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Morphometry of the invasive *Pomacea* spp (Gastropoda: Ampullariidae) from rice fields of Peninsular Malaysia

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Abstract
Morphometry is one of the methods that can be useful in establishing taxon identity. Morphometrics of *Pomacea* spp in paddy fields of Peninsular Malaysia has not been studied, hence, poorly understood. Morphometric measurement were carried out on shell and operculum characters for six populations of *Pomacea* spp from paddy fields of peninsula Malaysia to assess ecotype disparity for those characters among the different populations. Results revealed that significant disparity with respect to the assessed characters exists among the populations from different rice ecologies sampled. The highest value for shell length, body whorl height, spire height, aperture length, Aperture width, operculum length and Operculum width were recorded in the Tanjung Karang (Selongor) sampling locality. While the least value for most of these morphometric character were recorded from Tanjung Piandang locality (Perak). It could be concluded that the morphometric characters measured vary independently among the populations measured and is predicted among others factors to be governed by intensity of paddy cultivations rather than geographical gradients. The implications of this finding especially with respect to their management as pest of paddy were discussed.

Keywords: invasive pest, shell, operculum, spire, morphotypes

Introduction
Invasive *Pomacea* spp are a widespread and overwhelming pest of paddy in Southeast Asia in general and Peninsular Malaysia in particular (Anderson, 1993; Teo, 2003; Joshi and Sabastian, 2006) [3]. It has been reported to be present in all paddy fields of Peninsular Malaysia (Arfan *et al*., 2014) resulting in an estimated 46-100% decline in yield (Teo, 2003) [28].

Total land area under paddy cultivation in Malaysia is reported to be above 673, 745 ha (Harun and Arif, 2017) [15]. Added cost of production as a result of infestation in these fields by *Pomacea* spp is estimated to be RM82.5 million in 2009, as an amount expended on molluscides for its control (DOA, 2012). In spite of this huge monetary investment, infestation of paddy fields by invasive *Pomacea* ssp still remains widely distributed and a menace in all rice growing areas of Malaysia (Arfan *et al.,* 2014) [4]. This may pose a serious threat to economic production of paddy in Malaysia and hence can be regarded as one of the major factors that may hinder the attainment of the targeted 2025 self-sufficiency in paddy production set out in the National food security Agenda in the country. The status of *Pomacea* spp as a critical risk factor to agriculture and wetland ecosystem is also well documented (Rawlings *et al*., 2007, Joshi and Sabastian, 2006; Cowie and Hayes, 2012; Anderson, 1993) [23, 19, 10, 3].

Two major species of Pomacea (*P. canaliculata and P. maculata*) have been reported attacking paddy seedlings in Peninsular Malaysia (Teo, 2003; Arfan *et al*., 2014) [28, 4] with the latter being more abundant and widely distributed (Arfan *et al*., 2014) [4]. Precise identification of these two species have been reported to be problematic due to their enormous intraspecific differences and faint interspecific variations (Estebenet and Martin, 2003; Rawlings *et al*., 2007) [12, 23] resulting into poor understanding of its taxonomy (Torres *et al*, 2011; Cazzaniga, 2006) [29, 6]. Tentative diagnosis is crucial for the development and implementation of any meaningful pest control strategy for any pest species and pest scenario (Hyde *et al*., 2008; Rawlings *et al*., 2007) [18, 23] and of *Pomacea* spp pest complexes (Rawlings *et al*., 2007; Torres, 2011) [23, 29].

The first most important step in the control and management of invasive ampulariids starts with the tentative species delimitation, understanding temporal and spatial distribution, origin and population dynamics (Rawlings *et al*., 2007) [23]. This is because species specific differences are common in crop pest complexes with respect to breeding periods and attainment of temporal and spatial pest population peaks among others. This makes pest containment more complicated and continues over the entire production cycle. In addition, invasive species are known for population explosions, divergence and phenotypic plasticity in their new environment (Huey *et al*., 2005) [17] probably due strong founder effect, absence of their natural enemies and the combined influence of other new environmental selection pressures (Huey *et al*., 2005; Allendorf and Lundquist, 2003) [17, 2]. This makes the need for their tentative diagnosis of even more critical significance than endemic or local pest species. Taxonomic confusing in the genus Pomacea has delayed the development of management strategies for *Pomacea* spp both as pest and invaders (Cowie, 2002; Cowie *et al*., 2006) [8, 9].

