Merah, and possessing almost similar numbers (54 and 48) of *T. pahangensis*. To ensure that results of the statistical analyses could be interpreted confidently checks on assumptions that are associated with each test were performed (Figures not shown but available on request). For MANOVA, chi-square plots were used to confirm multivariate normality of the PC scores (Johnson & Wichern, 2002), while the assumption of equal covariance matrix (for the PC scores in MANOVA; and for the original variables in LD analysis) was checked using Box's test (see Timm, 2002).

RESULTS

***Total dataset*. –** The PCA scatterplot of all 448 individuals shows four distinct clusters which correspond to the four

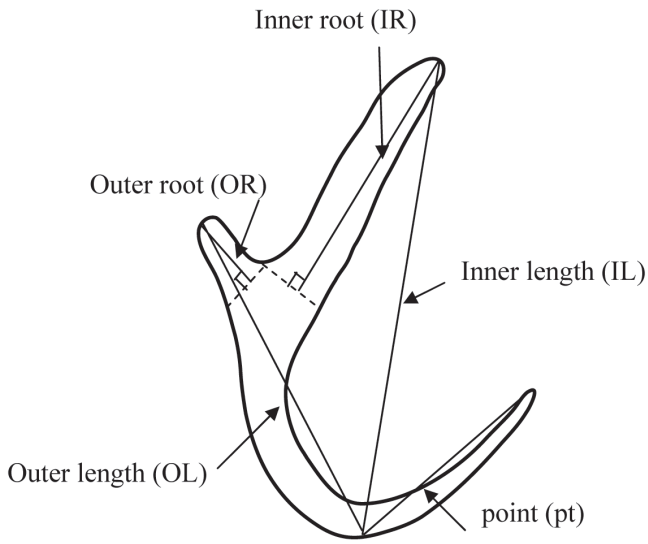

Fig. 1. A well-developed anchor of *Trianchoratus* species showing the basic measurements taken for morphometric analysis.

Table 1. The host and locality distribution patterns of the 448 individuals of *Trianchoratus* spp. used in the morphometric analysis.

***Trianchoratus* spp.**	**Host species**	**Locality**	**No. of individuals used in this study**
T. malayensis	*Channa lucius*	Bukit Merah	58
		Endau-Rompin	36
		Tasik Bera	25
Total			119
T. pahangensis	*Channa lucius*	Bukit Merah	113
		Endau-Rompin	4
		Tasik Bera	42
Total			159
T. longianchoratus	*Channa lucius*	Bukit Merah	25
T. ophicephali	*Channa striata*	Bukit Merah	95
		Tasik Bera	50
Total			145
Grand total			448

Trianchoratus species, essentially clustering 119 specimens as *T. malayensis*, 159 as *T. pahangensis*, 145 as *T. ophicephali* and 25 as *T. longianchoratus* (Fig. 2). The biplot shows that inner length and outer length of the well-developed dorsal anchor (IL3 and OL3) are the main distinguishing characters for the four species of *Trianchoratus* (Fig. 3). The first PC axis (PC1, x-axis), which accounts for 65% of total variation, is an index of the overall size of the three well-developed anchors; it separates the 448 individuals into two groups as shown by the horizontal bar plot in Fig. 2. The second principal component (PC2, y-axis), which explains 20% of total variance, is an index that contrasts the inner lengths (IL1, IL2), inner roots (IR1, IR2) and outer roots (OR1, OR2) of the ventral anchors against the other variables; it separates the *Trianchoratus* specimens into three groups as shown by the vertical bar plot in Fig. 2. Taken together, PC1 and PC2 resolve the 448 individuals into four groups. The Chernoff faces also show the distinctive features of these four species (Fig. 4), which indicate *T. malayensis* and *T. ophicephali* are most similar.

Fisher's LD analysis verifies results of the PCA (Fig. 5). The first LD function (LD1) provides clear separation of *T. longianchoratus* from the three other *Trianchoratus* species (65% of total variation), the second LD function (LD2) separates *T. pahangensis* from the *T. malayensis* – *T. ophicephali* cluster (21% of total variation) and the third LD function (LD3) differentiates between *T. malayensis* and *T. ophicephali* (14% of total variations) (Table 2). Lachenbruch's method verifies that benchmarking the present data set affords 100% correct classification.

