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Morphometric and meristic variation in two congeneric archer fishes *Toxotes chatareus* (Hamilton 1822) and *Toxotes jaculatrix* (Pallas 1767) inhabiting Malaysian coastal waters*

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Abstract: A simple yet useful criterion based on external markings and/or number of dorsal spines is currently used to differentiate two congeneric archer fish species *Toxotes chatareus* and *Toxotes jaculatrix*. Here we investigate other morphometric and meristic characters that can also be used to differentiate these two species. Principal component and/or discriminant functions revealed that meristic characters were highly correlated with pectoral fin ray count, number of lateral line scales, as well as number of anal fin rays. The results indicate that *T. chatareus* can be distinguished from *T. jaculatrix* by having a greater number of lateral line scales, a lower number of pectoral fin rays, and a higher number of anal fin rays. In contrast, morphometric discriminant analyses gave relatively low distinction: 76.1% of fish were ascribed to the correct species cluster. The observed morphometric differences came from the dorsal and anal spines lengths, with *T. chatareus* having shorter dorsal and longer anal spines than *T. jaculatrix*. Overall, meristic traits were more useful than morphometrics in differentiating the two species; nevertheless, meristics and morphometrics together provide information about the morphological differentiation between these two closely related archer fishes.

Key words: Archer fish, *Toxotes chatareus*, *Toxotes jaculatrix*, Species identification, Multivariate analysis
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1 Introduction

Toxotes chatareus and *Toxotes jaculatrix*, along with the rest of the family of Toxotidae, are well known for their impressive hunting techniques: they use jets of water to knock aerial insects into the water, where they can then be eaten (Gill, 1909; Allen, 2004). Despite this specialized hunting technique, they are opportunistic feeders and will consume a wide range of prey, including terrestrial insects, shrimps, and teleosts (Simon and Mazlan, 2008; 2010; Simon *et al*., 2008; 2009). Both species are euryhaline, inhabiting primarily the brackish mangroves of the South Pacific and Indian Oceans, although they can also be found far upstream in fresh waters and more saline coastal waters (Allen, 1978; 2001; Froese and Pauly, 2005; Temple, 2007; Temple *et al*., 2010). These fishes are relatively difficult to collect within the complex rooting systems of mangrove forests, together with their sharp vision and

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fast swimming speed (Blaber, 2000). Little is known about the biology and ecology of these fascinating fishes. Studies of population growth of *T. chatareus* and *T. jaculatrix* were recently performed by Simon *et al*. (2009), reporting that growth pattern by length-weight relationship for the sexes differed, and exhibited positive allometric growth (male, female, and combined sexes of *T. chatareus*; female and combined sexes of *T. jaculatrix*) and isometric growth (male samples of *T. jaculatrix* only). Allen *et al*. (2002) and Pethiyagoda (1991) reported that *T. chatareus* females are highly fecund, and release between 20000 and 150000 eggs. In addition, Simon *et al*. (2009) briefly described some biological aspects of *T. chatareus* and *T. jaculatrix*.

The genus *Toxotes*, particularly *T. chatareus* and *T. jaculatrix*, is very similar in appearance and until now external markings and/or number of dorsal spines (simple descriptive statistics) have been used to differentiate these two species (Allen, 2001; 2004). A detailed study using morphometric and meristic analyses of *T. chatareus* and *T. jaculatrix*, or any toxotid, has not been documented in Malaysia or elsewhere. Interspecies variation among fishes and other aquatic animals based on morphometric analyses are common (Luthy *et al*., 2005; Conde-Padín *et al*., 2007; Sin *et al*., 2009). Therefore, our aim was to find other morphological distinctions that may also be used to differentiate these closely related species more precisely along with Allen (2001; 2004)'s description.

