Traditional Morphometrics of Monogeneans (Metahaliotrema Spp.) from Scats Off Matang, Perak

Liew Zhe Aun, Michelle Soo Oi Yoon

Abstract: Monogenean was parasites that found on the marine or freshwater fish gill and skin as their host. They used their haptoral organs which are anchors, bars and hooks to attach the host gill or skin. In this study, Metahaliotrema species of monogenean was observed. Metahaliotrema species mostly found the fish called Scatophagus argus. In this study, the specimens of Metahaliotrema from the host Scatophagus argus at Matang, Perak was analyzed. There were three species of Metahaliotrema species could be found in the host fish i.e. Metahaliotrema mizellei, Metahaliotrema filamentosum and Metahaliotrema ypsilocleithrum. The morphometrics measurement of specimen's hard parts such as anchors, bars, copulatory organ and hook were be observed and measured using the software Leica QWin. The morphometrics information was analyzed based on morphometrics variables by using R to differentiate the Metahaliotrema species. The results show that the Metahaliotrema species were able to be differentiated by the morphometrics method. The measurement of all hard parts together was the best way to distinguish between the monogenean species. The measurementsof only the bars showed no differentiation at all.

I. INTRODUCTION

Parasite is an organism that lives in another organism, known as host. Parasite depend the host which they live for survival but it often harm its host by taking anything from the host [1]. It has to be live with host to grow and take advantage. Parasite can't live alone without host. Parasite rarely kills its host, if it kills its host which mean it uses the host too much strength and cause weaken the host.

Monogenean is a parasite that found in marine and freshwater fishes [2]. Monogenean is ectoparasites on the skin and gills of fish. It mouth is equipped with a sucker. It also has a posterior attachment organ armed with suckers and hooks calledhard part. The life cycle of monogenean is only involves one host for the adult. New host is located and infected by free-living larvae.

Revised Manuscript Received on March 08, 2019.

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Metahaliotrema is a genus rank of class monogenean classification. There are eight *Metahaliotrema* species according to World Register of Marine Species (WORMS) such as *Metahaliotrema arii* [3], *Metahaliotrema filamentosum* [4], *Metahaliotrema kulkarnii* [4] ,*Metahaliotrema mizellei* [4], *Metahaliotrema scatophagi* [3], *Metahaliotrema simile* [4], *Metahaliotrema yamagutii*[3] and *Metahaliotrema ypsilocleithrum*[4].

Metahaliotrema species is found on a fish called the spotted scat *Scatophagus argus*. *Scatophagus argus* is a fish from the family Scatophagidae. It occurs in two basic colour morphs which are called green scat and ruby or red scat. It also called spotted scat. It lives in coastal muddy areas, including estuaries, mangroves, harbours, and the lower courses of rivers [5].

Morphometrics can be defined as quantitative analysis of biological form [6] and approach to study of morphological variation which combines tools for many relevant biological questions description and statistical analysis [7,8]. It refers to form the quantitative analysis such as it encompasses both size and shape [9].

In this study presents the analysis of morphometrics variables of monogenean . The prime objective of this work was to determine the variability in size of the dorsal and ventral anchors of monogeneans from of the genus Metahaliotrema using traditional morphometrics to delineate the different species within the genus. In addition, analysis of the morphological characterization of monogenean based on the morphological similarity and differences was carried out.

II. METHODOLOGY

Data collection

Morphological data of Metahaliotrema species was collected from 19 slides with 108 specimens from the host (Scatophagus argus Linnaeus, 1766) is Matang, Perak.Species was identified based on the sclerotised hard parts which were their anchors and copulatory organ. Different species had different of their sclerotised hard parts. The length and shape of these sclerotised hard parts also differ across species.

Traditional Morphometrics (TM)

The morphometrics measurement of 108 specimens (n = 108) were taken from the sclerotised hard parts of the monogeneans consisting of anchors (two dorsal and two ventral), two bars (dorsal and ventral), 14 several marginal hooks and a copulatory organ. There are the commonly used morphometrics features monogeneans [10-12],For each anchor, there are 5 variables to measure: outer root (OR), inner root (IR), outer length (OL), inner length (IR) and point (Pt); 2 variables for both of bars: width (BW), height (BH); length of marginal hook (MHK) and length of copulatory tube (PL). The morphometrics measurements were done from digital image by using Leica images analysis software (QWin) from Leica Microsystems.

