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Full Length Research Paper

Characteristics of papillae in wild, cultivated and hybrid sea cucumbers (*Apostichopus japonicus*)

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We investigated the characteristics of papillae of living, boiled and dried specimens of three wild populations, cultivated and hybrid *Apostichopus japonicus* in the laboratory. Visual observation showed that the appearance of the papillae was obviously different among the populations. For the first time, we established a standard criterion for estimating papilla number (PL/L ≥ 0.05, PL: the length of the papilla, L: body length) in living, boiled and dried *A. japonicus*. According to the criterion, we estimated the papilla number in living, boiled and dried specimens of wild, cultured and hybrid *A. japonicus*. Results show that the number of both dorsal and ventral papillae was significantly different among the groups. The largest total number of papillae was found in the Russian population (66.25 ± 8.11), which was significantly larger than those in the other groups (*P*<0.01). It was followed by those in the Japanese population and two hybrid groups (53.64 ± 7.88 for JJ, 54.65 ± 4.74 for CR and 46.29 ± 6.91 for CK). The differences between the number of dorsal and ventral papilla in Russian, Japanese and Chinese♂×Russian♀ hybrid populations were significantly larger than those in the other groups. This study increases our understanding of papilla traits in different populations of *A. japonicus* and has a direct application potential for selective breeding and its aquaculture.

Key words: *Apostichopus japonicus*, papilla, criterion, hybrid.

INTRODUCTION

Papillae, conical prominences on the body wall of holothurians, are an important characteristic for the taxonomy (Liao, 1997). Within the six orders of Holothuroidea, papillae are obvious in the orders Aspidochirotida and Elasipodida and inconspicuous in the orders Molpadiida, Dendrochirotida, Apodida, and Dactylochirotida (Liao, 1997). *Apostichopus* japonicus (Selenka), which are distributed along the coasts of North China, Japan, Korea, and far East Russia in northeast Asia (Chang et al., 2009), is a typical species of Aspidochirotida with obvious papillae (Liao, 1997). *A. japonicus* is the most economically important species among the 20 edible species of sea cucumbers in China (Chang et al., 2004). High commercial demand has resulted in a great interest in aquaculture of *A. japonicus*. Production of *A. japonicus* reached 102159 tons in 2009 in China (Chinese fishery statistical yearbook 2010).

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Papillae are the most commercially important trait in marketability of *A. japonicus*, whether it is living, boiled or dried. The length, number and condition of papillae greatly affect their market price, ranging from US$ 500/kg-US$ 2000/kg (dried beche-de-mer) in China. This affects the commercial potential of investigations on papilla, especially the selective breeding for papilla traits. Standard and precise measurement or traits is essential for selective breeding of marine organisms. Imprecise and non-standard measurement might greatly reduce the efficiency of selective selection (Gjedrem and Baranski, 2009). In market of *A. japonicus*, poorly developed prominences are not considered as papillae, at least not as commercially important papillae.

In spite of the great commercial demands and potential for selective breeding, no standard criterion is available for evaluating the papillae of *A. japonicus*. This lack greatly hampers the industrial development of food science and aquaculture of *A. japonicus*. Although genetic structure of different populations among its distribution has been well documented (Kim et al., 2008;

Chang et al., 2009; Kang et al., 2011), phenotypic characteristics of *A. japonicus*, especially those of commercial interest, have rarely been studied. Wild, cultivated and hybrid *A. japonicus* have greatly different commercial value in the market. In addition to body color (Kanno and Kijima, 2003), papilla trait is another important factor affecting the great difference of market price. However, we know of no report on both observation and quantitative analysis of papilla traits of wild, cultivated and hybrid *A. japonicus*. This is a bottleneck to increased marketing and commercial globalization of *A. japonicus*.

In this study, we observed and analyzed characteristics of papillae in *A. japonicus*. The main purposes of this study were to investigate: 1) whether characteristics of papillae can be observed and analyzed in *A. japonicus*; 2) whether a standard criterion can be established for evaluation of the number of commercially important papillae in *A. japonicus*; 3) whether the established criterion is an effective and scientific method that can be used in marketing and aquaculture of *A. japonicus*; 4) whether different groups of *A. japonicus* have significant differences in papilla traits; 5) whether heterosis occurs in papillae of hybrid *A. japonicus* and 6) whether papilla number of Chinese wild populations and cultivated groups are significantly different.