2 Materials and methods

2.1 Sample collection

Sampling was carried out in the coastal waters of Matang (4°50′37″ N; 100°38′00.43″ E), the northern part of Peninsular Malaysia, from July 2007 to July 2008. A total of 128 fish (*T. chatareus*, *n*=63; *T. jaculatrix*, *n*=65) were collected using three-layered trammel, cast, and scoop nets. Nets were set up at random throughout the study areas. The mesh sizes (stretched length) were 4.2, 6.5, and 7.5 cm for the trammel net and 2.0 cm for the cast net, respectively. The mesh size of the scoop net was 1.5 cm. Net lengths were 2000 cm for the trammel net, and 250 cm for the cast net, and the scoop net diameter was 40 cm. Specimen identification was carried out in the field based on the external markings (bars and spots) and/or number of dorsal spines, according to Allen (2001; 2004). Samples were frozen soon after collection and defrosted for laboratory analyses, which took place about two months later to ensure that all fish were analyzed following a similar period of freezing. The weight of the fish was recorded with a precision balance to the nearest 0.01 g and sex was determined by internal gonad inspection, which was done after obtaining the meristic counts and morphometric measurements. Digital photographs were taken on the left side of each fish with a Canon Power Shot A640 (10.0 mega pixels; Canon, Tokyo, Japan) camera, and body coloration was recorded for species identification (Figs. 1a and 1b).

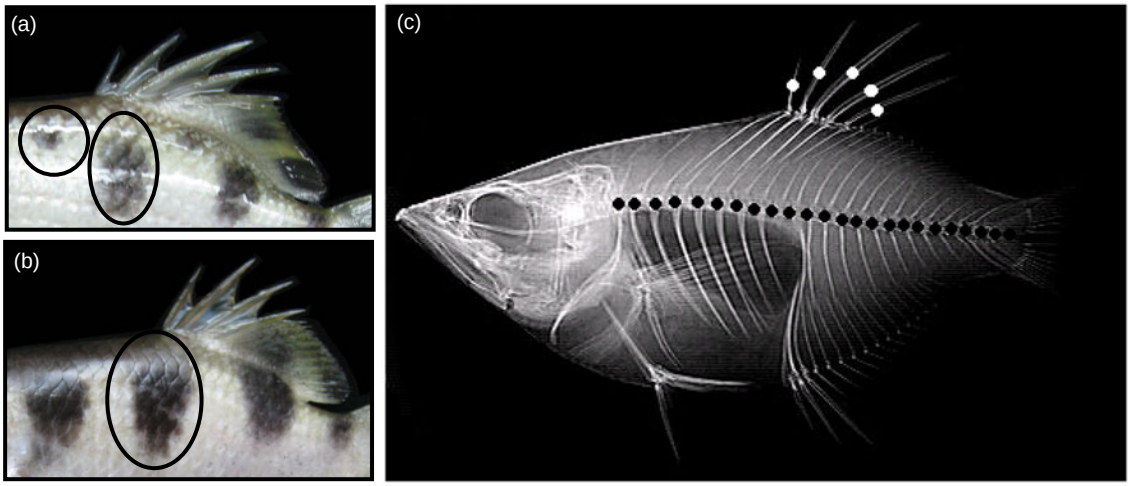

Fig. 1 Differences in body coloration between *Toxotes chatareus* (a) with 6–7 alternating vertical black bars and black spots and *Toxotes jaculatrix* (b) with 4–5 vertical black bars, and (c) radiographic image of *T. chatareus* showing number of vertebrae (black spots) and number of dorsal fin spines (white spots)

2.2 Morphometrics

In the laboratory, a total of 31 morphometric measurements were recorded for each fish. Measurements generally followed the description by Allen (2004) and Pouyaud *et al*. (1999). The 31 morphometric characters are given in Table 1 and illustrated in Fig. 2. Morphometric measurements were taken from the left lateral aspect, and measured to the nearest 0.01 cm using a digital caliper (absolute digimatic digital calipers, Mitutoyo, Japan).

