Genetic structure based on microsatellites and color variance analysis for the clam, *Paphia amabilis* (Philippi, 1847)

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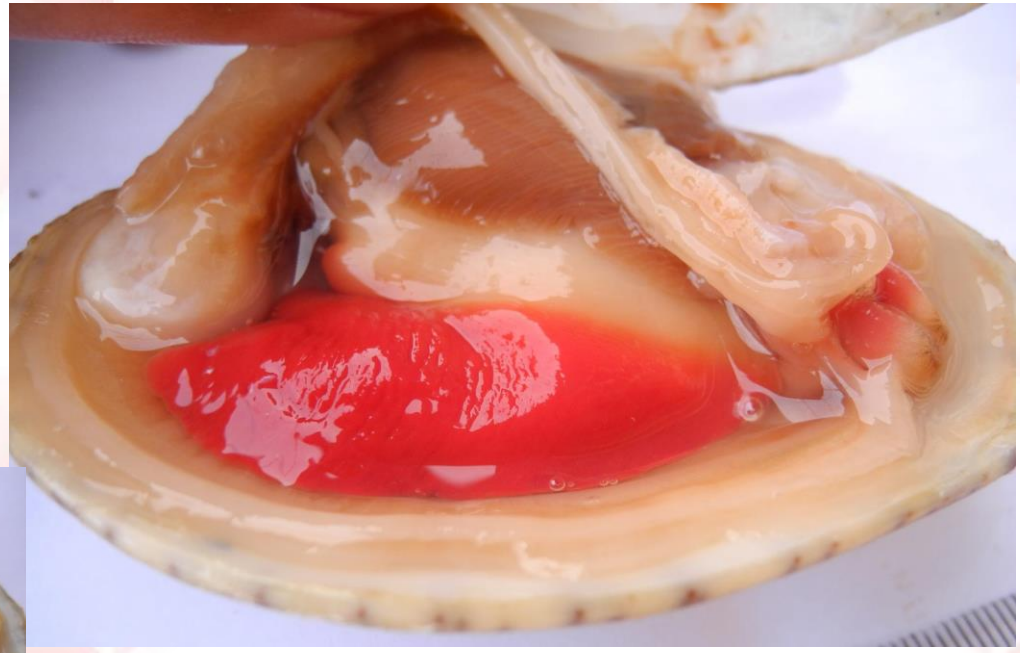
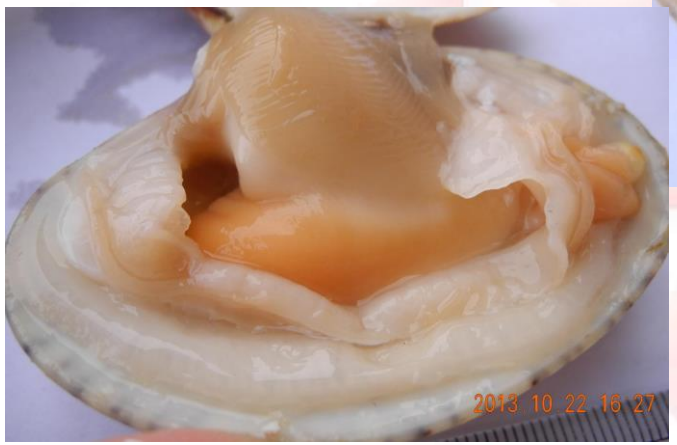
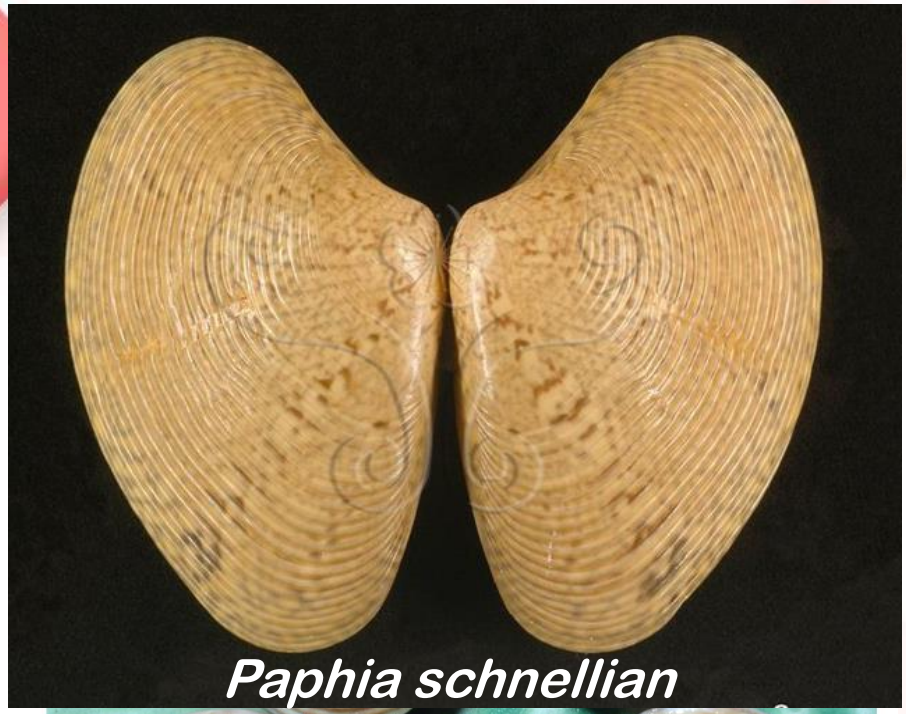
FEATURE