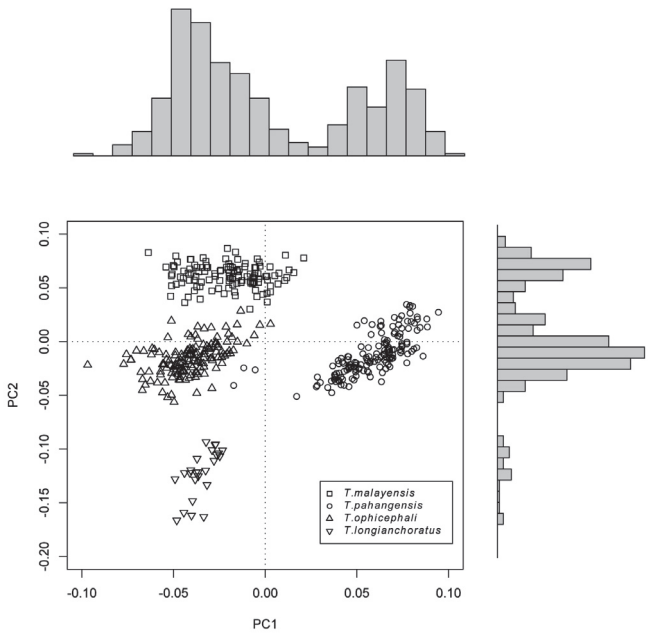

Fig. 2. PCA plot of four species of *Trianchoratus*. The horizontal and vertical barplots indicate one-dimensional summary of the PC axes.

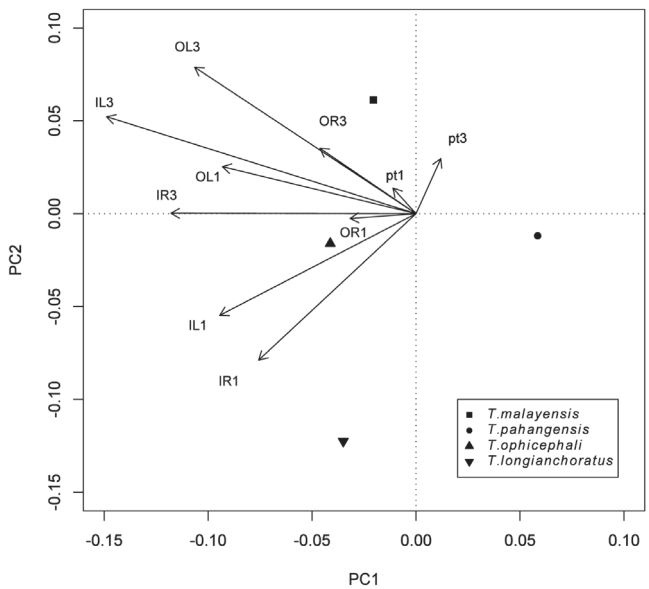

Fig. 3. Biplot of the first two principal components for the four species of *Trianchoratus*, with mean coordinates of the species indicated. Only vectors (IL1, OL1, IR1, OR1 and pt1) from one ventral anchor are shown.

Table 2. Diagnostic morphometric characters of the four *Trianchoratus* species from channid hosts based on Fisher's Linear Discriminant analysis. (IR3, inner root of dorsal anchor; OR3, outer root of dorsal anchor; OL1, outer length of ventral anchor; OL3, outer length of dorsal anchor, the numbers attached to the parameters denote the anchors, 1 and 2 for the two ventral anchors and 3 for the dorsal anchor).

Linear discriminant functions (LD)	Contribution to total variation	LD index	Character traits with large loading magnitude (in µm)	Species distinguished
LD1	65%	LD1 > 5	IR3 ≥ 30 OR3 = 0	*T. longianchoratus*
		LD1 < 5	IR3 ≤ 30 OR3 > 0	other 3 spp.
LD2	21%	LD2 < −1.5	IR3 < 20 OL1 < 30	*T. pahangensis*
		LD2 > −1.5	IR3 > 20 OL1 > 30	other 2 spp.
LD3	14%	LD3 < 0	OL3 > 35	*T. malayensis*
		LD3 > 0	OL3 < 35	*T. ophicephali*

Morphometric variations within each Trianchoratus spp. **–** The results of the PCA and MANOVA for the different individuals of the four species are provided below.

Trianchoratus malayensis**. –** The PCA scatterplots show that the 119 *T. malayensis* could be separated into 3 groups along the PC1 axis with no separation along PC2 (Fig. 6). The scatterplot result is confirmed by the MANOVA which shows all three groups have significantly different mean PC1 scores (p-value < 10^{-13}; Box's test p-value = 0.01), but not for PC2 scores. PC1 accounts for 63% of the total variation.