2.3 Meristics

The total number of vertebrae (TV) was counted from the radiographic images (Fig. 1c) taken with a microradiographic unit (M60, Softex, Tokyo, Japan). A stereo microscope (Stemi DV4/DR, Zeiss, Oberkochen, Germany) was used for counting fin rays. All meristic characters were counted twice on the same day by the same observer. The number of dorsal fin spines (DS) was excluded in the present study because this trait is constant in the two species

Table 1 Definitions of morphometric measurements and meristic counts of *Toxotes chatareus* and *Toxotes jaculatrix* used in this study

Character	Description	Acronym
31 morphometric measurements		
Standard length	Tip of the upper jaw to the tail base	SL
Pelvic fin length	From base to tip of the pelvic fin	PVFL
Pelvic spine length	From base to tip of the pelvic spine	PVSL
Pectoral fin length	From base to tip of the pectoral fin	PFL
Caudal fin length	From tail base to tip of the caudal fin	CFL
Pre-dorsal length	Front of the upper lip to the origin of the dorsal fin	PDL
Pre-anal length	Front of the upper lip to the origin of the anal fin	PAL
Pre-pectoral length	Front of the upper lip to the origin of the pectoral fin	PPCL
Pre-pelvic length	Front of the upper lip to the origin of the pelvic fin	PPVL
Length of dorsal fin base	From base of first dorsal spine to base of last dorsal ray	LDFB
Length of anal fin base	From base of first anal spine to base of last anal ray	LAFB
First dorsal spine length	From base to tip of the first dorsal spine	LDS1
Second dorsal spine length	From base to tip of the second dorsal spine	LDS2
Third dorsal spine length	From base to tip of the third dorsal spine	LDS3
Fourth dorsal spine length	From base to tip of the fourth dorsal spine	LDS4
Fifth dorsal spine length	From base to tip of the fifth dorsal spine	LDS5
First anal spine length	From base to tip of the first anal spine	LAS1
Second anal spine length	From base to tip of the second anal spine	LAS2
Third anal spine length	From base to tip of the third anal spine	LAS3
Length of soft dorsal ray	From base to tip of the soft dorsal ray	LSDR
Length of soft anal ray	From base to tip of the soft anal ray	LSAR
Mouth height	Maximum vertical measurement of the mouth when completely open	MH
Upper jaw length	Straight line measurement between the snout tip and posterior edge of maxilla	UJL
Lower jaw length	Straight line measurement between the snout tip and posterior edge of mandible	LJL
Length of caudal peduncle	From base of the last anal fin ray to middle of caudal fin fold	LCP
Body depth	Maximum depth measured from the base of the dorsal spine	BD
Body width	The greatest width just posterior to the gill opening	BW
Snout length	The front of the upper lip to the fleshy anterior edge of the orbit	SNL
Eye diameter	The greatest bony diameter of the orbit	ED
Head length	From the front of the upper lip to the posterior end of the opercular membrane	HL
Depth of caudal peduncle	The least depth of the tail base	DCP
9 meristic counts		
Dorsal fin ray	Number of soft fin rays in dorsal fin	DFR
Anal fin ray	Number of soft fin rays in anal fin	AFR
Anal spine	Number of spine in anal fin	AS
Caudal fin ray	Number of caudal fin rays	CFR
Pectoral fin ray	Number of soft fin rays in pectoral fin	PCFR
Pelvic fin ray	Number of soft fin rays in the pelvic fin	PVFR
Pelvic spine	Number of spine in pelvic fin	PVS
Total vertebrae	Total number of vertebrae in vertebral column	TV
Lateral line scale	Number of scale along lateral line	LLS

