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Inner shell as variation key of local hard clam *Meretrix* spp.

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Abstract

The morphology and 12 shell morphometric features proportionate to shell length were analysed between local hard clam; *Meretrix lyrata, M. meretrix* and *M. lusoria* from Sarawak, Malaysia. *Meretrix* spp. was observed to comprise a unique feature of a pallial sinus scar for each species. Analysis of variance revealed significant differences among *Meretrix* spp. using proportion ratios of SL for SW; LL; AL; LCT; AW; PW and PS ($p<0.05$). Cluster analysis among morphometric features of *M. lyrata, M. meretrix* and *M. lusoria* were discriminated at 98.5% similarities and supported by the principal component analysis. The present study suggests that pallial sinus scar shape, together with interior and exterior morphometric features, were suitable as identification keys for *Meretrix* spp. Hence, the present study emphasizes on the application of interior, rather than exterior morphology and morphometric features in hard clam identification before further investigation can be performed through genetic identification means.

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Introduction

Approximately, 1000 species of marine bivalve from 82 different families are currently known throughout the world. There are two common species of hard clams in Sarawak, Malaysia; *Meretrix lyrata* and *M. meretrix* (Hamli *et al.*, 2012). These species are distributed in the Indo-West Pacific region from east Africa to Philippines, north to Japan and south to Indonesia (Poutiers, 1998). Meanwhile, *Meretrix lusoria* and *M. petechialis* are common *Meretrix* spp. that are often misidentified as *M. meretrix* (Yamakawa and Imai, 2012). However, Chen *et al.* (2009) suggested that *M. lusoria* and *M. petechialis* should be categorised as junior synonyms to *M. meretrix*. Furthermore, Torii *et al.* (2010) suggested that hybridization was occurring between *M. lusoria* and *M. petechialis*, since the later species has been an introduced alien species from China and Korean Peninsula into the natural habitats of *M. lusoria* in Japan (Yamakawa and Imai, 2012). Therefore, cross-breeding between these two species may cause ambiguous identification.

The morphological features between different *Meretrix* species and locality has been described by Lin *et al.* (2007) and are well supported with molecular works. Shell colour, periostracum character, shell length and outer shell shape are the main morphological components for *Meretrix* spp. classification (Yamakawa and Imai, 2012; Yoon *et al.*, 2012). Moreover, linear dimension between umbo, pallial line scar, anterior and posterior margin of *M. casta* has been explored by Durve and Dharmaraja (1970) as additional features. More quantification of morphological features are used in order to differentiate species as applied by Torii *et al.*

Table 1 : Shell morphometric analysis of one way ANOVA with Tukey's HSD test for three *Meretrix* species

Shell	Morphometric characteristic	*M. lyrata* (mm) N=194	*M. meretrix* (mm) N=35	*M. lusoria* (mm) N=25	F Value	P
Outer	SW/SL	0.618 ± 0.003^{a}	0.624 ± 0.005^{a}	0.596 ± 0.005^{b}	3.261	0.04064*
	SH/SL	0.873 ± 0.003^{a}	0.880 ± 0.005^{a}	0.885 ± 0.006^{a}	1.387	0.2526^{ns}
	UL/SL	0.839 ± 0.003^{a}	0.819 ± 0.005^{a}	0.822 ± 0.005^{a}	5.383	0.05365^{ns}
	LL/SL	0.348 ± 0.006^{a}	0.331 ± 0.011^{ab}	0.298 ± 0.012^{b}	4.082	0.01848*
	AL/SL	0.504 ± 0.003^{a}	0.533 ± 0.005^{b}	0.540 ± 0.006^{b}	15.34	<0.0001*
	PL/SL	0.771 ± 0.007^{a}	0.780 ± 0.005^{a}	0.785 ± 0.008^{a}	1.074	0.3532^{ns}
Inner	LPAS/SL	0.599 ± 0.003^{a}	0.601 ± 0.007^{a}	0.581 ± 0.005^{a}	22.74	0.1058^{ns}
	LCT/SL	0.262 ± 0.002^{a}	0.238 ± 0.007^{b}	0.221 ± 0.004^{b}	16.12	<0.0001*
	PVM/SL	0.185 ± 0.006^{a}	0.179 ± 0.004^{a}	0.187 ± 0.003^{a}	0.6202	0.5426^{ns}
	PS/SL	0.157 ± 0.005^{a}	0.109 ± 0.005^{b}	0.195 ± 0.008^{c}	33.36	<0.0001*
	AW/SL	0.121 ± 0.001^{a}	0.104 ± 0.003^{b}	0.102 ± 0.003^{b}	35.33	<0.0001*
	PW/SL	0.164 ± 0.001^{a}	0.149 ± 0.003^{b}	0.159 ± 0.006^{a}	11.85	<0.0001*