MATERIALS AND METHODS

Sea cucumber collection and maintenance

Three wild populations, cultivated and hybrid *A. japonicus* were investigated in the laboratory. Wild sea cucumbers were collected from Vladivostock, Russia (RR, 43°7′ N, 131°52′ E), Aomori, Japan (JJ, 40°59′ N, 140°16′ E), Zhangzi Island, China (ZC, 39°3′ N, 122°42′ E) and Heishijiao (HC, 38°52′ N, 121°34′ E), China. Cultivated individuals were collected from Wafangdian (LC, 39°41′ N, 121°24′ E), Zhuanghe (CC, 39°35′ N, 122°55′ E) and Donggang (DC, 39°47′ N, 124°6′ E), China, respectively. One hybrid (Chinese♂×Korean♀) was bred and cultivated at Xingcheng (CK, 40°31′ N, 120°46′ E), China and the other hybrid (Chinese♂×Russian♀) was bred and cultivated at Zhuanghe (CR, 39°35′ N, 122°55′ E), China. Their Korean and Russian parents were transported from Kimchaek, North Korea and Vladivostok, Russia, respectively. The cultivated and hybrid sea cucumbers were three years old. The age of the wild sea cucumbers was unknown.

Twenty individuals were randomly collected from each population (19 individuals from CC population). All individuals were temporarily maintained at the Key Laboratory of Mariculture, Ministry of Agriculture, Dalian Ocean University in China for 24 h under ambient light, sea water temperature (15°C), salinity (30‰) and pH (8.0).

Morphological observations of papillae

Three sea cucumbers from each group were randomly chosen and placed into individual trays with seawater. The morphological characteristics of the papillae were observed under natural light after they naturally elongated. After the morphological observation, the sea cucumbers were dissected for observation of structure and papillae.

Product preparation and trait measurement

A. japonicus are marketed live, boiled and dried. We evaluated the papillae of forms of living, boiled and dried sea cucumbers of the three wild populations and cultivated and hybrid *A. japonicus*. The papillae were evaluated first in living *A. japonicus* and then in the same specimens after boiling and then drying. The number of commercially important papillae was counted by an investigator. Body length and length of papillae were measured using digital calipers without touching sea cucumbers after they naturally elongated. The largest side length was considered as the papilla length. The specimens were weighed using an electronic balance and their volume measured using a graduate cylinder after 10 min of exposure to air.

After measuring the living *A. japonicus*, we dissected specimens to prepare boiled and dried products. The body wall was weighed after removal of the organs and coelomic fluid. The body walls were then boiled in sea water for 40 min, afterwards, papillae number, body length, papilla length, body volume and weight of the body wall were measured by the same investigator. After cooling also, body walls were placed into a container and dried at 65°C for 72 h. Papillae number, body length, papilla length and weight of the dried body wall were measured by the same investigator.

Criteria for commercially important papillae

Ten parameters of the living, boiled and dried sea cucumbers were measured or weighed: body weight (W), body wall weight (W_B), body length (L), body volume (V), papilla length (PL), papilla length/ body weight (PL/W), papilla length/body wall weight (PL/W_B), papilla length/ body length (PL/L), papilla length/ body volume (PL/V) and the number of papillae. The key to establish criteria for the number of commercially important papillae is to decide what is the smallest papilla of *A. japonicus*. Obviously, the decision of the smallest commercially important papilla in *A. japonicus* depends on body size. For each specimen, therefore, we divided the smallest length of papillae of a specimen by its body size traits (body weight, body length, body volume and body wall weight) to establish criteria for evaluation of the papilla number. Consequently, 11 criteria were established for living, boiled and dried *A. japonicus*.

Evaluation of the criteria

We randomly chose 25 individuals (12 female and 13 males; from different provinces of China) to estimate by observation the number of papillae of all the specimens. To choose the optimal criterion from the 11 established criteria, we used a paired design strategy to analyze the difference between observed (by 25 individuals) and calculated (11 criteria) values in living, boiled or dried specimens.

Comparison of papilla traits in nine populations

We re-estimated papilla traits of the three wild populations and cultivated and hybrid *A. japonicus* according to the optimal criterion ($PL_L/L_L \geq 0.05$). The total number of dorsal and ventral papillae and the difference between the number of dorsal and ventral papillae were re-estimated in living, boiled or dried specimens.

Statistical analysis

Statistical analysis was done with SPSS 16.0, one-way ANOVA and Dunnett multiple comparisons to detect the differences between observational and estimated number and variables among the nine