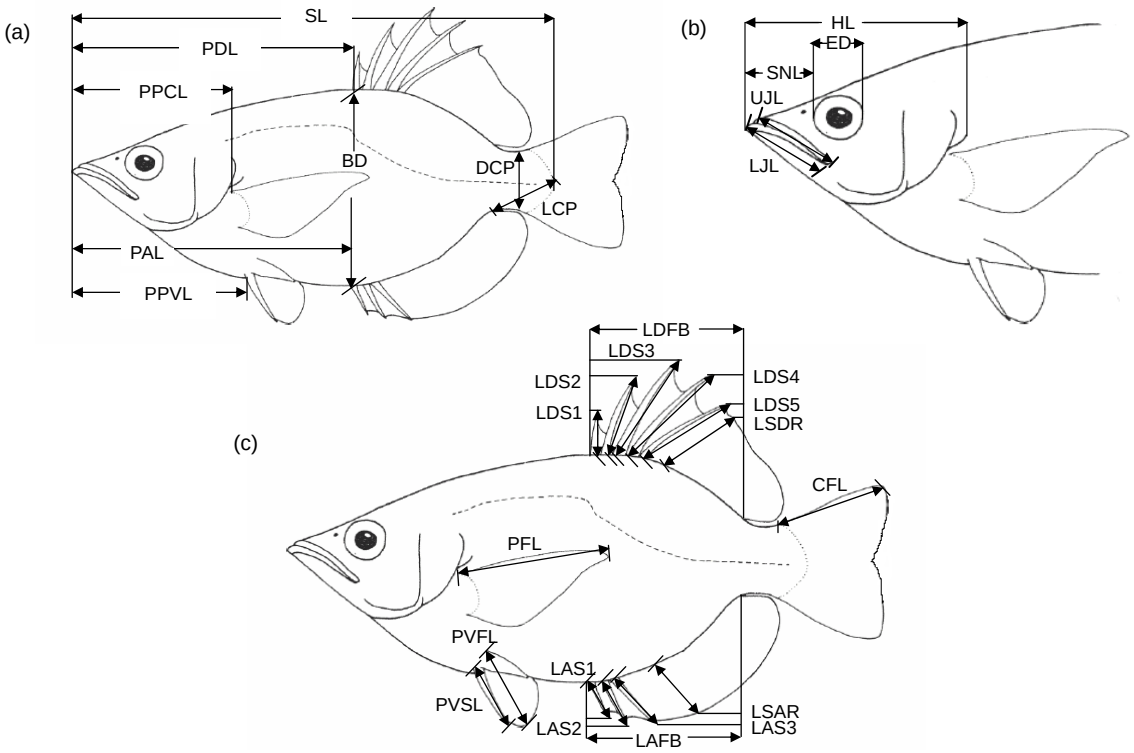

Fig. 2 Morphometric measurements of archer fish
(a) Body measurement; (b) Head measurement; (c) Fin measurement

(*T. chatareus*, DS=5; and *T. jaculatrix*, DS=4), as documented by Allen (2004).

2.4 Statistical analysis

Box plot analysis was performed to examine the distribution and to detect the presence of extreme outliers. Extreme outliers are defined as those which lie more than three times the inter-quartile range to the left or right of the first and third quartiles, respectively. Separate statistical analyses were conducted on morphometric and meristic data since morphometric data are continuous and more susceptible to the environmentally induced variability, while meristic data are discrete and fixed early in development (Ihssen *et al*., 1981; Hermida *et al*., 2005; Turan *et al*., 2006). Spearman's rank correlation indicated that there was a low association between meristic characters and standard length (SL) of the samples. Thus, the meristic characters were not adjusted for size differences. In contrast significant correlations were observed between size and morphometric characters, and allometric effects may accentuate such differences. Therefore, transformation of absolute measurements to size-independent shape characters was performed before the final analysis. In order to eliminate any variation resulting from allometric growth, all morphometric characters were therefore adjusted to an overall mean standard length of 12.63 cm according to the following equation (Reimchen *et al*., 1985; Senar *et al*., 1994):

$$Y'_{ij} = \log Y_{ij} - b_j(\log \mathrm{SL}_i - \log \overline{\mathrm{SL}}),$$

where Y'_{ij} is the adjusted value of character j for individual i, Y_{ij} is the original value, b_j is the pooled regression coefficient of log$Y$ on logSL, SL_i is the standard length of individual i, and $\overline{\mathrm{SL}}$ is the overall mean standard length.