Data analysis- principle component analysis (PCA)

The software R was used to perform the analyses four scripts were prepared for implement PCA in R. The first script was ran from the excel sheet data which included all the sclerotised hard parts of the monogeneans consisting of each anchor, there are 5 morphometrics variables: outer root (OR), inner root (IR), outer length (OL), inner length (IR) and point (Pt); 2 variables for both of bars: width (BW), height (BH); length of marginal hook (MHK) and length of copulatory tube (PL) as well as separate into 3 species into 3 different excel sheet file. Besides that, the second scripts only included 5 variables for each anchor. Moreover, the third script included 2 variables for both of bars. The last script included ventral bar only. All the scripts were loaded into R and generate scatterplots for results.

III. RESULT AND DISCUSSION

Measurement of all hard parts (copulatory organ, bars and anchors)

Figure 1 shown that the scatter plot of morphometrics data from all the hard parts were comprised copulatory organ, hook, anchors and bars. 108 specimens included three monogenean species. Out of the 108 specimens shown, 106 specimens belong to *M. mizellei*. Another two specimens were *M. filamentosum* and *M. ypsilocleithrum* respectively. Most of *M. mizellei* were clustered tightly together in the scatter plot. Except *M. filamentosum* and *M. ypsilocleithrum* were located far away the cluster.

103 specimens of *M. mizellei* could be found in the middle of the clusters. The 3 red circle specimens of *M. mizellei* were out of the middle of the cluster. Probably these 3 specimens size were different with the 103 specimens. Then, the morphometrics measurement values were different with the others. Therefore,the overall information also had difference.

One specimen of *M. filamentosum* (blue circle) and one specimen of *M. ypsilocleithrum* (green circle) were separated and far from the main cluster. These two specimens were positioned away from the main cluster that means they were different species with the main cluster species.

*M. filamentosum*and *M. ypsilocleithrum* also located far way with each other. Both of them were proved which they were different species between them. Some specimens were missing some hard parts measurement then the measurement values were empty. The respective missing measurements valueswere replaced with mean values and assumed that these mean values contributed being to the plots were located different on the scatter plot even though they belong to same species.

Measurement of ventral anchors and dorsal anchors

Figure 2 was the scatter plot of morphometrics data which comprised ventral anchors and dorsal anchors.According to the scatter plot, most of the *M. mizellei* were clustered tightly together. Except*M. filamentosum*and*M. ypsilocleithrum* were located far away from the main cluster of *M. mizellei*.

Besides that, 100 specimens of *M. mizellei* could be found in the middle of the clusters. The other 6 specimens of *M. mizelle i*with red circle were out of the middle of the cluster. Probably these 6 specimens anchors size were different with the 100 specimens. The morphometrics measurement values were different with the others. It was assumed that the specimens with larger anchors had full-grown adults compared to juvenile monogeneans that hadn't fully grown.

One specimen of *M. filamentosum* and one specimen of *M. ypsilocleithrum* were separated and far away from the main cluster. These two specimens were positioned away from the main cluster because they were different species with the main cluster of *M. mizellei* species. *M. filamentosum*and *M. ypsilocleithrum* also located far way with each other. Mean both of them were also proved which they were different species. Some specimens at the slides were missing some anchorsof which the measurement values were empty. The respective missing measurements values were replaced with mean values and assumed that these mean values contributed being to the plots were located different on the scatter plot even though they belong to same species.

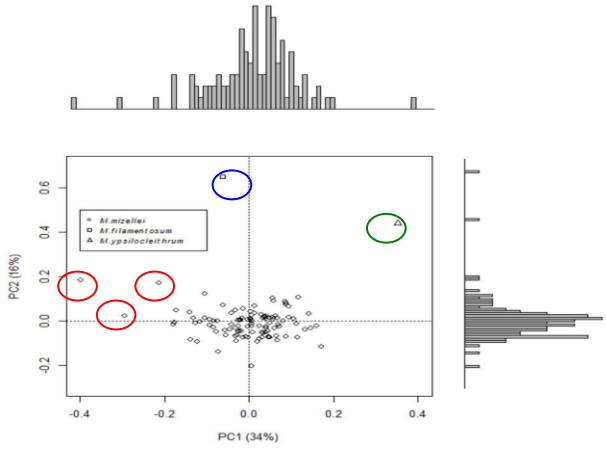

Fig. 1 PCA scatter plot for Metahaliotrema species based on the morphometry of hard parts which were ventral and dorsal anchors, ventral and dorsal bars, hook and copulatory organ. The centroid of each species is indicated as a shape