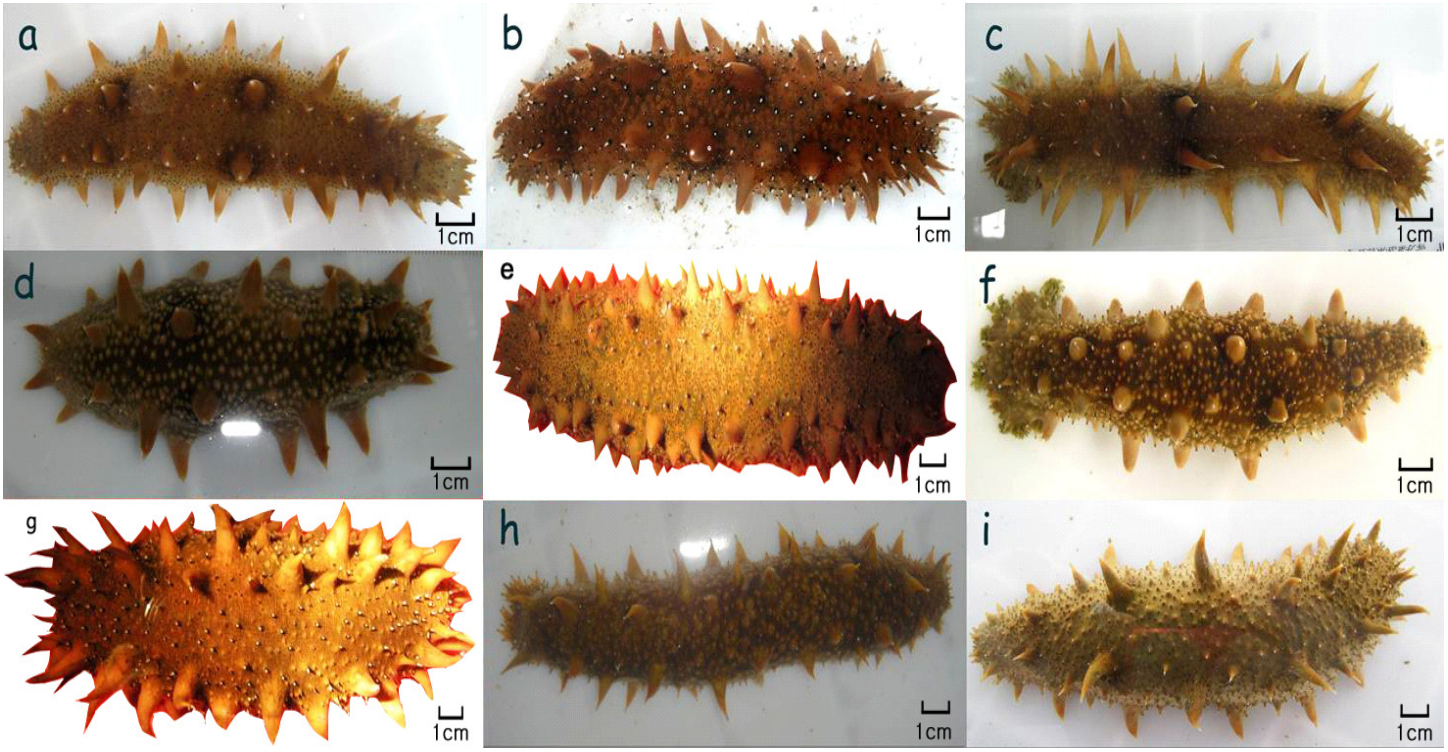

Figure 1. Appearance of specimens of *Apostichopus japonicus*. (a) CC, (b) CR, (c) DC, (d) HC, (e) JJ, (f) LC, (g) RR, (h) CK, (i) ZC. CC, Chinese pond cultured sea cucumber from Zhuanghe; CR, Chinese♂×Russian♀ hybrid; DC, Chinese pond cultured sea cucumber from Donggang; HC, Chinese sea cucumber from the population at Heishijiao; JJ, Japanese sea cucumber; LC, Chinese pond cultured sea cucumber from Wafangdian; RR, Russian sea cucumber; CK, Chinese♂×Korean♀ hybrid; ZC, Chinese sea cucumber from the population at Zhangzi Island.

populations of *A. japonicus*. A probability level of $P<0.05$ was considered statistically significant.

RESULTS

Morphological characteristics of papillae

A. japonicus had five ambulacra and five interambulacra (Figure 2). Four rows of tube feet were distributed in the ventral ambulacra, including 2 rows in the 2nd ambulacruma, 1 row in the 4th ambulacrum and 1 row in the 10th ambulacrum (Figure 2). The tube feet were dense in the 2nd ambulacrum and sparse in the 4th and 10th ambulacra. Papillae, on the other hand, were distributed in the dorsal and part of ventral ambulacra, including all of the 6th and 8th ambulacra and part of the 4th and 10th ambulacra. Characteristics of papillae were conspicuously different among the different groups (Figure 1). Chinese populations of *A. japonicus* had 4 rows of papillae (2 rows on the dorsal side and 2 rows on the ventral side), while Russian and Japanese populations had 6 rows of papillae (4 rows on the dorsal side and 2 rows on the ventral side).

More also, a white, semitransparent conical prominence was found on the top of the papilla when *A. japonicus* was naturally elongated. The main morphological structure and location of papillae of *A. japonicus* is showed in Figure 2.