SL=Shell length, SH=Shell height, SW=Shell weight, UL=Umbo length, LL=Ligament length, AL=Anterior length, PL=Posterior length, PVM=Pallial line to ventral margin, LPAS=Length of posterior adductor scar to anterior adductor scar, AW=Anterior adductor scar width, PW=Posterior adductor scar width, LCT=Length of cardinal tooth, PS=Pallial sinus open scar

Note: *different superscripts between column indicate a significantly difference at level p<0.05 using Tukey mean comparison, ns: not significantly difference

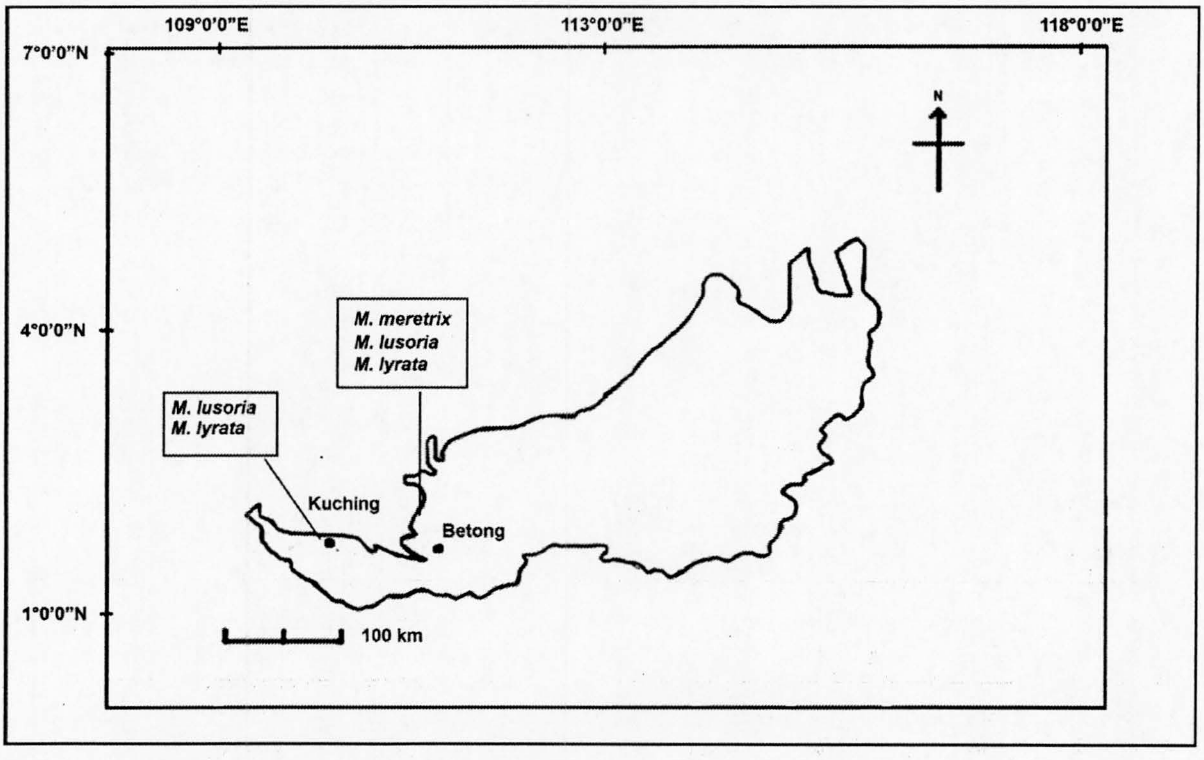

Fig. 1: Distribution of *Meretrix* spp. at two division of Sarawak, Malaysia

(2010) in which 9 shell features were used to differentiate *M. lusoria* and *M. petechialis* form Japan and Korea.