Locality labels account for slightly over half of the total variations in PC1 (R^2 = 52%), but contribute almost nothing to PC2 (R^2 = 0.6%). In other words the size of the anchors are different in three localities: *T. malayensis* from Bukit Merah has the largest overall size (negative scores) of the three well-developed anchors, followed by *T. malayensis* from Tasik Bera with medium size anchors and *T. malayensis* from Endau-Rompin, which has the smallest size anchors. The distinguishing character for these three locality-dependent groups is the inner length of the well-developed dorsal anchor (IL3) (Table 3).

Trianchoratus pahangensis**. –** The 159 individuals of *T. pahangensis* are separated into three groups in the PCA scatterplot (Fig. 7). Along the PC1 axis, the 159 *T. pahangensis* are divided into two groups. PC1 accounts for 72% of the total variation and separates the monogeneans with large anchors from those with smaller anchors. The group with smaller size anchors is further sub-divided into

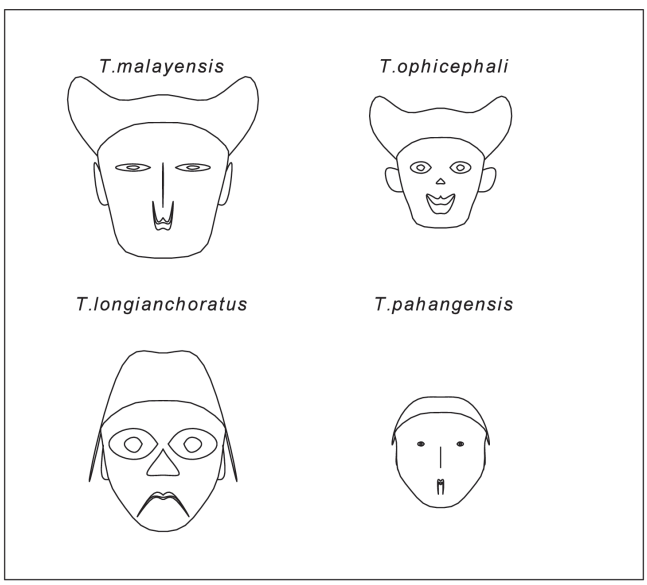

Fig. 4. Chernoff faces: graphical summary of the mean of each of the 15 variables in the four *Trianchoratus* species represented as different facial features.

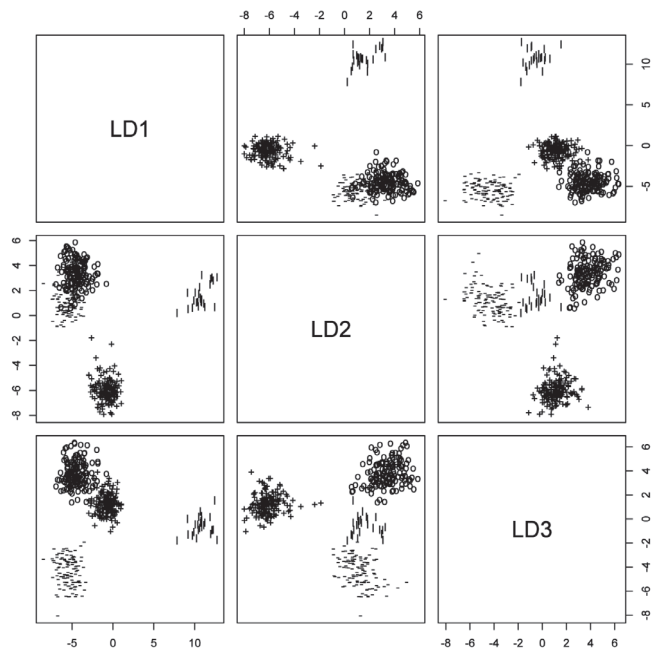

Fig. 5. Fisher's linear discriminant analysis plots of the first three LD functions, which account for 65%, 21% and 14% of the total variation, respectively. Indicators: *Trianchoratus malayensis* (-); *T. pahangensis* (+); *T. ophicephali* (o); *T. longianchoratus* (l).

two groups based on the inner length (IL1 and IL2) and inner root (IR1 and IR2) of the ventral anchors along the PC2 axis. The biplot indicates that the outer length and inner root of the ventral anchors (OL1 and IR1) (Table 3) are the characters which strongly influence the PC1 and PC2 coordinates of one individual on the PCA scatterplot.