Criteria for evaluating the number of papillae

According to the ratio of smallest length of papillae and the different body traits, we established 11 criteria ($PL_L/W_L \geq 0.05$, $PL_L/L_L \geq 0.05$, $PL_{SL}/L_{SL} \geq 0.05$, $PL_{DL}/L_{DL} \geq 0.05$, $PL_L/V_L \geq 0.05$, $PL_{SL}/W_L \geq 0.025$, $PL_{DL}/W_L \geq 0.02$, $PL_L/W_{LB} \geq 0.01$, $PL_{SL}/W_{SB} \geq 0.15$, $PL_{SL}/V_{SL} \geq 0.15$ and $PL_{DL}/W_{DB} \geq 0.5$) to estimate the number of commercially important papillae in the living and the boiled or dried body wall of *A. japonicus*. According to these criteria, we found the greatest difference using PL/WB and least difference using PL/L (Table 1). The ratios of the smallest length of papillae and body length (PL/L) were not significantly different among the three groups ($P>0.05$). The number of papillae in the body wall of the same individual, whether living, boiled or dried, was almost the same when evaluated by the PL/L criterion (Table 1).

To find an optimal criterion for evaluating the number of commercially important papillae, we used a paired design strategy to compare the number of papillae estimated by

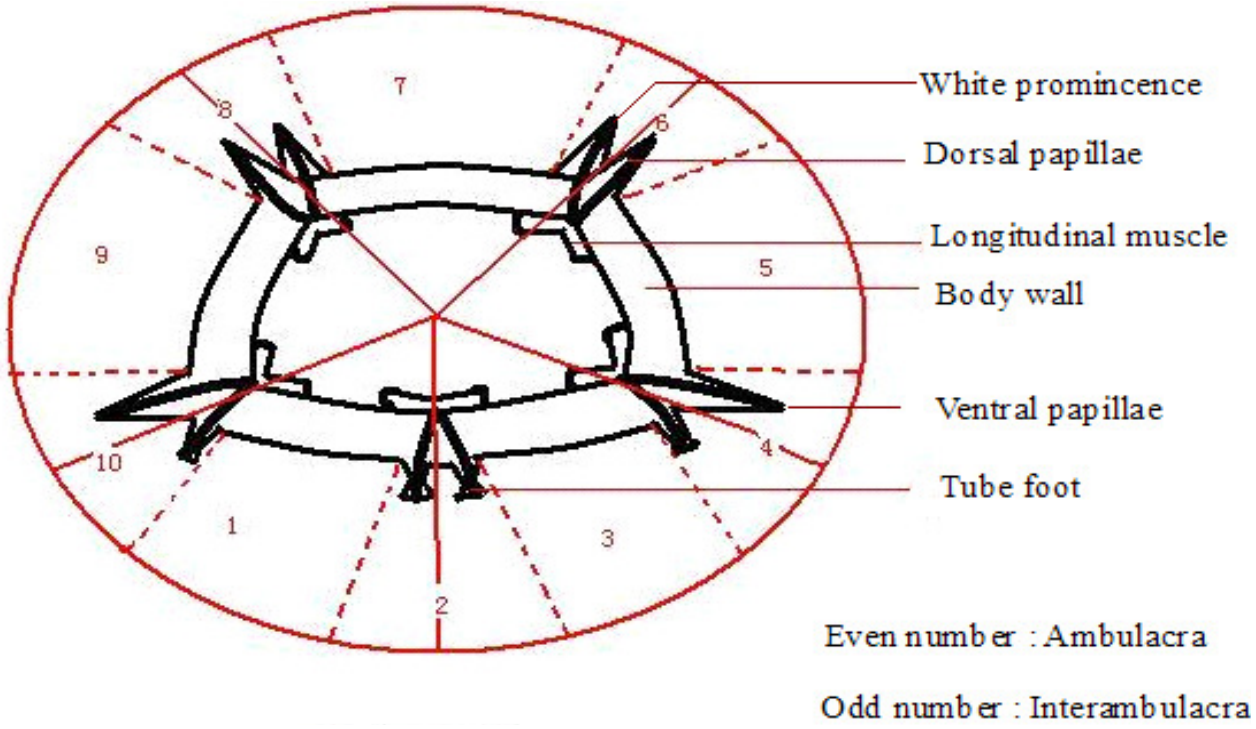

Figure 2. Drawing of *Apostichopus japonicus* in transverse section showing the location of the papillae and numbered sequence of ambulacra and interambulacra (odd number refers to 1, 3,…, 9; even number refers to 2, 4,…, 10).

Table 1. The ratios of the smallest length of papillae and four body size related traits (body weight, body wall weight, body length and volume).

Parameter	Smallest length of papillae /body weight	Smallest length of papillae / body wall weight	Smallest length of papillae / Body length	Smallest length of papillae /body volume
Living individuals	0.0524 ± 0.0080	0.1036 ± 0.0198	0.0511 ± 0.0122	0.0547 ± 0.0083
Boiled body wall	0.0310 ± 0.0066	0.1617 ± 0.0171	0.0527 ± 0.0057	0.2033 ± 0.0239
Dried body wall	0.0227 ± 0.0034	0.5299 ± 0.1018	0.0538 ± 0.0073	-

25 individuals with the number calculated by the criteria. The number of papillae did not differ significantly between the observed and calculated values for all three groups according to PL/W, PL/L and PL/V ($P<0.05$) but did differ significantly according to PL/BW ($P>0.05$, Figure 3). Considering precision, convenience and marketability, we chose PL/L ≥ 0.05 as the optimal criterion to evaluate the number of papillae in living, boiled and dried *A. japonicas* (Table 2).