Several published studies regarding *Meretrix* spp. have been performed in the East Asian region, but a detailed morphological study from Southeast Asia is limited. Inadequate published material of *Meretrix* spp. identification and an earlier morphological study from Southeast Asia region is not well defined and unable to support the postulation concerning hybridization among *Meretrix*

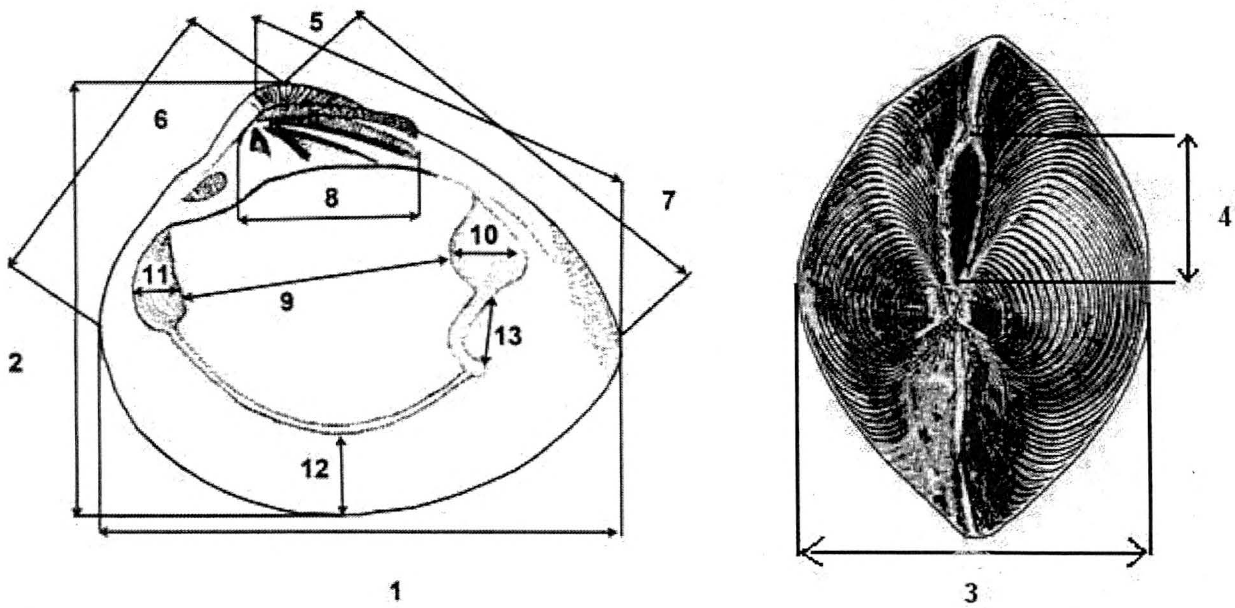

Fig. 2 : Measurements of shell characters for *Meretrix* spp. Note: 1(SL), 2(SH), 3(SW), 4(LL), 5(PL), 6(AL), 7(UL), 8(LCT), 9(LPAS), 10(PW), 11(AW), 12(PVM), 13(PS)

species (Purchon and Purchon, 1981; Hamli *et al.*, 2012). Therefore, a morphological study of *Meretrix* spp. from this region was carried out to provide additional information in malacology.