- ***P. amabilis*' muscles (f'oot and siphons) have a bright red color due to the presence of carotenoids which makes it worth rose.**





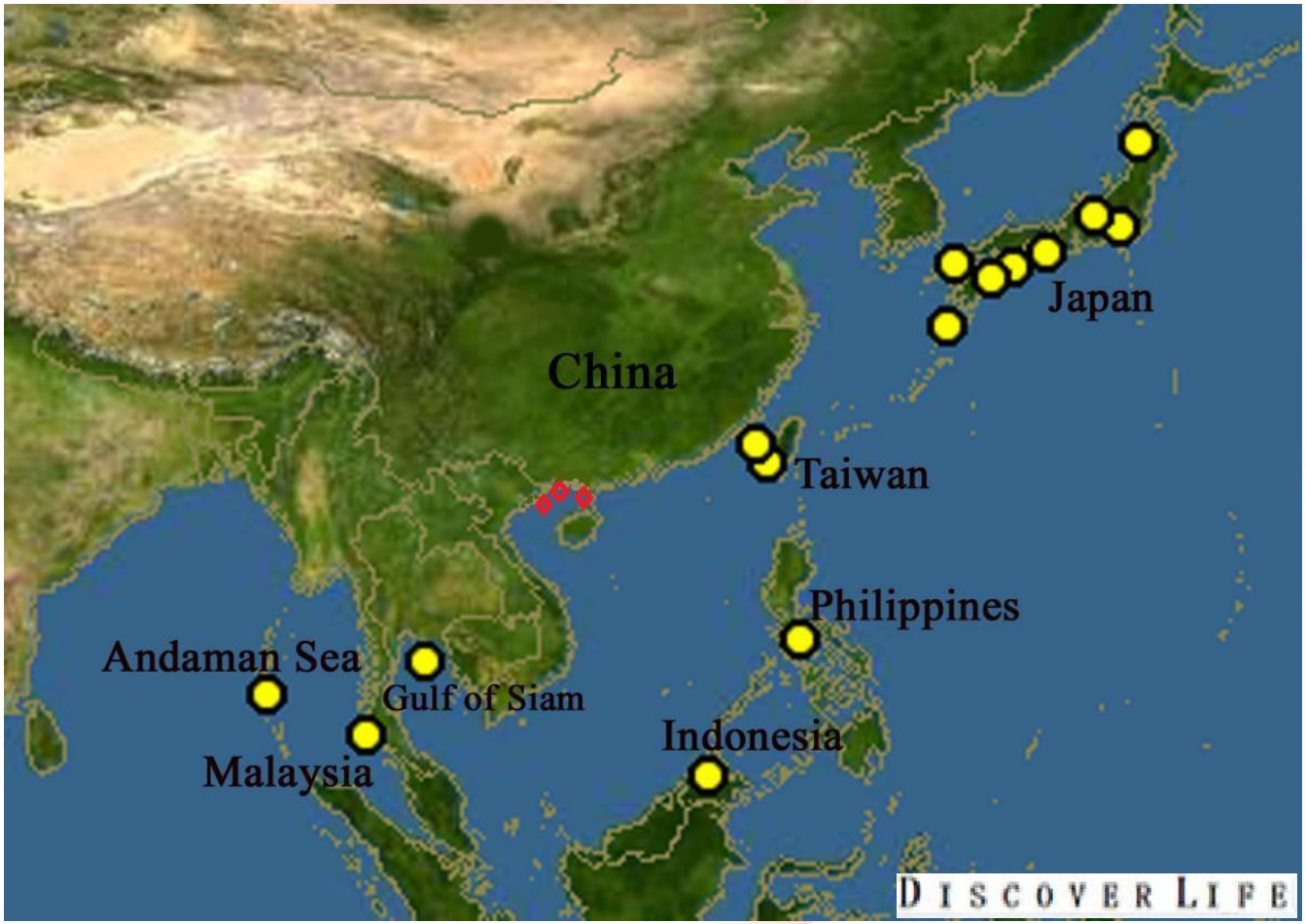


Paphia schnellian

P. amabilis

- **The shell color is reddish brown.**
- **The growth wheel is round striated, and undulated.**
- **The ventral margin of its shell is flat. (*Paphia schnellian*)**

DISTRIBUTION



- Inhabit sandy and muddy bottoms.
- From the subtidal zone to 70 m in depth.
- It is an important edible shellfish in southern Japan and China

P. amabilis is mainly distributed at the southeast coasts of Asia, from Japan to Andaman Sea- Nicobar Islands.

AQUQCULTURE AND PROBLEMS

- In recent years, its natural populations have been declining significantly due to overfishing and environmental changes, which highlights the urgency of its aquaculture.

------In 1980's, the resources of *P. amabilis* were rich in four provinces of China: Fujian, Guangdong, Guangxi and Hainan. But now the fishing of this clam is only available in several regions of Guangdong and Guangxi.

- Aquaculture and hatchery spats production of this species raised a pressing demand for microsatellites to evaluate the genetic diversity of the natural populations and to assist the selection of founder populations.