Papilla number in different populations

We used $PL_L/L_L \geq 0.05$ (Table 2) as a standard criterion to re-evaluate the papilla number in the three wild populations and cultivated and hybrid *A. japonicus* (Figures 4 to 7). As shown in Figure 4, papilla number was significantly different in the nine involved populations ($P<0.01$). The largest total number of papillae was found in the Russian population (66.25 ± 8.11), which was significantly larger than those in other groups ($P<0.01$). It was followed by those in the Japanese and two hybrid groups (53.64 ± 7.88 for JJ, 54.65 ± 4.74 for CR and 46.29 ± 6.91 for CK). In Chinese populations, the average number of papillae in the three cultivated groups was 35.16 ± 1.57; larger than the average number of papillae in the two wild populations (29.57 ± 3.31).

The number of both dorsal and ventral papillae showed significant differences among groups (Figure 5 and 6; <0.01). The number of both dorsal and ventral papillae of the Russian population (37.95 ± 3.66 and 28.30 ± 3.60) was significantly larger than those in other groups ($P<0.01$). The numbers in hybrid specimens were significantly larger than those in both wild populations

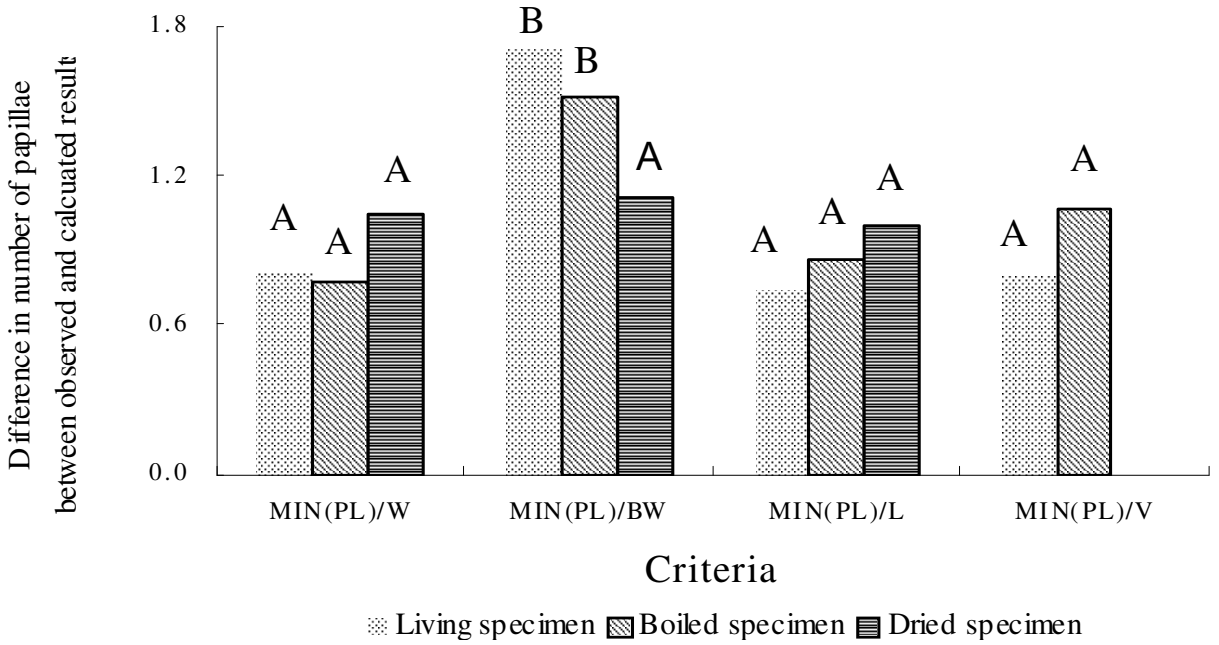

Figure 3. Differences in number of papillae estimated by observations of 25 people and calculated by the four kinds of criteria. Letters above bars refers to significant difference among specimens.

Table 2. Four criteria for evaluating the number of papillae.

Parameter	Length of papillae / body weight	Length of papillae / body wall weight	Length of papillae / body length	Length of papillae / body volume
Living individuals	0.05	0.10	0.05	0.05
Boiled body wall	0.03	0.15	0.05	0.20
Dried body wall	0.02	0.50	0.05	-