Past attempts at diagnosis and identification of these species in Malaysia have relied solely on morphological characters (Arfan *et al*., 2014) [4] perhaps due dearth or paucity of categorical information on other quick fields and laboratory techniques for their identification in literature. The categorical identities of such snails in most case remain skeptical (Estebenet and Martins, 2003) [12]. One of the means of addressing the taxonomic impediment posed by reliance on morphological characters to delimit species biodiversity in apple snails populations can be achieved through the use of morphometrics (Tumbari and Martin, 2013) which is believed to be an indispensable technique in not only the identification of species but also in quantifying the nature of morphological variations within a species (Roth and Mercer, 2000; Adams *et al*., 2004).This therefore, call for the need to incorporate morphometry of basic features as means of increasing the scope of our knowledge base on this species in peninsular Malaysia. This study therefore has the

Objective

of assessing shell and Operculum morphometric characters variations among *Pomacea* spp eco-populations from rice fields of Peninsular Malaysia.

Materials and Methods

Study area and sampling

This study was conducted in paddy fields from five randomly selected states of Peninsular Malaysia. Field samplings were conducted from six randomly selected farms from these states. These field locations are Tanjung Karang (N03^{0}27.385' E101^{0}09.541') (Selangor), Kampung Golok (N05^{0}50.989' E102^{0}28.932') (Kelantan), Parit buntar (N05^{0}3.968' E100^{0}22.931') (Perak), Tanjung Piandang (N05°04.057' E100°23.116') (Perak), Kampung Tebengu (N06^{0}05.968' E100 19·780') (kedah) and Permatang Kuang (N05°31.414' E100°24.709') (Penang) (Fig 1).

Sampling at all locations was carried out using random sampling procedure where, paddy fields to be sampled were randomly selected. Each randomly selected paddy field was geo-referenced and further divided into four blocks according to the position of the field and from each block two random samplings were carried out using a 0.5m^2 quadrate. The snails falling within each quadrate were carefully collected by hand or by using strainer to avoid any damage to the rice plants in the field (Arfan *et al.*, 2014) [4].

Fig 1: Map of Malaysia showing the states of the sampled localities

All collections from particular paddy fields were termed as a population. Snails collected were put into plastic aquarium with sufficient water so that the snails can be fully immersed but with sufficient air above the water to permit the water to remain oxygenated and for the snails to breathe air when they wish to (Cowie *et al.*, 2006) [9] and transported to the Faculty of Agriculture, Universiti Putra Malaysia for cleaning. Snails were properly washed with tap water to remove dirt particles from their shell to ease field identification.

Morphological identification was done according to the external morphology of the apple snail's shells as described by Marwato and Nur (2012), Hayes *et al.*, (2012) [16] and Arfan *et al.*, (2014) [4] to separate apple snails from other species of aquatic snails. After identifications, all the snails were kept in separate plastic aquarium with proper labels identifying collection site and taken to the laboratory. Specimens brought to the laboratory were packed in plastic bags and finally kept in -20^0 C freezer to preserve them until morphometric studies.

Morphometric Measurements

Morphometric measurement were taken on 30 randomly selected individuals from each population as described by Chiu *et al.*, (2002) [7] with slight modifications. Ten (10) variables: Shell length, Shell width, Body whorl height, Spire height, Apertural length, Apertural width, Aperture wall thickness, Operculum length, Operculum width, and Operculum wall thickness shown in literature as being vital to *Pomacea* spp and other snails conchology (Marwato and Nur 2012; Estebenet *et al.*, 2006; Chiu *et al.*, 2002; Burch, 1980) [13, 7] were measured on each individual.

All the ten continuous shell and operculum metric measurements were taken with digital calipers (0.1 mm precision) along imaginary straight lines on both the shell and operculum as shown in Figure 2. Shell length (SL) was measured along an axis passing through the apex (a) to the bottom (i) of the shell. Shell width (SW) were measured as the maximum width perpendicular to the shell length distance (d'-g'). The body whorl length (WL) was measured from the intersection of the axis passing through the whorl apex (b) to the bottom of the shell (i). Spire height (SPH) was measured from the beginning of the 1st suture (c) to the apex of the Shell (a).