Materials and Methods

Study area: Three *Meretrix* species were collected from two study areas of Sarawak, Malaysia (Fig. 1) and transferred to laboratory for morphological and morphometric analysis. The visceral mass in each sample was removed and only shells were used for morphological study.

Morphological and morphometric features : A total of 194, 35 and 25 individuals of *Meretrix lyrata*, *M. meretrix* and *M. lusoria*, respectively, were used in the present study. The samples were preserved in ice and transferred to laboratory for analysis. Morphological structures of *Meretrix* spp. were identified based on Poutiers (1998). A total of 13 shell characters, such as shell length (SL), shell height (SH), shell width (SW), umbo length (UL) ligament length (LL), anterior length (AL), posterior length (PL) (Mass *et al.*, 1999), length of cardinal tooth (LCT), length of posterior adductor scar to anterior adductor scar (LPAS), pallial line scar to ventral margin anterior (PVM), adductor scar width scar (AW), posterior adductor scar width scar (PW) (Hamli and Idris, 2014), and an additional character of pallial sinus open scar (PS) shape were analysed (Fig. 2).

Statistical analysis : The morphometric ratio on variables were tested using one-way-analysis of variance (ANOVA) with general linear model (GLM) using statistical analysis computer software (SAS) version 9.1 (SAS Institute 2004). If significant differences were detected ($p<0.05$), comparison between morphometric ratios of *Meretrix* spp. were determined by Tukey's mean comparison test. Significant differences of morphometric ratios between *Meretrix* spp. was tested for similarity by Principal Component Analysis (PCA), and hierarchical cluster analysis was carried out by computer program PRIMER version 5 (Plymouth Routines In Multivariate Ecological Research) (Clarke and Gorley, 2001).

Results and Discussion

Exterior shell : Three *Meretrix* species had identical trigonal shape of the shell, which was an unreliable criteria for species identification. However, each *Meretrix* sp. had their own unique colour and pattern on the shell. The easiest to identify was *Meretrix lyrata* due to undulate pattern on the shell surface (Fig. 3). According to Poutiers (1998), *M. lyrata* has a concentric grove at the shell surface, as compared to the

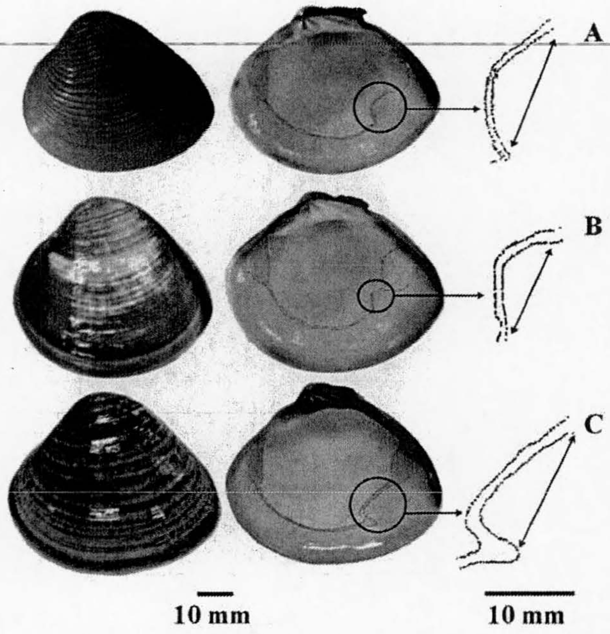

Fig. 3 : Outer shell and pallial sinus scar pattern for (A) *Meretrix lyrata;* (B) *M. meretrix;* (C) *M. lusoria*

smooth surface of *M. meretrix*. A smooth and shiny shell surface is unique to *M. meretrix* and *M. lusoria*. The shell color of *M. meretrix* was light brownish to dark brownish while for *M. lusoria*, the color was brownish to whitish. Furthermore, *M. lusoria* had a distinct "W" like pattern that could be seen on the shell as compared to *M. meretrix*.