Adjusting for overall mean standard length removes size effects from morphometric data, and has been shown to be an appropriate procedure for objective analysis of the data when there is size overlap among the groups (Claytor and MacCrimmon, 1986).

All statistical analyses were performed for combined sexes since all measurements were transformed and the effect of size removed (Karakousis *et al*., 1993; Mamuris *et al*., 1998). The collected samples were predominantly male in both species (*T. jaculatrix*, male n=60, female n=5; and *T. chatareus*, male n=60, female n=3); therefore, sex effects were not considered in the present study.

The efficacy of size transformation was determined from the coefficient of determination (R^2) values of the logY' vs. logSL regression. Some of the morphometric characters could be considered as redundant, as the same part of the body is measured by two or more characters. Therefore, stepwise discriminant function analysis (DFA) was performed to extract the most important characters for differentiating species, using the F-value criterion (F-entry, 3.84; F-removal, 2.71). Selected characters were then subjected to principle component analysis (PCA) to reveal patterns of the species, and followed by DFA to compute the classification success. Misclassification rates of DFA were calculated using holdout cross validation procedures proposed by Lachenbruch (1967). The kappa (κ) statistics was used to determine the improvement over chance of the percent-correct classification rates (Titus *et al*., 1984). All statistical analyses were performed with SPSS Version 15, MINITAB Version 14, and PAST Version 1.34 software.

3 Results

Descriptive statistics for each of the morphometric characters are given in Fig. 3. Twelve fish, six from each species, were identified as extreme outliers and therefore were excluded from the analyses. The indication of the transformed characters being free from the influence of size was provided by R^2 values. Prior to transformation almost 50% of the characters show R^2 values above 0.50, whereas after transformation all the characters registered R^2 values of zero. Stepwise discriminant analysis identified 14 of the initial 31 morphometric characters as the most important characters for differentiating species; therefore, these characters were incorporated into PCA and DFA analyses.

The first four principle components cumulatively account for 60.7% of the total morphological variations (Table 2). Almost all the loadings on PC1 (22.8%) are negative with no clear pattern. PC2 described 17.7% of the total variance, with length of dorsal fin base (LDFB), depth of caudal peduncle (DCP) and head length (HL) (large positive loadings) and the first anal spine length (LAS1) and length of soft dorsal ray (LSDR) (large negative loadings) being most highly correlated with PC2. PC3, which accounted for 10.9% of the total variance, contrasted with the first and third lengths of dorsal spines (LDS1 and LDS3; large negative loadings) and the third anal