- We found 10-19% individuals with yellowish muscles in natural populations, and wondered its genetic mechanism.

DEVELOPMENT OF MICROSATELITE MARKERS

- A microsatellite-enriched library was constructed using a selective hybridization and magnetic bead enrichment method.
- Of the 44 sequenced clones, 30 microsatellite sequences were suitable for primer design.
- 20 primer pairs were amplified consistently in Beihai wild population.

Locus	GenBank No.	Motif	Primer sequence (5'–3')	Ta (℃)	Mg^{++} (mM)
Pam01	KF649189	$(CG)_7$	atatgactttctgccgtgacc tttcattggctaacacgacttg	50	2.0
Pam03	KF649190	$(CT)_{13}A(TC)_{14}$	tgtctgcatcatgattggttg tggcaaatgccgaaattaac	60	2.0
Pam05	KF740483	$(AC)_5$	gagggctcagatttgtgtttc atagggataagattggggagtc	60	1.5
……	……	……	……		
Pam 32	KF649200	$(AC)_6(ACA)_6$	catgcccatattttggtgaag tttgtgcatgttatcattgtcg	58	1.5
Pam 34	KF649201	$(CT)_{18}(TTAA)_3$	cttgattgaagcaaaatgcaaa ttcttgacaaccagtgccata	58	1.5
Pam36	KF740502	$(AG)_{24}$	gcaggtgtttgggagtatgtc cacgcagatcacagaaagtca	60	1.5
Pam38	KF740504	$(CT)_{23}$	gaggtgtaagtgaagctgatgc ggcatcttaatgtgtccctca	60	1.5