The shell width (SW) and anterior length (AL) was unique to *M. lusoria* and *M. lyrata*, respectively, which was significantly shorter ($p<0.05$) in length. Furthermore, *M. lusoria* could be differentiated with *M. lyrata* ($p<0.05$) for ligament length (LL) and no differences ($p>0.05$) compared

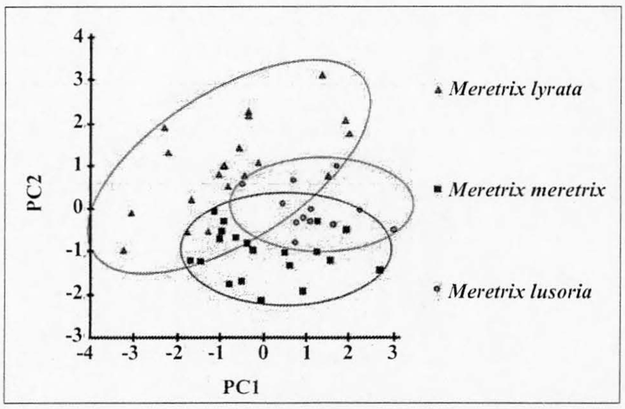

Fig. 5 : Principal Component Analysis (PCA) between three local hard clams morphometric shell from Sarawak, Malaysia

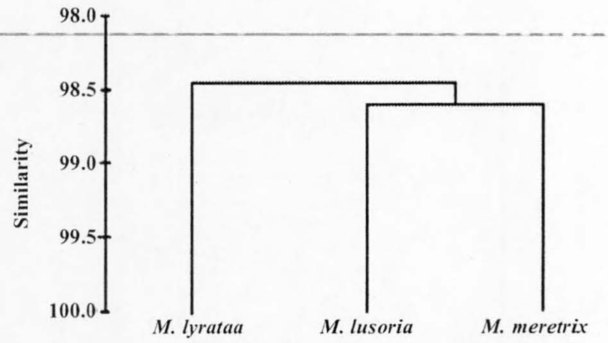

Fig. 4 : Hierarchical cluster between three local hard clams from Sarawak, Malaysia

to *M. meretrix*. However, this feature was frequently misidentified due to the eroded shell surface, particularly for synonym species. Variable environmental factors are known to influence shell morphology and morphometric of many bivalves such as latitude (Beukema and Meehan, 1985), depth (Claxton *et al*., 1998), shore level (Franz, 1993), currents (Fuiman *et al*., 1999), water turbulence (Hinch and Biley, 1988), wave exposure (Akester and Martel, 2000) and type of substratum (Claxton *et al*., 1998). Confusion in species identification was due to hybrid and is discussed in several literature (Torii *et al*., 2010; Yamakawa and Imai, 2012), which necessitated both molecular and morphological works for species identification. Nevertheless, the sole use of morphology, particularly on the exterior shell, can lead to misidentification since these can change based on the environment. Hence, if this is used without any other supported identification key to represent particular species can be argued.

Interior shell : The interior shell feature is the best solution to overcome the environmental effects for species identification. The pallial sinus scar shape, that is located near to the posterior adductor muscle scar, is unique for *M. lusoria* because of a "L" pattern. The "L" pattern was due to the protruding of pallial line scar from the pallial sinus scar (Fig. 3). However, this pattern was not observed in *M. lyrata* and *M. meretrix*. The pallial sinus scar of both species were hyperbola in shape, but was larger in size for *M. lyrata* and smaller in size for *M. meretrix*. The pallial sinus scar can be used as a key identifier due to non-overlapping feature between *Meretrix* spp.