Aperture length (AL) was measured as the length from the apex of the aperture (e) to the bottom of the Aperture (i), Aperture widths (AW) were measured as the maximum diameter perpendicular to the Aperture length (f-h), Aperture wall thickness (AT) were taken from three random points on the edges of the aperture and summed up to get the average thickness for each individual. Operculum length (OL) were measured as the maximum longitudinal length of the operculum (j'-o'). Operculum width (OW) were measured as the maximum length measured at 90^0 to the operculum length (m'-n'). Operculum wall thickness (OT) was taken from three random points on the edges of the operculum and summed up to get the average thickness for each individual.

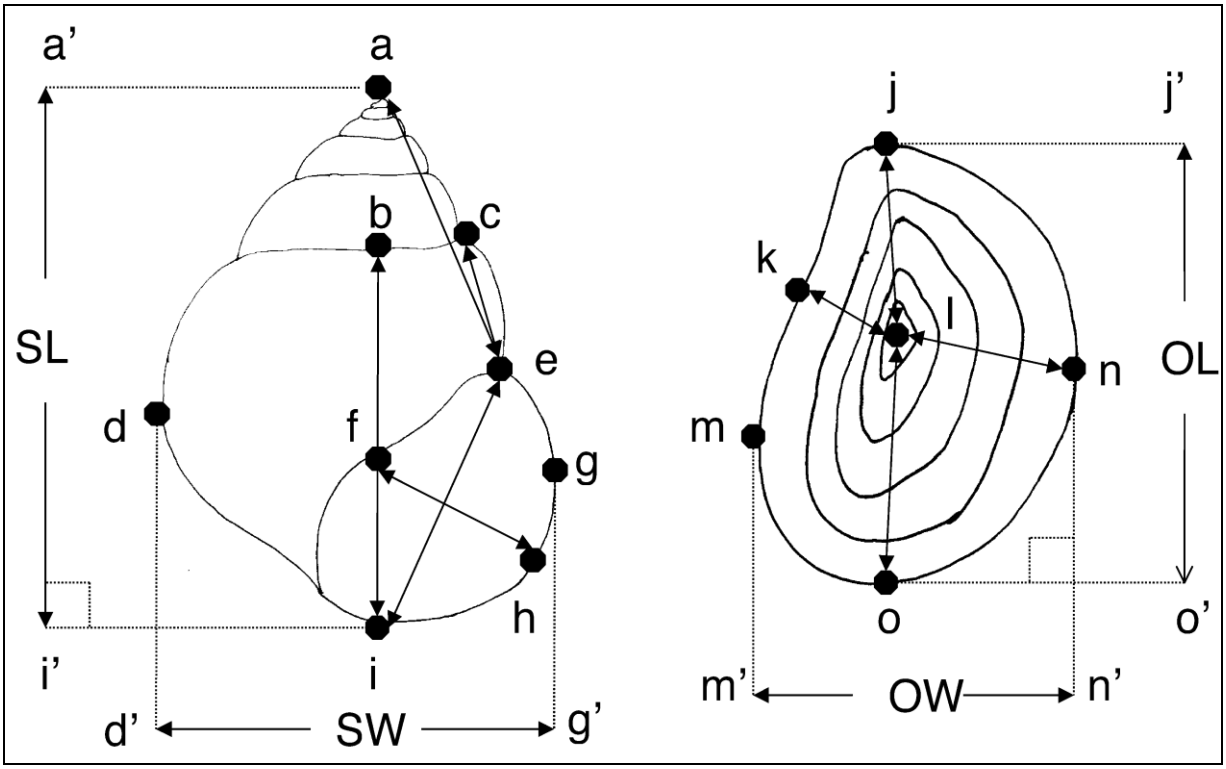

Fig 2: Measurements of variables and points on shells (left) and Operculum (right) of viviparid snails (Chiu *et al.*, 2002) [7].