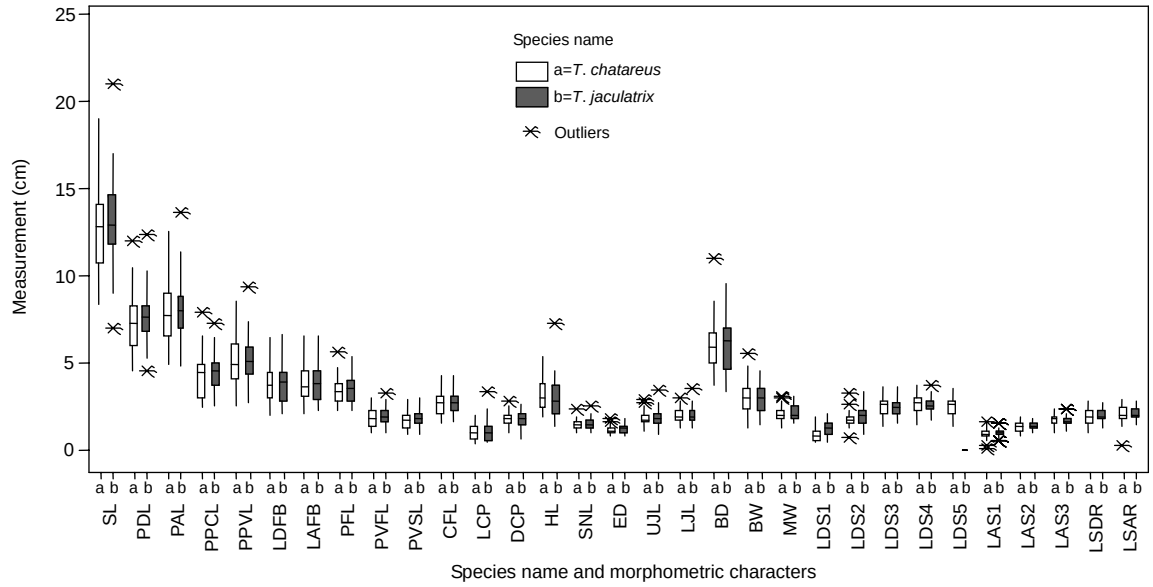

Fig. 3 Box plot of 31 morphometric characters of *Toxotes chatareus* and *Toxotes jaculatrix*

spine length (LAS3; large positive loading). The two species appeared to differ on PC3, but with some overlap (Fig. 4), *T. chatareus* having on average a shorter dorsal spine length and longer anal spine length than *T. jaculatrix*.

Table 2 Principal component loadings for the morphometric characters

Morphometric character	PC1	PC2	PC3	PC4
PDL	−0.244	0.228	−0.245	−0.214
PPCL	−0.211	0.229	−0.138	−0.551
LDFB	−0.285	0.421	0.208	−0.051
PVFL	−0.461	0.062	0.134	0.144
DCP	−0.091	0.406	−0.270	0.136
HL	0.010	0.437	0.258	0.145
ED	−0.265	0.221	0.042	0.418
MH	−0.203	0.107	−0.097	−0.261
LDS1	−0.242	−0.245	−0.439	−0.039
LDS3	−0.114	0.005	−0.419	0.565
LAS1	−0.309	−0.306	0.112	0.099
LAS3	−0.178	−0.123	0.568	0.060
LSDR	−0.424	−0.310	−0.002	−0.043
LSAR	−0.321	−0.183	0.071	−0.093
Eigen value	3.191	2.475	1.530	1.299
Proportion	0.228	0.177	0.109	0.093
Cumulative	0.228	0.405	0.514	0.607

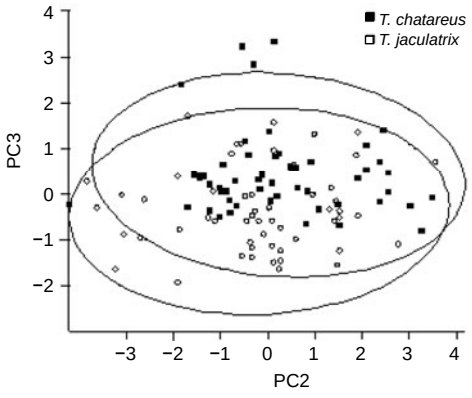

Fig. 4 Scatter plot of PC2 vs. PC3 scores and 95% confidence ellipses of the scores for PCA using morphometric characters

The discriminant function tested using Wilks' λ statistic was significant (λ=0.59, $\chi^2_{14}=54.8$, P<0.001), indicating a relatively high degree of interspecies variance and that the means of the discriminant scores for the two species are different. The DFA picks out LDS1 and LDS3 as important discriminating characters, similar to the pattern seen on PC3. In addition, DFA also picks out LDFB (Table 3).