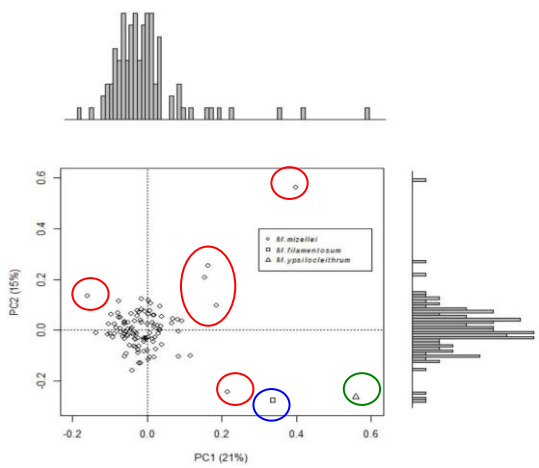

Fig. 2 PCA scatter plot for Metahaliotrema species based on the morphometry of hard parts which wereventral and dorsal anchors. The centroid of each species is indicated as a shape

Measurement of ventral bar and dorsal bar

In Figure 3, the scatter plot of morphometrics information constructed with measurement of ventral and dorsal bars. According to the scatter plot, most of the *M. mizellei* were clustered tightly together. Except *M. filamentosum* were located far away the main cluster of *M. mizellei*.

101 specimens of *M. mizellei* could be found in the middle of the clusters. The 5 red circle specimens of *M. mizellei* were out of the middle of the cluster. Probably these 5 specimens ventral and dorsal bars size were different with the 101 specimens. Then, the morphometrics measurement values were different with the others. It is assumed that the specimens with larger ventral and dorsal bars had full-grown adults compared to juvenile monogeneans that hadn't fully grown.

*M. filamentosum*was separated far from the main cluster. This specimen was positioned away from the main cluster that meansit was different species with the main cluster species.

*M. ypsilocleithrum*was located in the main cluster with *M. filamentosum*. The specimen contained both bar measurement and it still clustered closely together with *M. filamentosum*, this shown that the measurement can't differentiate them. Some specimens were missing some bars measurement then the measurement values were empty. The respective missing measurements values were replaced with mean values and assumed that these mean values contributed being to the plots were located different on the scatter plot even though they belong to same species.

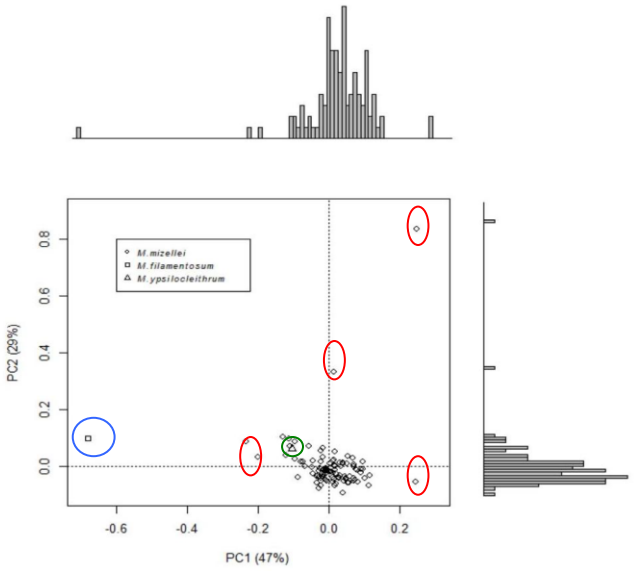

Fig. 3 PCA scatter plot for Metahaliotrema species based on the morphometry of hard part which was ventral bar. The centroid of each species is indicated as a shape

Measurement of ventral bar

As shown in Figure 4, the scatter plot of morphometrics information based on measurement of ventral and dorsal bars. According to the scatter plot, most of the *M. mizellei* were clustered tightly together. Except *M. filamentosum* was located far away the main cluster of *M. mizellei*.

