Aquaculture 491 (2018) 205–209

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Identification of a species diagnostic character for instar and juvenile mud crabs (Genus Scylla)

Chona Camille Vince Cruz-Abeledo*, Kenbert Ting, Ma. Carmen Ablan-Lagman

De La Salle University, 2401 Taft Avenue, Malate, Manila, Philippines

ARTICLE INFO

Keywords:
Molecular biology
Taxonomy
Crustaceans
Aquaculture
Fisheries

ABSTRACT

A species diagnostic character in instar and juvenile *Scylla* spp. allows for more focused use of resources in farms, enhanced research in wild populations and improved culture practices into preferred species. Species identification using molecular methods entails extraction of soft tissue that can be fatal to early developmental morphs. This prevents downstream applications that require live samples. In this study, a potential species diagnostic character from the frontal lobe spine shapes of 177 wild-caught *Scylla* crablets was generated. The species of the samples were identified using the molecular internal transcribed spacer 1 and 16S rDNA markers. Fourier transformation was employed on the images of their spines using the software SHAPE and the results underwent discriminant and principal component analyses. This method was able to assign 92.4% of *Scylla serrata*, 96.2% of *S. olivacea* and 90.1% of *S. tranquebarica* to the right species. The frontal lobe spine shapes of each species group were then traced and used as a means for species identification on 50 cultured and 100 wild-caught *Scylla* crablets through Resemble.js. This method was then able to assign 85.9% of the instars and 84.7% to the correct species, verified using molecular markers. Results of this study show great potential for the use of the frontal lobe spines as a morphological diagnostic character for instars and juveniles. Increased accuracy can be achieved by expanding the reference shape database and inclusion of more *Scylla* populations across the region.

1. Introduction

Mud crabs (Genus *Scylla*) are valuable brackish-water commodities exhibiting fast growth rates and high flesh content. Three out of the four species can be commonly found in the Philippines: the mud crab *Scylla serrata*, the purple mud crab *S. tranquebarica*, and the orange mud crab *S. olivacea*. Improvement in culture practices have reduced reliance on wild-caught adults to 7.2% but grow out of wild-caught juveniles is still the predominant practice (Quinitio and Parado-Estepa, 2008). Crab growers use late instar to early juvenile mud crabs, called crablets, with carapace widths ranging from 10 to 60 mm and weighing less than 6 g, for grow-out in fish ponds.

One means to increase the production of mud crabs in grow-out ponds and maximize limited resources is to focus on the rearing of the preferred species, *Scylla serrata*, that grow bigger and faster compared to the other species. In the Philippines, however, any batch of wild-caught crablets may be a mix of any of the three species because of their overlapping ranges (Walton et al., 2006). Fishers compensate for projected loss in yield caused by farming mixed species by increasing the number of captured crablets.

There is no morphological diagnostic marker for *Scylla* species at early developmental morphs (Fushimi and Watanabe, 1999). The species diagnostic markers for the genus, including the inner carpus spines, the frontal lobe spine shape and the polygonal shapes on the swimming and walking legs, only become evident during late juvenile stages when the carapace width is at least 80 mm (Keenan et al., 1998). Molecular markers are available for use in species identification of mud crabs but the amount of tissue needed for nucleotide extraction and amplification are fatal for the crablets, preventing the grow-out of identified individuals (Imai et al., 2004). Developments in the use of image analysis and mobile computing have helped in the search for morphological diagnostic markers in other organisms. Graphical analysis programs, such as SHAPE, convert geometric images into mathematical values, called Fourier components (Iwata and Ukai, 2002). These values can be analysed using multivariate statistics to find similarities not easily detected using visual comparisons. With the combination of molecular and imaging techniques, it is possible to detect morphological similarities that may not be detectable through simple visual inspection.

In this study, the use of image analysis and molecular markers were combined to determine if the frontal lobe spine shape, a species diagnostic character in adult *Scylla*, can be used to identify the species of instar and juvenile mud crabs.

* Corresponding author.
E-mail address: chona.abeledo@dlsu.edu.ph (C.C. Vince Cruz-Abeledo).

https://doi.org/10.1016/j.aquaculture.2018.03.035
Received 12 October 2017; Received in revised form 9 March 2018; Accepted 19 March 2018
Available online 21 March 2018
0044-8486/ © 2018 Elsevier B.V. All rights reserved.

2. Materials & methods

2.1. Collection of samples

One hundred seventy seven crablets from the wild in Buguey, Cagayan, Philippines (18.2585° N, 121.7879° E) were gathered in 2015 to check if the frontal lobe spine shapes can be used for species assignment and to develop reference shapes for species identification. The site was chosen because of its pristine conditions, minimizing the existence of developmental defects on the crablets that may be caused by exposure to pollutants.

Another set of 50 crablets were sourced from a mud crab hatchery, also in 2015, in Iba, Zambales (15.3330° N, 119.9758° E) and 100 wild-caught crablets from Orani, Bataan (14.7933° N, 120.4761° E). These were used to check the effectiveness of the generated reference frontal lobe spine shapes for species identification.

All crablets had a minimum carapace width of 20 mm to ensure clarity in images and enough tissue is available for molecular analysis. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This included the use of market-standard transportation of the organisms in a well-aerated, moistened box, and the flash freezing in the sacrifice of animals.