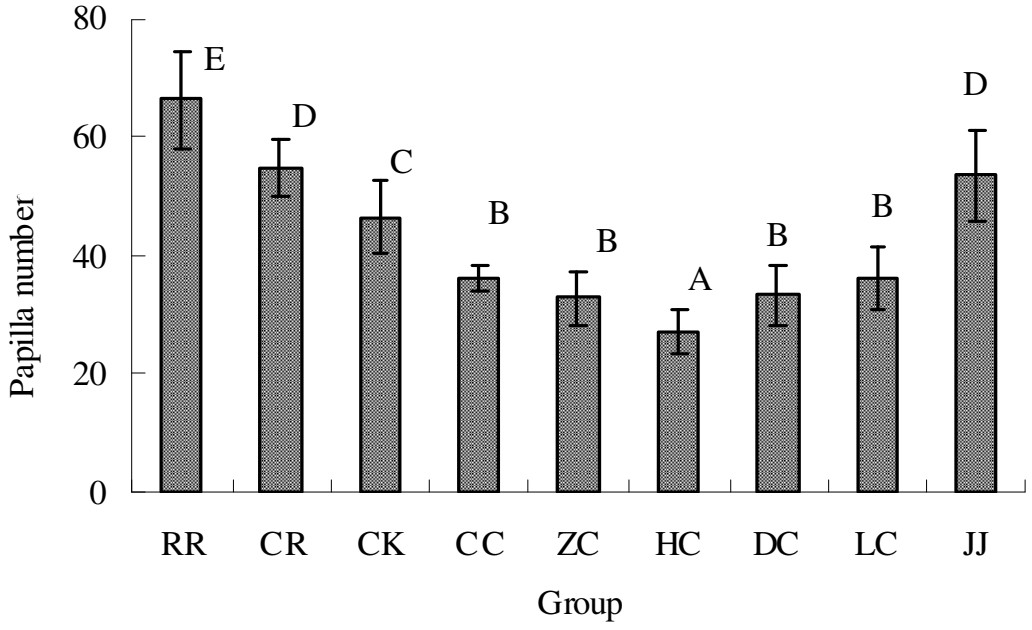

Figure 4. Total number of papillae in different groups of *Apostichopus japonicus*. Letters above bars refers to significant difference among groups.

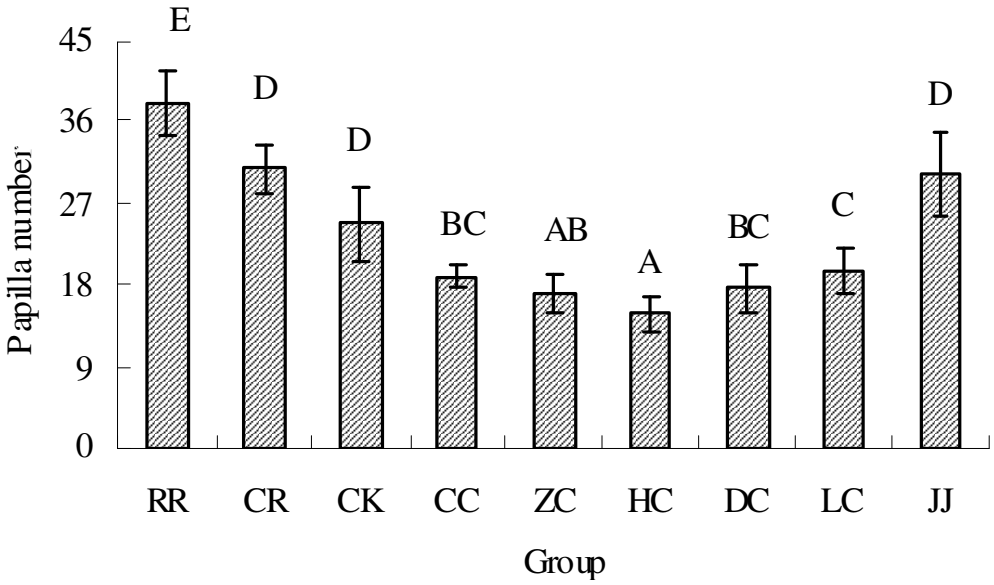

Figure 5. Number of dorsal papillae in different groups of *Apostichopus japonicus*. Letters above bars refers to significant difference among groups.