101 specimens of *M. mizellei* could be found in the middle of the clusters. The 5red circle specimens of *M. mizellei* were out of the middle of the cluster. However, probably these 5 specimens ventral and dorsal bars size were different with the 101 specimens. The morphometrics measurement values were different with the others. It was assumed that the specimens with larger ventral and dorsal bars had full-grown adults compared to juvenile monogeneans that hadn't fully grown.

The specimen of *M. filamentosum* was separated far from the main cluster. This specimen was positioned away from the main cluster that meantit was different species with the main cluster species.

*M. ypsilocleithrum*was located in the main cluster with *M. filamentosum*. *M. ypsilocleithrum*contained both bar measurement and it still clustered closely together with *M. filamentosum*, showing that the measurement can't differentiate them. Some specimens were missing some bars measurement then the measurement values were empty. The respective missing measurements values were replaced with mean values and assumed that these mean values contributed

being to the plots were located different on the scatter plot even though they belong to same species.

IV. GENERAL DISCUSSION

According to the four scatter plots, the most significant result to differentiate the monogenean species was using all the hard parts measurement which included ventral and dorsal anchors, ventral and dorsal bars, hook and copulatory organ . All hard parts measurement could be used to distinguish between each monogenean species. The specimens in scatter plot separate to cluster clearly with their own species. On the other hand, the most not significant result was using ventral and dorsal bars measurement . The ventral and dorsal bars measurement couldn't be used to distinguish between each monogenean species. The reason was the cluster of specimens in scatter plot was spread out and not accurate.

Some missing hard parts in specimens could cause error and inaccurate clustering in the scatter plot. Errors came from the mounting specimens on slides were no good and the lack of experience to mount the specimens properly without damaging too much the organs. Most of dorsal bar from specimens were not seen because their dorsal bar were covered by themselves ventral bar.

In overall, the scatter plots showed three different clustering of monogenean species which were *M. mizelle, M. filamentosum* and *M. ypsilocleithrum*. The results shown that by using morphometrics analyses could differentiate monogenean species. Different monogenean species formed different clusters.

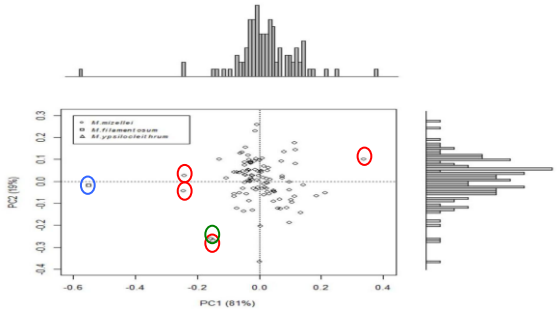

Fig. 4 PCA scatter plot for Metahaliotrema species based on the morphometry of hard part which wasventral bar. The centroid of each species is indicated as a shape

V. CONCLUSION

Total numbers of 108 *Metahaliotrema* monogeneans were observed from host fish *Scatophagus argus*from Matang, Perak. Three species of *Metahaliotrema* were separated under Kritsky*et al*. [4] which were *M. mizellei*, *M. ypsilocleithrum* and *M. filamentosum.* Four scatter plots with the three different species were built to visualize the result and differentiate the species with the measurements of the specimen's anchors, bars, copulatory organ and hook. From the four scatter plots built, the best result was the morphometrics measurement of all the hard parts which was the most accurate way to differentiate the species. On the other hand, the bad result was the scatter plot built with only morphometrics measurement of ventral bar and dorsal bar. Comparison between all the hard parts and bars, all the hard parts had more measurements than ventral bar and dorsal bar. The ventral bar and dorsal bar of specimens only contained two measurements then there was very easily mistaken. The missing hard parts morphometrics measurement of specimens contributed error and inaccurate to make result from the inconsistent scatter plots that same species far away from the clusters. In general, the results were still consistent and difference species could be separated clearly through the scatter plots. In this study, all the hard parts were good to be used to differentiate the monogenean species.

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