Table 3 Standardized and unstandardized canonical discriminant function coefficients for morphometric characters

Morphometric character	Standardized function coefficient	Unstandardized function coefficient
PDL	0.332	10.884
PPCL	0.143	2.797
LDFB	−0.465	−8.496
PVFL	0.184	1.965
DCP	0.232	4.121
HL	−0.173	−2.169
ED	0.214	3.637
MH	0.250	4.288
LDS1	0.829	4.994
LDS3	−0.591	−8.601
LAS1	0.276	3.130
LAS3	−0.175	−2.590
LSDR	−0.438	−5.508
LSAR	0.132	1.121
Constant		−1.023

The discriminant function managed to assign correctly 86.7% of the fish to the species and 76.1% after cross-validation, 73% (κ) better than what would have occurred by chance. The relatively high classification success provides support for the morphometric differences between the species (Table 4).

Table 4 Classification results of discriminant function analysis using morphometric characters of *Toxotes chatareus* and *Toxotes jaculatrix*

Discriminant function analysis	Species	Predicted count*		Total
		T. chatareus	*T. jaculatrix*	
Original	*T. chatareus*	47 (87.0%)	7 (13.0%)	54
	T. jaculatrix	8 (13.6%)	51 (86.4%)	59
Cross-validated	*T. chatareus*	41 (75.9%)	13 (24.1%)	54
	T. jaculatrix	14 (23.7%)	45 (76.3%)	59

* Figures in parentheses indicate percentage of classification

For the meristic characters, pelvic fin ray (PVFR), caudal fin ray (CFR), anal spine (AS), pelvic spine (PVS), and total vertebrae (TV) were constant between the species and were excluded in the analyses. The remaining meristic characters, dorsal fin ray (DFR), pectoral fin ray (PCFR), anal fin ray (AFR), and lateral line scale (LLS) were subjected to PCA. Univariate comparisons of these characters between species were significant (P<0.05) except for DFR (Table 5).

Table 5 Univariate comparisons of meristic characters of *Toxotes chatareus* and *Toxotes jaculatrix*

Meristic character	Value*	
	T. chatareus	*T. jaculatrix*
DFR	11.96±0.07[a]	11.88±0.07[a]
PCFR	12.76±0.06[a]	14.49±0.09[b]
AFR	16.19±0.07[a]	15.81±0.09[b]
LLS	32.50±0.45[a]	28.73±0.13[b]

* Values are expressed as mean±SE. [a, b] Different superscript letters at the same row indicate significant difference at $P \leq 0.05$

Three PCs were extracted, accounting for 94% of the total variation. PC1 accounted for 52.5%, and the loadings were all positive. Thus, this is not particularly informative, probably describing general size axis. The loadings on PC2 (32.0%) and PC3 (10.3%) are both positive and negative. PC2 contrasts between PCFR (negative loading) and LLS (positive loading) (Table 6). Bivariate plot of PC1 and PC2 scores revealed that separation of *T. chatareus* and *T. jaculatrix* was evident on PC2. *T. chatareus* has positive scores while *T. jaculatrix* has negative scores, albeit with slight overlap (Fig. 5).

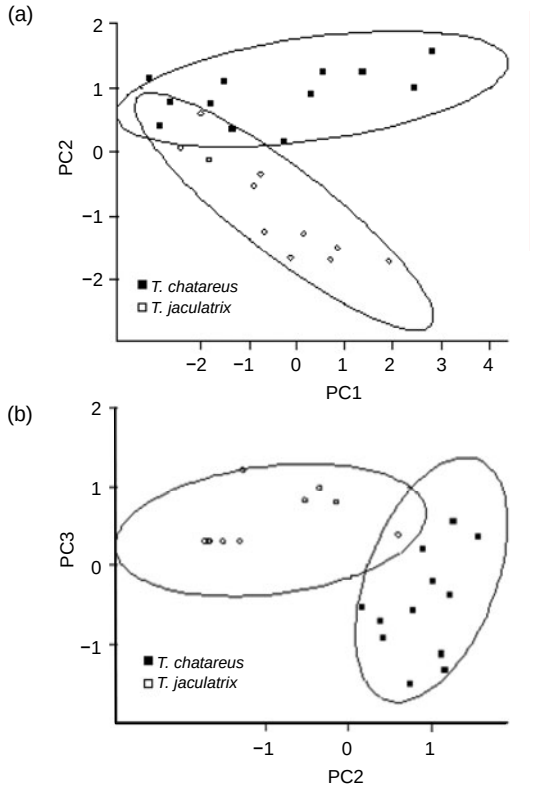

Fig. 5 Scatter plots of PC1 vs. PC2 (a) and PC2 vs. PC3 (b) scores, and 95% confidence ellipse of the scores for PCA using meristic characters