Statistical Analysis

Data for all sampled location were subjected to One-way analysis of variance using Proc GLM in SAS to assess whether there is difference between the different populations with respect to the measured morphometric characters. Data for each population was thereafter analysed using simple descriptive statistic of means, standard error, range and standard deviation by PROC UNIVARIATE in SAS. Prior to analysis, correlation between sexes of snails and measured mopho character were analysed. No significant correlation existed between gender of snails and all of the measured variables (Table1). Therefore, individual were not separated into male and females throughout the analysis.

Table 1: Correlation estimates between sex of snails and measured morphometric variables

Variables	*r*	*p*
Species	0.01850	0.8625
Shell Length	0.06484	0.0617
Shell Width	-0.16861	0.1000
Body Whorl Height	0.14521	0.3012
Spire Height	-0.08511	0.0561
Aperture Length	0.06873	0.4510
Aperture Width	0.1687	0.0584
Aperture Thickness	-0.18471	0.1800
Operculum Length	0.08556	0.0613
Operculum Width	-0.16795	0.06423
Operculum Thickness	-0.18520	0.4133

Results

ANOVA and Descriptive statistics results for all the measured morphometric characters for all the sampled localities are presented in Table 2. Significant ($P< 0.05$) variations occurred among the locations for all the 10 measured morphometric variables. Significantly (F=4.51; P=0.001; DF=5,174; LSD= 2.33 mm) highest mean shell length (38.18±0.86 mm) was recorded from Tanjung Karang (Selengor) sampling locality, while the least (33.84±0.64 mm) was recorded from Tanjung Padiang (Perak) locations. The mean shell width (28.46±0.67 mm) was significantly (F=4.07; P=0.002; DF=5,174; LSD=1.94 mm) highest at Parit Bundar (Perak) sampling locality, whereas, the least mean shell width (25.12±1.04 mm) was obtained from Permatang Kuang (Penang) populations. Mean body whorl height was significantly (F=4.03; DF=5,174; P=0.001; LSD= 2.14 mm) highest (34.94±0.78 mm) from Tanjung Karang (Selengor) populations, while the least (31.27±0.63 mm) was gotten from Tanjung Padiang (Perk) populations. Spire height was significantly (F=2.44; DF=5,174; P=0.037; LSD=0.47 mm) tallest (3.28±0.21 mm) in the Tanjung karang (Selengor) populations and the Tanjung Padiang (Perk) populations recorded the least mean spire height (2.51±0.15 mm) (Table 2).

The difference for mean spire height was statistically significant for some populations (F=4.31; DF=5,174; P=0.001; LSD=1.73 mm). Mean aperture length of 27.24±0.67 mm was obtained from the Tanjung Karang (selongor) populations and the lowest mean length of 24.02±0.46 mm was also recorded from Tanjun Padiang (Perak) populations. Also, statistically significant (F=2.96; DF=5,174; P=0.013; LSD=1.26 mm) mean widest aperture (18.46±0.56 mm) was obtained on Tanjung karang (Selangor) populations, whereas the narrowest mean aperture (16.71±0.32 mm) was from Tanjung Padiang (Perak) populations. Apartural wall thickness was significantly (F=5.70; DF=5,174; P=<0.0001; LSD=0.03 mm) thickest (0.28±0.01 mm) in ang Permatang Kuang (Pennang) populations, whereas the thinnest (0.21±0.00 mm) was from Tanjung Padiang (Perak) populations (Table 2).

The highest mean value for operculum length (25.01±0.59 mm) was found in the Tanjung Karang (Selangor) collection locality and was significantly (F=5.41; DF=5,174; P=0.0001; LSD= 1.03 mm) different from the least mean value (21.97±0.49 mm) recorded from Tanjung padiang (Perak) paddy fields. Also, the widest mean Operculum (16.27±0.42 mm) was recorded from Tanjung karang (Selangor) population and was significantly (F=3.99; DF=5,174; P=0.02; LSD=1.09 mm) different from the least mean value (14.81±0.43 mm) from (Kampung Golok) Kelantan populations. Similarly, the lowest significant (F=12.17; DF=5,174; P=<0.0001; LSD=0.03 mm) operculum thickness (0.19±00 mm) was recorded in Tanjung padiang (Perak) sampling locality and the highest mean value (0.27±0.01 mm) was recorded from Tanjung Karaneg (Selangor) locality (Table 2).

