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A Comparison of Protein Content and Vitellogenin Sequences of *Scylla serrata* Sexes

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Abstract: The Philippines is one of the most prolific king mud crab (*Scylla serrata*) producers. Locally, pricing of mud crab harvests depend on weight and sex, with fishers pricing a sex- category called the "baklang alimango" higher compared to adult males and females. This type of crab is preferred as its meat boasts lower cholesterol levels compared to the sexually mature adult female, and its size is bigger than the sexually mature adult male. Its general morphology suggests it is an adult-sized immature female. To generate baseline information on the possible hormonal differences across the sex categories, the protein profile of the hepatopancreas and the vitellogenin sequences of the identified sexes were compared. No significant difference in protein concentrations were found across all sexes in the eleven *Scylla serrata* representatives of a wild population from Pangasinan. Alternatively, the SDS-PAGE profiles done on the same samples show the adult-sized immature females having significantly different protein profiles from the normal adult males and females for the supernatant, soluble, and microsome fractions of the hepatopancreas. Lastly, the PCR-amplified vitellogenin sequences showed that although normal females and adult-sized immature females have more similar sequences compared to the male sequences, they form separate subgroups. It is recommended to identify the different proteins in the SDS-PAGE profiles to aid in determining the physiological events that may be related to the phenotype, and to compare the protein profiles of the intermediate with both immature males and females.

Key Words: sex development; vitellogenin;, protein profiling; mud crabs

1. Introduction

The king mud crab, *Scylla serrata*, is a valuable resource in commercial fisheries and aquaculture industries (Imai et al, 2004). It is a mud crab widely distributed throughout the Indo-Pacific, stretching from east Africa to the Pacific (Keenan et al, 1998). It is a sought after delicacy because of its size, meat yield, and flavor. In the Philippines, local fishers distinguish a sex-category called the "baklang alimango". It is priced higher over sexually mature adult males and adult females as it is believed to be a healthier option due to its lower cholesterol level, while being larger in size.

The quickest means to differentiate sexes in

mud crabs is the shape of their abdominal flaps. The male crabs exhibit a deep narrow V-shaped abdomen, and the females have a wide U-shaped abdomen that is near to semi-circular in shape. A typical "baklang alimango" is differentiated by a wide V-shaped abdomen halfway in appearance between the adult male and female. Morphological examination of the "baklang alimango" indicates that it is most likely an adult-sized sexually immature female.

The sexual morphology of these adult-sized immature females may be brought about by altered hormonal signaling. This can be detected through the comparison of the protein profile of the hepatopancreas, where bulk of sex hormones is produced, of the different sexes. Alterations in hormonal signaling may be environmental or genetic in nature. A candidate hormone responsible for female maturation is vitellogenin, produced by sexually-maturing females in their livers, together with other the endogenous estrogen which are released into the bloodstream (Warrier et al, 2001). Differences in the vitellogenin gene sequence may affect the structure and functionality of the hormone.

2. METHODOLOGY

2.1. Sampling

A total of 67 *Scylla serrata* were collected from Dagupan, Pangasinan (16.0333° N, 120.3333° E). All samples were adults, with carapace widths of at least 100 mm.

2.2. Species and sex identification

From the 67 samples, observations on the shape of the forward-lobe spines (FLS), dactyl shape, propodus size and shape, and polygonal patterning on the legs were noted down to determine the samples' species (Keenan et al, 1998). Sexing was done based on shape of abdominal flap.

Confirmation of the morphological species identification was done through the amplification of the 16S rDNA gene from DNA extracted in muscle tissue following the protocol of Imai et al (2004).

2.3. Comparison of protein profiles

Pancreatic tissue, weighing 350 mg, was acquired from each individual representing each sex category. Cells were lysed using the freeze-thaw method and fractionated through differential centrifugation. Protein content in each fraction was measured using the Bradford assay (Kruger, 1994). Protein profiles were generated by running the isolated fractions in SDS-PAGE (Schägger, 2006).