Based on the shell length (SL) proportion ratio, from 12 morphological features, only 3 exterior and 4 interior features showed significant difference among the *Meretrix* spp. *Meretrix lyrata* had significantly longer ($p<0.01$)

cardinal tooth (LCT) as compared to *M. meretrix* and *M. lusoria*. The LCT feature was unique for *M. lyrata* but not for *M. meretrix* and *M. lusoria*, which was comparable. According to Hamli and Idris (2014), the LCT between *Polymesoda expansa* was distinct to *P. bengalensis*. Similarities between *M. meretrix* and *M. lusoria* also occurred for anterior adductor muscle scar (AW) as *M. lyrata* had significantly larger ($p<0.01$) AW. The posterior adductor muscle scar width (PW) was significantly smaller ($p<0.01$) for *M. meretrix* as compared to PW ($p>0.05$) of *M. lyrata* and *M. lusoria*. Idris *et al.* (2009) distinguished *Pinna deltodes* and *P. bicolor* by the length of the posterior adductor muscle scar to posterior dorsal nacreous layer and width of sulcus, whil Scheltema (1983) used the posterior adductor muscle scar position to the nacreous lobe as a key to differentiate these species. In a recent study on *Meretrix lusoria* and *M. petechialis* morphology was able to distinguish both species using socket size (Torii *et al.*, 2010). The present study was able to differentiate among *M. meretrix*, *M. lusoria* and *M. lyrata* using pallial sinus open scar (PS) as a unique morphological feature for each species. This character was protected at the inner shell from outside physical effects. *Meretrix lusoria* was represented as significantly ($p<0.01$) longer PS as compared to *M. lyrata* and *M. meretrix* which had moderate and shorter PS sizes, respectively. All the key features used in bivalve species discrimination that were mentioned in published articles referred to the interior part of shell. Rosewater (1961) in Pinnidae classification emphasized the application of the internal shell feature for species identification. This indicated the reliability of interior shell feature in morphometric studies. The sole application of exterior shell feature causes the ambiguous species differentiation as reported by Mass *et al.* (1999).

Multivariate analysis : The present study signified the advantages of inner and outer shell feature as species identification. Therefore, the proportional ratio of morphometric features (SW, LL, AL, LCT, AW, PW, PS) that are unique for a particular species was used in cluster and PCA analyses. Results on cluster analysis showed that three hard clams were grouped together at 98.5% similarity (Fig. 4). *M. meretrix* and *M. lusoria* formed a cluster at 98.5% similarity, while *M. lyrata* was discrete at this level. *Meretrix lusoria* and *M. meretrix* were discrete at 98.6% similarity. Results from the cluster analysis was also supported by PCA indicated three separate groups of *Meretrix lyrata*, *M. meretrix* and *M. lusoria* based on SW, LL, AL, LCT, PS, AW and PW (Fig. 5). A clear separation was demonstrated by *M. lyrata*, while *M. meretrix* and *M. lusoria* showed overlapping patterns between these. The application of PCA and cluster analysis have been practiced for freshwater clams (Hinch and Bailey, 1988), *Corbicula* clam (Pigneur *et al.*, 2011), zebra mussel (Lajtner, 2004) and mangrove clam (Hamli *et al.*, 2015). This analysis is suitable to analyse multiple parameters in order to discriminate between samples. However, application of PCA or cluster analysis becomes consistent when the parameters used have high variation between the samples. Therefore, the analysis will be able to show differences between species that are investigated.

Application of interior morphology and morphometric shell characteristics (LCT, AW, PW, PS) as identification keys can be used to distinguish between three local hard clams (*Meretrix* spp.). The interior morphometric shell becomes tangible if the exterior morphometric shell (SW, LL, AL) and morphological (pallial sinus shape) feature implemented together for species identification. It is important to apply this feature since it is fast, cheap and efficient. Furthermore, it is important to reaffirm the morphological and morphometrics before genetic identification can be used to reveal the species identity to avoid species misidentification.

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