Table 6 Principal component loadings for the meristic characters

Meristic character	PC1	PC2	PC3	PC4
DFR	0.639	−0.126	0.114	−0.750
PCFR	0.201	−0.794	0.437	0.371
AFR	0.608	−0.022	−0.675	0.419
LLS	0.427	0.594	0.584	0.353
Eigen value	2.099	1.279	0.412	0.209
Proportion	0.525	0.320	0.103	0.052
Cumulative	0.525	0.845	0.948	1.000

The squared canonical correlation of the discriminant function was 0.86, suggesting that a high proportion of the total variance of meristics was attributable to differences between species (Table 7). Discriminant analyses manage to correctly classify 100% of the samples.

Table 7 Standardized and unstandardized canonical discriminant function coefficients for meristic characters

Meristic character	Standardized function coefficient	Unstandardized function coefficient
DFR	0.296	0.495
PCFR	−1.441	−2.250
AFR	0.757	1.066
LLS	0.431	0.162
Constant		2.792

4 Discussion

Morphometric character analysis demonstrated that although the two species are less distinct, 76.1% of fish were ascribed to the correct species cluster. The LDS1, LDS3, and LAS3, and to some extent, LDFB were found to be important discriminating morphometric characters in the present study with *T. chatareus* in general having a shorter dorsal spine length and longer anal spine length than *T. jaculatrix*.

Analyses of meristic characters revealed that PC2 and the discriminant function are highly correlated with PCFR (negative loading), LLS (positive loading), and AFR (positive loading). *T. chatareus* can be distinguished from *T. jaculatrix* by having greater number of LLS, lower PCFR, and a slightly higher AFR counts.

Comparison of meristic and morphometric traits showed the ability of the morphometric discriminant function to correctly classify individuals, in agreement with the results obtained by discriminant function analyses with other fish species (Meng and Stocker,

1984; Tudela, 1999; Murta, 2000). This study has demonstrated that both morphometric and meristic variations exist between the two species of archer fishes. The morphometric and meristic results indicate that they should be considered as complementary, not alternative approaches, to the same problem.

T. chatareus and *T. jaculatrix* are aggressive in nature and they sometimes lose their fins and spines because of their hostile behavior (personal observation). Moreover, body coloration, which was previously used as one of the identifying characters of these two species, usually depends on the habitat. The present study has uncovered some morphological (i.e., morphometric and meristic) distinctions between the two closely related archer fishes, using multivariate techniques as reported for other marine vertebrates and invertebrates (Fridriksson, 1958; Boetius, 1980; Pierce *et al*., 1994a; 1994b; Tudela, 1999; Bolles and Begg, 2000). This study demonstrates that *T. chatareus* and *T. jaculatrix* from Matang coastal waters were different from one another in both morphometric and meristic traits. The best statistical classifications of these groups using multivariate discriminant analyses were obtained using meristic characters, while morphometric characters provided comparatively less evidence of differentiation. Therefore, the findings of the present study can be used to identify these two species more precisely as color can be regarded as a plastic character. There could also be a possible link between the observed meristic variability with differences in habitat and prey-predatory relationship. However, the true reasons for the observed meristic and morphometric variation should be studied further using appropriate sampling design that includes different localities as well as the predatory behavior of the fishes.

In summary, this study has provided important morphological information that can be used to differentiate these congeneric species more precisely. The authors hope that the information obtained from the present study will be helpful for fisheries, biologists, and taxonomist concerned with these two fascinating fishes.

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