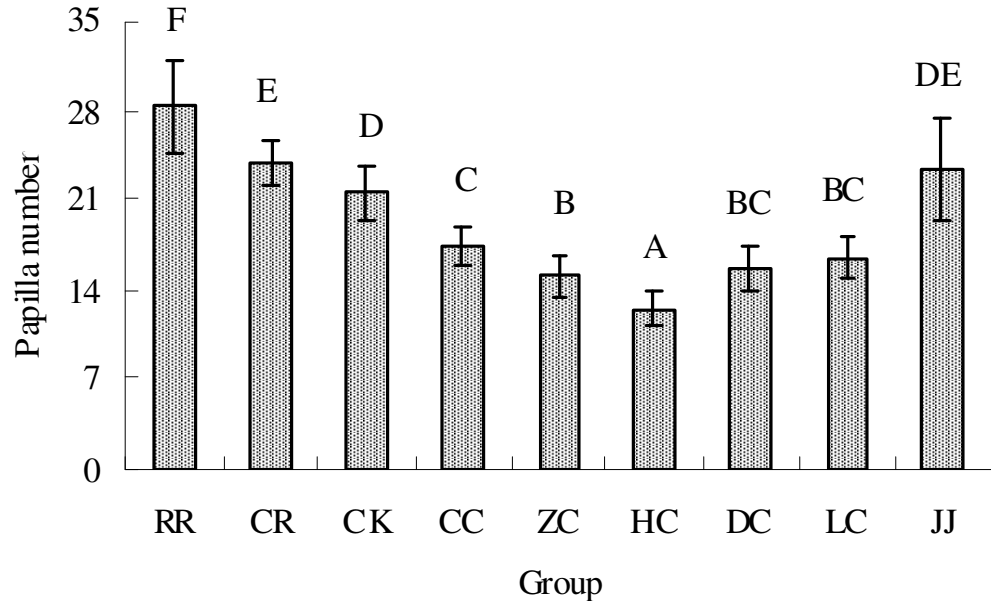

Figure 6. Number of ventral papillae in different groups of *Apostichopus japonicus*. Letters above bars refers to significant difference among groups.

and the cultivated Chinese groups (P<0.01). Between the wild populations and the cultivated Chinese groups, however, we found no significant difference in the number of either dorsal or ventral papillae (P>0.05). The number of ventral papilla in the hybrids (Chinese♂×Russian♀) was significantly larger than the other hybrids (Chinese♂×Korean♀) (P<0.05). The number of dorsal papillae, however, showed no significant difference between the two hybrids (P>0.05).

The number of dorsal papillae is obviously larger than that of ventral papillae. The differences between the number of dorsal and ventral papillae in Russian, Japanese populations and the hybrid (Chinese♂×Russian♀) were significantly larger than those in other groups, with averages of 9, 7 and 7, respectively (Figure 7). The average difference was 3 in the hybrid (Chinese♂×Korean♀) and 2 in the five Chinese groups. There was no significant number

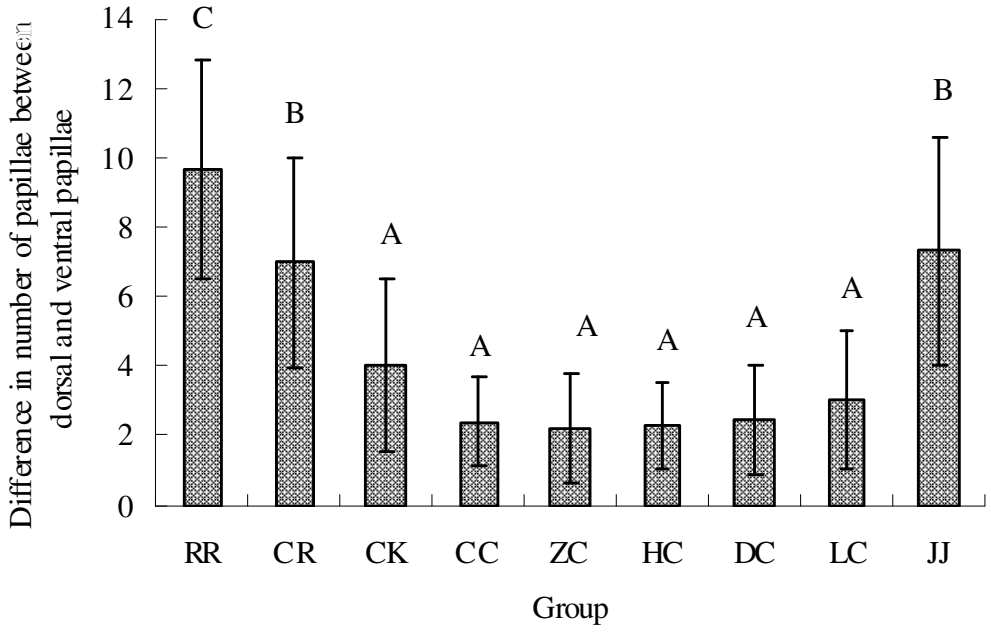

Figure 7. Difference between the number of dorsal and ventral papillae in different groups of *Apostichopus j*aponicus. Letters above bars refers to significant difference among groups.

difference between dorsal and ventral papillae among the hybrid (Chinese♂×Korean♀) and five Chinese groups (Figure 7).

DISCUSSION

Characteristics of papillae

Papillae have different morphological characteristics in different species of holothurians. Thelenota ananas Jaeger has 3 to 11 warts on a same base. In Stichopus variegateus Semper, many small tubercles are distributed on the body. In the genera Peniagone and Scotoanassa, some papillae together are like a velum (Liao, 1997). In contrast, *A. japonicus* has conical and large papillae (Chang et al., 2004). Difference in papilla characteristics (Figure 1) affects the commercial values of different populations of *A. japonicus*. The reason of the characteristics difference of papillae of *A. japonicus* remains unclear, although both genetic and environmental factors might be responsible.