The locality labels only explain a very small fraction of total variations in PC1 (R^2 = 2%) and PC2 (R^2 = 0.2%). This indicates that differences in the multivariate means of PC1 and PC2 scores in populations from the different localities are unlikely to be biologically significant, even though the MANOVA result is statistically significant (p-value = 0.02). In fact the latter needs to be cautiously interpreted since the equal covariance assumption in MANOVA is violated (Box's test result: p-value = 0.03) This suggests that the variants are not dependent on locality and this is supported by the presence of individuals from different localities in the three groups generated (Fig. 7).

It should be noted that in Fig. 7 we have colour-coded all the monogeneans belonging to two *C. lucius* (Host 1 and Host 2) from the Bukit Merah. The scatterplot (Fig. 7) shows that Host 1 possesses variant 1 and variant 3 while Host 2 has variant 2 and variant 3. The MANOVA results (p-value < 0.001; Box's test p-value = 0.06) indicate significant differences in the mean of PC1 and PC2 scores of *T. pahangensis* from Host 1 and Host 2. The above analyses suggest that intraspecific morphometric variants appear to be only partially dependent on host factors.

***Trianchoratus ophicephali*. –** The PCA scatterplot of the 145 individuals of *T. ophicephali* (Fig. 8) does not show obvious separations, although PC1 and PC2 account for 51% and 10% of total variation, respectively. However, the MANOVA result indicates that the Tasik Bera and Bukit Merah populations have significant differences in mean PC1 scores (p-value < 0.001), but not in the mean PC2 scores. Locality labels account for about one-fifth of total variations (R^2 = 21%) on PC1 indicating some degree of locality influence. The morphometric variants of *T. ophicephali* in the two localities are distinguished by two main traits: IL1 and IL3 (Table 3).

***Trianchoratus longianchoratus*. –** The PCA scatterplot for the 25 individuals of *T. longianchoratus* (Fig. 9) indicates the presence of two clusters along PC1 axis, one in the direction of negative PC1, and another in the opposite direction. The cluster in the direction of the negative PC1 has more individuals which are further separated into two clusters by PC2. Biplot indicates that IR1 is the important distinguishing character for the 25 individuals. Although there appears to be three clusters in the PCA scatterplot of *T. longianchoratus* (Fig. 9), it would be premature to declare the number of intraspecific morphometric variants present until more samples of *T. longianchoratus* are analysed.

DISCUSSION

Both PCA and Fisher's LD analysis are able to separate the 448 individuals of *Trianchoratus* into groups which correspond to their respective species, 119 *T. malayensis*, 159 *T. pahangensis*, 145 *T. ophicephali* and 25 *T. longianchoratus* (Fig. 2) based on morphometric differences of their anchors, particularly the overall size of three well-developed anchors. Fisher's LD provides diagnostic morphometric characters for the four *Trianchoratus* spp., which are summarised in Table 2. These analyses indicate the suitability and effectiveness of using the present set of morphometric variables for

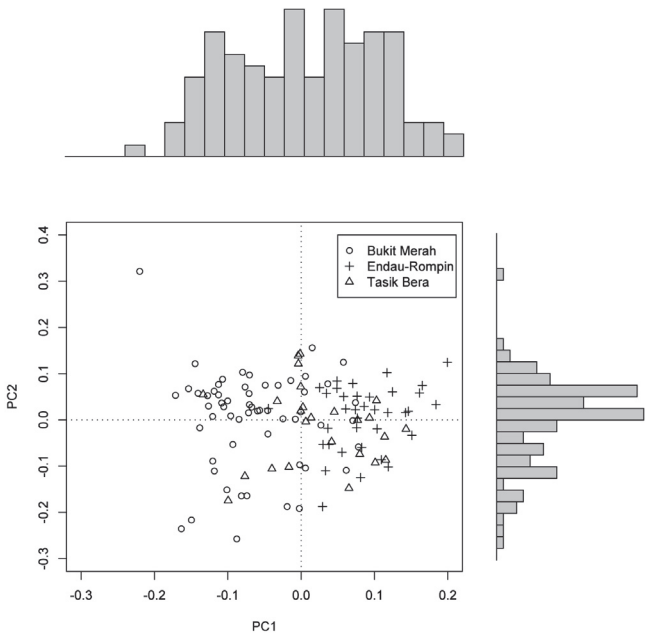

Fig. 6. PCA plot of *Trianchoratus malayensis*, with geographical origin of data indicated. The horizontal and vertical barplots indicate one-dimensional summary of the PC axes.