Table 2: Descriptive statistics and ANOVA parameters for all the measured morphometric variables across the sampled localities

	Kampung Golok (Kelantan)	**Tanjung karang (Selangor)**	**Parit Buntar (Perak)**	**Localities Tanjung Piandang (Perak)**	**Kampung Tebangu (Kedah)**	**Permatang Kuang (Penang)**			
Morphometric Characters	**Mean ±SE (Range) Stdv (mm)**	**Mean ±SE (Range) Stdv (mm)**	**Mean ±SE (Range) Stdv (mm)**	**Mean ±SE (Range) Stdv (mm)**	**Mean ±SE (Range) Stdv (mm)**	**Mean ±SE (Range) Stdv (mm)**	***F***	***P***	**LSD**
Shell Length	34.89±0.78 (28.19-45.39) 4.27	38.18±0.86 (31.33-50.88) 4.61	37.86±0.87 (30.39-49.23) 4.79	33.84±0.69 (27.25-46.22) 3.77	36.25±1.19 (22.77-51.22) 6.53	34.69±0.47 (30.04-40.28) 2.59	4.51	0.001	2.33
Shell width	25.69±0.57 (20.79-31.43) 3.13	27.97±0.75 (20.33-37.41) 4.10	28.46±0.67 (22.29-37.81) 3.57	25.30±0.63 (20.09-37.64) 3.42	26.41±1.04 (15.44-39.76) 5.71	25.12±0.39 (21.08-30.10) 2.18	4.07	0.002	1.94
Body whorl height	31.88±0.73 (25.55-41.06) 3.99	34.95±0.78 (28.47-45.77) 4.27	34.67±0.82 (28.47-45.88) 4.94	31.27±0.63 (26.24-43.43) 3.44	33.23±1.08 (19.94-46.74) 5.92	31.93±0.46 (27.57-36.92) 2.54	4.03	0.001	2.14
Spire height	3.08±0.18 (1.41-5.52) 1.01	3.28±0.21 (1.51-6.21) 1.17	2.97±0.17 (1.05-4.82) 0.91	2.52±0.15 (0.95-4.51) 0.84	3.04±0.19 (1.04-5.58) 1.04	2.73±0.13 (1.49-4.2) 0.72	2.44	0.037	0.49
Aperture length	25.13±0.67 (19.05-37.61) 3.66	27.24±0.67 (22.69-38.31) 3.68	26.65±0.56 (21.48-33.70) 3.09	24.02±0.46 (20.64-31.64) 2.49	26.65±0.89 (16.29-36.56) 4.88	24.66±0.35 (21.53-27.94) 1.94	4.31	0.001	1.73
Aperture width	16.75±0.48	18.46±0.56	18.31±0.41	16.71±0.32	18.02±0.59	17.26±0.29	2.96	0.013	1.26

	(11.97-22.41) 2.62	(14.36-27.82) 3.05	(15.09-23.94) 2.27	(13.80-21.56) 1.74	(11.59-25.55) 3.22	(14.81-20.09) 1.61			
Aperture thickness	0.24±0.01 (0.13-0.43) 0.07	0.23±0.02 (0.16-0.70) 0.10	0.22±0.01 (0.1-0.31) 0.04	0.21±0.00 (0.17-0.26) 0.02	0.26±0.01 (0.11-0.40) 0.08	0.28±0.01 (0.13-0.38) 0.05	5.70	<0.001	0.03
Operculum Length	22.23±0.53 (14.91-20.03) 2.93	25.01±0.59 (19.17-33.81) 3.21	24.65±0.63 (18.11-33.07) 3.42	21.97±0.49 (17.65-29.01) 2.69	24.73±0.81 (15.27-33.32) 4.46	22.96±0.37 (26..93-18.46) 2.05	5.41	0.0001	1.63
Operculum width	14.81±o.43 (10.13-18.90) 2.32	16.63±0.42 (13.67-21.36) 2.28	16.27±0.35 (13.77-21.51) 1.93	14.84±0.28 (11.91-18.93) 1.54	16.29±0.53 (9.95-23.66) 2.93	15.49±0.30 (12.52-18.33) 1.67	3.99	0.002	1.09
Operculum Thickness	0.20±0.01 (0.11-0.43) 0.01	0.20±0.01 (0.16-0.44) 0.05	0.20±0.01 (0.16-0.38) 0.04	0.19±0.00 (0.17-0.22) 0.01	0.25±0.01 (0.14-0.39) 0.07	0.27±0.01 (0.17-0.32) 0.05	12.17	<0.0001	0.03