To our knowledge, the white and semitransparent conical prominence on the top of the papillae in the naturally elongated *A. japonicus* has not been reported before and its function is unknown. Vanden-Spiegel et al. (1995) reported that the conical papillae of the sea cucumber *Holothuria forskali* are topped by a hemispherical bud. We observed that contraction in response to external stimulation first occurred in the white prominence and then in the whole papilla. The response to stimulation was much less when it was applied to the papilla rather than the white prominence. This suggests the white prominence is more sensitive to external stress. This is supported by the study by Vanden-Spiegel et al. (1995), who reported that the epidermis of the bud of the papilla in H. forsakali has a ciliated cell that intimately contacts the nerve plexus and has the classical structure echinoderm sensory cells. From our experiment with aquaculture of *A. japonicus*, moreover, rotting of the dermis, which is always the result of disease and environmental stresses, first happens in the papillae with disappearance of the white prominence. This provides new insight into environmentally friendly aquaculture of *A. japonicus*.

Criterion for evaluating papilla number

Papilla number is one of the most commercially important traits in marketability of *A. japonicus*. However, the lack of a standard criterion to evaluate the number of commercially important papillae greatly hampers the development of selective breeding and aquaculture in *A. japonicus*. In this study, we calculated ratios of the smallest length of papillae and four body size traits and established 11 criteria for evaluation of papillae in living, boiled and dried *A. japonicus*. Of the four kinds of criteria, we found no significant difference in number of papillae between estimated by observation by 25 people and calculation by the criterion PL/L ≥ 0.05. Moreover, the number of papillae in different specimens (living, boiled and dried) of the same individual was almost the same when evaluated by the PL/L criterion. This suggests that

PL/L ≥ 0.05 is the optimal criterion to evaluate the number of papillae of *A. japonicus*. For the first time, we reported an effective and scientific criterion (PL/L≧0.05) for the evaluation of papilla number in living, boiled and dried *A. japonicus*. This criterion provides the first insight into the standardization of evaluation of papilla number in *A. japonicus*. It has direct potential in selective breeding, aquaculture and marketing of *A. japonicus*.

Papilla number trait in the nine wide, cultivated and hybrid groups

For the first time, this study reports that both dorsal and ventral papilla number were significantly different among the three wild populations and cultivated and hybrid *A. japonicus*. This enriches our understanding of papilla traits in wild populations, and cultivated and hybrid individuals of *A. japonicus*. Kanno et al. (2006) reported that *A. japonicus* populations with different colors are reproductively isolated, suggesting that the phenotypes of different populations of *A. japonicus* have a genetic basis. Our results suggest that number of papillae is also a genetically based population-specific characteristic in *A. japonicus*. This study therefore provides fundamental information for the future selective breeding programs based on papilla characteristics in *A. japonicus*.

The widths of the 5 ambulacra and 5 interambulacra of *A. japonicus* were unequal. The width of ambulacra was larger than that of interambulacra on the ventral side. In contrast, the width of the interambulacra was greater than that of the ambulacra on the dorsal side. Each ambulacrum contained 2 rows of papillae. Together with the two rows of ventral papillae, there should be 6 rows of papillae in all populations of *A. japonicus*. We observed that Russian and Japanese populations had 6 rows of papillae (4 rows the dorsal side and 2 rows on the ventral side), but the Chinese population had 4 rows of papillae (2 rows on the dorsal side and 2 rows on the ventral side). However, we found that Russian population had significantly more papillae on both dorsal and ventral sides. This might be due to the presence of four more rows of well developed dorsal papillae of Russian population of *A. japonicus*. Although the mechanism remains mostly unknown, it suggests that expression of papilla involved genes that are more active in the Russian population. Further studies are therefore needed to find papilla genes with differential expression in different populations (groups) of *A. japonicus*.

Hybridization is an effective breeding method in marine organisms (Rahman et al., 2005; Ding et al., 2007). Jiang et al. (2009) reported an improvement of traits related to growth in hybrid F1 individuals from crossing Chinese and Russian populations of *A. japonicus*. However, characteristics of papillae in hybrids of *A. japonicus* have not been investigated. Although its mechanism is been widely achieved through hybridization in commercially important marine organisms (Rahman et al., 2005; Brady and Scheibling, 2006; Ding et al., 2007; Zhang et al., 2007). Heterosis of the papilla characteristic has never been reported in *A. japonicus*. We found that papilla number of the hybrid (Chinese♂×Russian♀) was significantly larger than Chinese populations, but significantly lower than the wild Russian population. This suggests heterosis of papilla traits is complex in *A. japonicus*.

In markets, wild and cultivated sea cucumbers obviously have different commercial values. Chen et al. (2008) reported that wild and cultivated *A. japonicus* have different genetic structure. However, no report is available on the difference in characteristics of papillae between wild and cultivated sea cucumbers. We found no significant difference in number of either dorsal or ventral papillae of wild populations, cultivated and hybrid *A. japonicus.* This therefore confirms that the number of papillae is a genetically based trait.

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