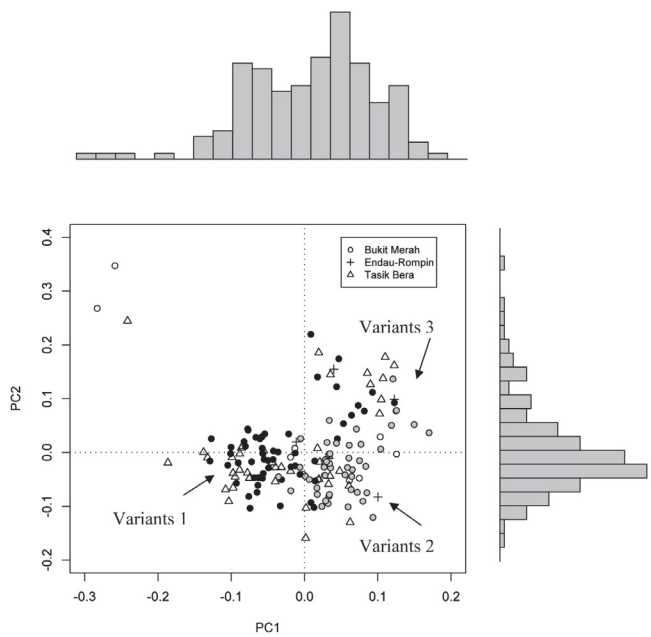

Fig. 7. PCA plot of *Trianchoratus pahangensis.* The horizontal and vertical barplots indicate one-dimensional summary of the PC axes. (Black dots = Host 1; Grey dots = Host 2).

Table 3. Distinguishing morphometric traits of the intraspecific variants of the four *Trianchoratus* species from channid hosts. (The abbreviations for the measured parameters are as in the text).

***Trianchoratus* spp.**	***Channa* spp.**	**Intraspecific variants**	**Mean (standard deviation) of major variables (in µm)**	**Possible Factors**
T. pahangensis (n = 159) PC1 index = overall size of 3 anchors PC2 index = IL1 + IL2 + IR1 + IR2	*C. lucius*	1	OL1 = 24.3 (0.1) IR1 = 26.1 (0.2)	Host-dependent (Partial)
		2	OL1 = 22.6(0.1) IR1 = 23.1(0.2)	
		3	OL1 = 24.4(0.2) IR1 = 20.4(0.2)	
T. malayensis (n = 119) PC1 index = overall size of 3 anchors PC2 index = no separation on PC2	*C. lucius*	1	IL3 = 50.9 (2.9)	Locality-dependent (Bukit Merah)
		2	IL3 = 47.3 (3.0)	Locality-dependent (Endau-Rompin)
		3	IL3 = 43.8 (2.5)	Locality-dependent (Tasik Bera)
T. ophicephali (n = 145) PC1 index = overall size of 3 anchors PC2 index = IL1 + OL1 + IR1 + OR1 against IL3 + OL3 + IR3	*C. striata*	1	IL1 = 49.5 (0.2) IL3 = 43.6 (0.2)	Locality-dependent (Bukit Merah)
		2	IL1 = 52.3 (0.3) IL3 = 45.2 (0.3)	Locality-dependent (Tasik Bera)
T. longianchoratus (n=25) PC1 index = IR1 + IR2 + OL3 + IR3 + OR3 + pt3 against other variables PC2 index = OL3 + OR3 against other variables	*C. lucius*	3* (needs confirmation)		

discriminating species of *Trianchoratus* (see Lim, 1986; Tan & Lim, 2009). Fisher's LD analysis (Fig. 5) using the current dataset gives excellent classification results as indicated by the 0% estimated actual error rate by Lachenbruch's method, thus providing a powerful species classification tool for the future. The automated assignments of unknown specimens of *Trianchoratus* can be directly done using Fisher's LD analysis with the current data set as reference.