2.2. Identification of developmental morphs

Principal Component Analysis (PCA) was performed on standardized morphometric ratios to give rise to clusters equivalent to developmental morph (Le Vay, 2001). The ratios were computed by dividing the weight with the carapace width, the carapace width with the carapace length and the abdominal flap width with the abdominal flap length. The use of standardized ratios and multivariate analysis was necessary as overlaps in published ranges of carapace width and body weight made these parameters insufficient as a means of identifying developmental stage on their own.

As majority of the immature mud crabs in the study were taken from the wild, the exact age and number of molts could not be determined. The expected developmental morphs range from late instar to early juvenile. The instar is the earliest morph where the carapace aalready exhibits its serrated oblate shape, the carapace width ranges from 10 to 50 mm with weights ranging from 0.5 to 3.5 g (Quinitio and Parado-Estepa, 2008). The juvenile morph is characterized by rapid weight gain and repeated molting events. The expected carapace widths can range from 40 to 60 mm with a weight range of 3.2–5.0 g (Quinitio and Parado-Estepa, 2008).

2.3. Molecular species identification

DNA was extracted from the muscle tissue of all 327 crablets using the KAPA Express Extraction Kit (Rehbein and Schiefenhövel, 2012). DNA from ten adults each of *Scylla serrata*, *S. tranquebarica* and *S. olivacea*, with mean carapace widths and weights of 123.4 mm and 436.5 g, 116.4 mm and 386.7 g, and 105.6 mm and 342.5 g respectively, served as positive controls. PCR amplification of the nuclear internal transcribed spacer 1 (ITS-1) region and restriction digestion using *Hha* I endonuclease at least twice for all crablets following the protocol of Imai et al. (2004) was done. The digested PCR products were visualized in 0.7% agarose gel stained with Sybr® Safe referenced with a 1000 bp ladder and used Gel Analyzer 2010 to determine the sizes of digested PCR products (Farrag et al., 2013). The sizes of the digested PCR products were used to identify the species of the samples.

To verify the accuracy of the species identification, 16S rDNA sequences of five crablets per species from Buguey, Iba, and Orani and from the adults that served as positive control (Imai et al., 2004) were amplified and then sent for sequencing through First Base Malaysia. The results were aligned with NCBI reference sequences using the online BLASTn tool.

2.4. Finding the corresponding morphological marker from molecular data

High-resolution images of the dorsal carapace for all the 177 crablets from Buguey, Cagayan were taken using a mobile phone camera (Mobile phone model: Apple iPhone 6). Each sample was laid flat on a white background to maximize contrast with the dark colored frontal lobe spines. Photographs were taken at a vertical distance of 135 mm directly on top of the sample in such that the whole carapace is visible in the photo and its outline clearly traceable. The auto focus feature of the mobile phone camera was used. The photographs were cropped to show only the frontal lobe spines and collaged into a single Bitmap strip image. Separate bitmap strips for each developmental stage were created. Separate analyses for the instars and juveniles by loading the corresponding bitmap strip independently onto the free software SHAPE (Iwata and Ukai, 2002) were done. SHAPE is an image analysis tool that flattens bitmap images and converts geometric shapes into Fourier components.

Discriminant (DA) and principal component (PCA) analyses were used to process the twenty four (24) Fourier components of each crablet within each developmental stage using the software STATISTICA. The Fourier components are derived from a mathematical transformation of the sinusoidal conformation of the shape of the frontal spines. Discriminant analysis is a multivariate analysis used to check for significant differences between assigned groups, and to check if the set of measured variables of an individual characterizes it as part of one group or another (Manel et al., 1999). For discriminant analysis, initial assignment of species of the crablets was based on their molecular identification. The usefulness of the frontal lobe spine shape was gauged based on percent assignment done using the Fourier Components. Principal component analysis, on the other hand, is a multivariate analysis that condenses the measured information into principal components that may be plotted on a factor plane (Overton et al., 1997). Individuals with more similar features cluster together in the graph and reveal groupings based on the continuous parameters that were recorded.

The outlines of the frontal lobe spines of crablets of the same species were traced and merged to form the species reference shapes.

2.5. Testing effectiveness of reference shape as a means of species identification

The reference frontal lobe spine shapes were tested on the 100 wild-caught crablets from Orani, Bataan, and on the 50 cultured crablets from Iba, Zambales. Photographs of the dorsal carapace of the crablets in each developmental stage were taken using the same conditions of the crablets from Buguey, Cagayan. The images were cropped to show only the frontal lobe spines and were compared to the reference frontal lobe spines using the free online image comparison tool Resemble.js (Zorrilla et al., 2013). Resemble.js is capable of comparing two images and provide the percent difference (%d) based on color and shape. For this study, the software was set to ignore the color values and to execute antialiasing to eliminate the contribution of image imperfections brought about by low resolution or lighting on the edges of the geometric shapes in the comparisons.

The species of each crablet was identified by determining which reference frontal lobe spine shape had the least percent difference (%d) to its own. The accuracy of this method was tested by comparing the results with the molecular species identification done. Multiple *t*-tests were also performed to check if the percent difference (%d) between the reference shapes and the frontal lobe spine shapes of the crablets with the same species is significantly less compared to the percent difference (%d) with the frontal lobe spine shapes of crablets with a different species.

Download English Version:

https://daneshyari.com/en/article/8493230

Download Persian Version:

https://daneshyari.com/article/8493230

Daneshyari.com