2.4. Comparison of vitellogenin sequences

DNA was extracted from pancreatic tissue and the vitellogenin gene was amplified following the protocol of Warrier et al (2001). Sequences were aligned and differences visualized through a Neighbor-Joining Tree constructed using MEGA.

3. RESULTS AND DISCUSSION

3.1. Species and sex identification

From the 67 samples, only 11 were confirmed to be *S. serrata*. From the 11 samples, 4 were adult-sized immature females, 4 were adult males, and 3 were adult females.

3.2. Comparison of protein profiles

No significant difference in protein concentration was found in all fractions following ANOVA (Table 1).

Table 1. Average protein concentrations (ug/uL) of the whole hepatopancreatic tissues, and their fractions.

Fraction	Adult-sized immature females	Male	Female
Whole hepatopancreas	14.11	25.14	17.44
Supernatant	13.63	13.90	15.82
Nuclei	3.60	3.04	2.39
Microsome	1.38	1.19	1.42
Soluble	0.32	0.21	0.65

SDS-PAGE analysis shows that adult-sized immature females have significantly different protein profiles than the adult males and females (Fig. 1).

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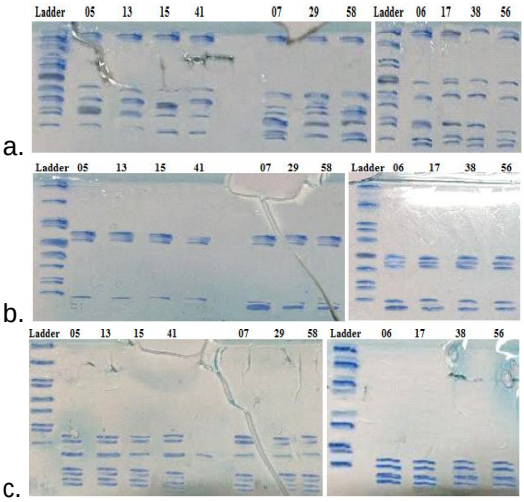

Fig. 1. Protein profiles of (a) supernatant, (b) soluble, and (c) microsome fractions. The 1st four lanes represent the males (05, 13, 15, 41), lanes 6, 7 and 8 represent females (07, 29, 58), while the four lanes of the second gel represent the adult-sized immature females (06, 17, 038, 056).

3.3. Comparison of vitellogenin sequences

Neighbor-joining analysis using MEGA program shows that the male crabs, which are boxed blue, and the female crabs, which are boxed pink, are in the extremities of the phylogenetic tree. The intermediate, which are boxed violet, can be seen being related closer to the females than that of the males even though they are clustered between the 2 sexes. The bootstrap values are low, with iterations set at 100 (Fig. 2).

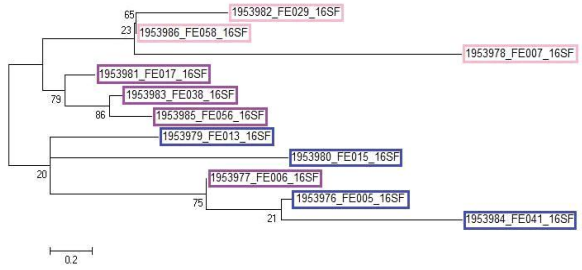

Fig. 2. A neighbor-joining tree showing the sequence similarities of the vitellogenin gene of the adult male (boxed blue), adult female (boxed pink), and adult-sized immature females (boxed violet).

4. CONCLUSION

This study shows no significant difference in the amount of protein production can be observed in the hepatopancreas of adult male and female mud crabs from the adult-sized immature females. The SDS-PAGE of the fractions, however, shows that the protein compositions are very different. The vitellogenin sequences across the sex categories show there is enough difference between the normal adult females and the adult-sized immature females to form two sub-groups.

Identity of the different proteins in the SDS-PAGE profiles will aid in determining the physiological events that distinguish the adult-sized immature females from normal males and females. Comparison of the protein profiles of this phenotype with normal immature males and females would supplement analysis if indeed these individuals are differentiated just because of their sexual development.

5. ACKNOWLEDGMENTS

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