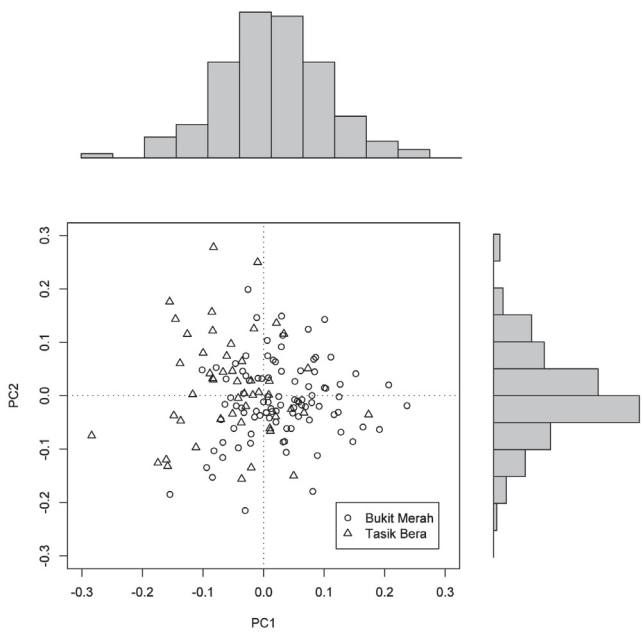

Fig. 8. PCA plot of *Trianchoratus ophicephali* with geographical origin of data indicated. The horizontal and vertical barplots indicate one-dimensional summary of the PC axes.

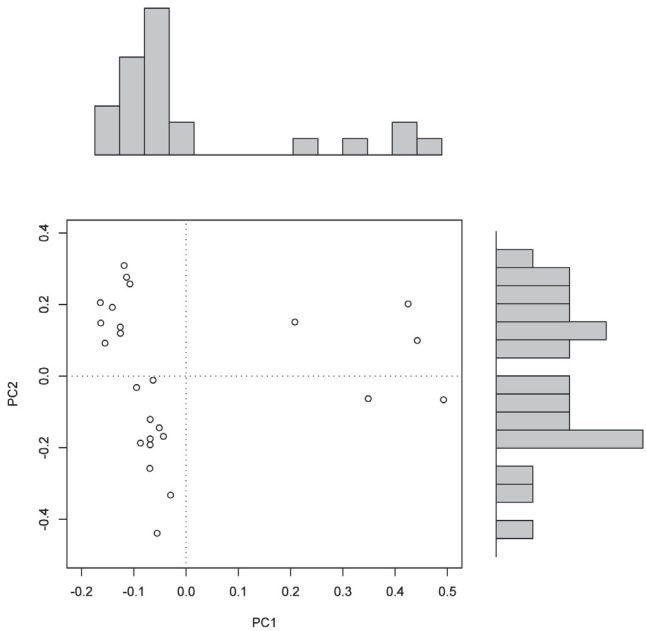

Fig. 9. PCA plot of *Trianchoratus longianchoratus*. The horizontal and vertical barplots indicate one-dimensional summary of the PC axes.

For the present study, PCA has been successful in detecting the presence of groupings or intraspecific morphometric variants within *T. malayensis*, *T. pahangensis* and *T. ophicephali* and possibly in *T. longianchoratus* (Figs. 6, 7, 8 & 9) and the numbers of groups vary according to the species. The morphometric characters defining these intraspecific morphometric variants are also different among the four *Trianchoratus* species (Table 3). Our preliminary analyses indicate that the factors affecting intraspecific morphometric variations vary according to species: *T. malayensis* and *T. ophicephali* are affected by locality differences while *T. pahangensis* is influenced by host factors. It should be noted, however, that the locality labels explains only 21 % of the variations in *T. ophicephali* (R^2 = 21%) compared to 52% in *T. malayensis* (R^2 = 52%). Host and locality factors are common explanations for observed intraspectific morphometric variations in other investigations (Rohde, 1991; Ergens, 1991; Shinn et al., 2001), but it is difficult to explain why intraspecific morphometric variants of congeneric species (*T. malayensis* and *T. pahangensis*) are influenced by different factors, viz. locality and hosts, respectively. One explanation is that the inherent genotypic variations in the cross-fertilising hermaphroditic monogeneans (see Lim, 2002) respond differently to different factors or interactions of factors.

The morphometric variants in *T. ophicephali* are less obvious compared to those in *T. malayensis* which could be due to the lack of congeneric competitors in *T. ophicephali* and hence a lack of impetus to change. Even though intraspecific morphometric variants appear to be present within the scatterplot for *T. longianchoratus*, it is difficult to define the variants due to small sample size (25 specimens) (Fig. 9). Larger sample size is a necessary requirement to statistically define intraspecific morphometric variants within species populations.

Improved sampling designs are needed to ascertain the factors (environmental conditions, host factors and inherent genotypic variations) responsible for the intraspecific morphometric variations observed here. Adequate samples from more localities with similar and different environmental conditions as well as hosts of different sizes (age groups) are needed. Locality-dependent intraspecific morphometric variants could be used as potential locality indicators to determine the geographical origin of their fish hosts, but should be used with caution since this requires understanding of the role of each species within its community.

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