Discussion

In this study, we used shell and operculum character metric measurement to analyse for possible variations among six population/ecotypes of *Pomacea* spp from paddy fields of peninsular Malaysia. Morphometric analysis serves as a vital tool in biological studies for estimating huge discrepancies of shape, which represents collective outcome of complex interactions of the organism's ontogenetic components (Stone, 1998; Estebenet and Martin, 2003) [25, 13] and its environmental impacts on those components (Estebenet *et al*., 2006; Estebenet and Martin, 2003) [13, 12]. It can be used for differentiating Mulluscan species by contrasting overall features of shell measurement (Stone, 1998) [25].

The result of ANOVA for the morphometric characters from this particular study demonstrate that *Pomacea* spp in paddy fields from Tanjung Karanag (Selangor) are generally much larger than those from other sampling locations with respect to mean value for shell length, body whorl height, spire height, aperture length, Aperture width, operculum length and Operculum width. While the least value for most of these morphometric character were recorded from Tanjung Piandang locality (Perak). Intra-specific morpho-variations among parapatric populations of Mulluscan species in terms of some morphological characters are known. For instance, shell allometric growth inter-population difference was found in *P. canaliculata* from laboratory studies in Argentina (Tumbari and Martin, 2011) [27] and in field populations of Vivparids snails *Cipangopaludina chinensis* sampled four locations in Taiwan (Chiu *et al*, 2002) [7]. Similarly, shell band and band width variations were reported in *P. canaliculata* from selected aquatic habitats from the Philipines (Galan *et al*., 2015) [14], spire height, shell whorl, and apertural area among population differences were found from Madino in the Philippines (Mahilum and Demayo, 2014a) [20]. Furthermore, dissimilarity in shell shape among sexes was established in *P. canailiculata* population from the Philippines (Mahilum and Demayo, 2014 b) [20]. These differences is believed to arise as a response to ecological variants such as water depth (Galan *et al*., 2015) [14], food availability (Tumbari and Martin, 2011) [27] and as a result of environmental resistance in form of disturbances imposed on resident population which may consequently alter its ontogenetic development (Mahilum and Demayo, 2014a, b) [20, 21] among others. Phenotypic appearance of any biological extant is a product of functional interplay between its genomic makeup and its environmental elements (Estebenet *et al*. 2006) [13]. Enormous intraspecific differences for *Pomacea* spp have been elucidated in literature (Estebenet and Martin, 2003; Rawlings *et al*., 2007) [12, 23]. This perhaps explains the reason why the variation seen for most of the morphometric parameters in this our study are not ordered along defined spatial gradients or population clusters and their proximity. Some closely neighboring localities are seen to have differed statistically in their mean shell length. For instance, Parit Bundar is significantly longer than those of Tanjung Piandang both in Perak. Similarly, all the other measured morphometric characters of *Pomacea* spp from paddy fields of Pennisular Malaysia varied significantly without following a defined geographical spread and this made the designation of a standard size that applies to all the localities evaluated impossible.

Conclusion

The highest value for shell length, shell width, body whorl height, operculum width and spire height were recorded in the Tanjung Karang sampling locality. While the least value for some of these morphometric character were recorded from Tanjung Piandang (Perak) locality. The latter being significantly lower than those recorded on individuals from Parit Bundar, also in Perak, thus, making the assigning of a standard size that spread over to all the localities evaluated difficult. However, the result still strongly suggest that Ecotypes of *Pomacea* spp exist in Peninsular Malaysia which is somewhat distinguishable along the paddy production output of the locations. These findings will be vital in planning control measure as lethal doses (LD_{50} and LD_{90}) of mollucicides and other pesticides are mostly dependent on the live bodyweight, which in turn will depend on the metrics of the morphological features assessed. To this end, higher application rates of Mollucicides will be required in localities with bigger apple snails relative to where the sizes are smaller.

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