- The 20 microsatellite markers were used to analyze the genetic structure of the three wild *P. amabilis* populations in Beibu Bay, South China Sea.

COLLECTION OF SAMPLES

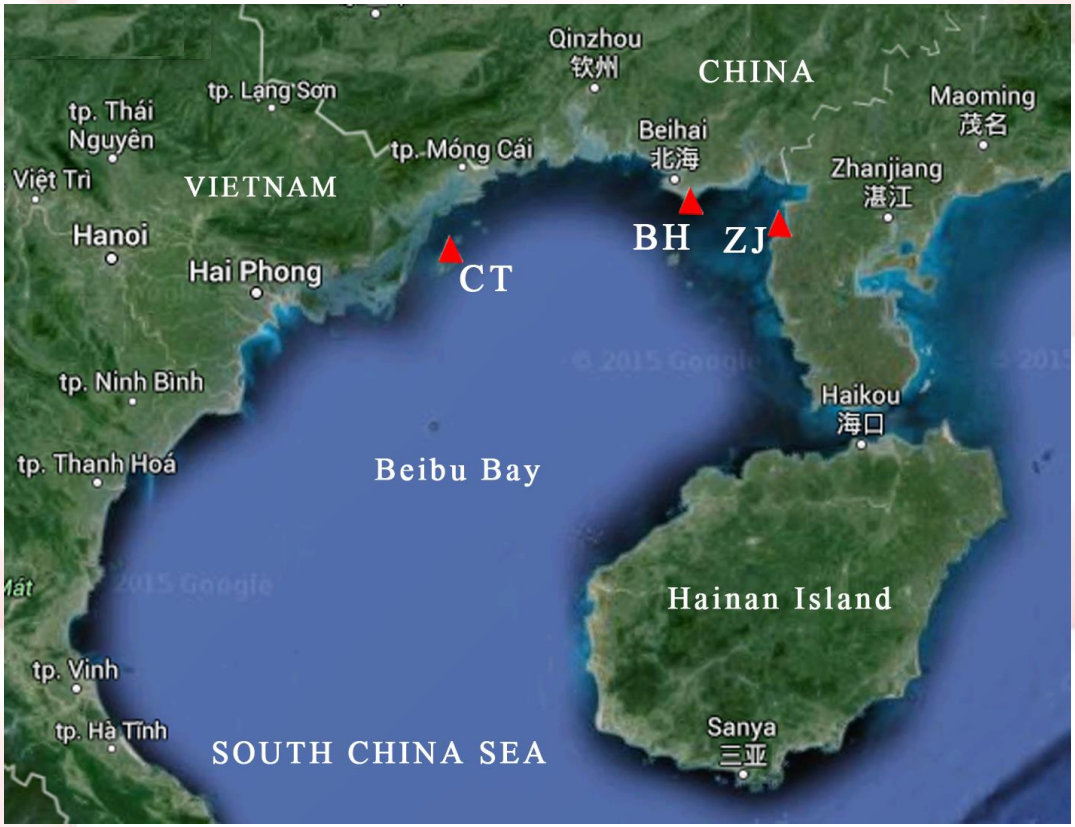
Three *P. amabilis* wild populations (n=32/population for genetic analysis) :

CT: from Co To Island, Vietnam in winter, N=57;

ZJ: from the west of Zhanjiang, Guangdong, China, in winter, N=43.

BH: from Beihai, Guangxi, China, in winter,N=60;

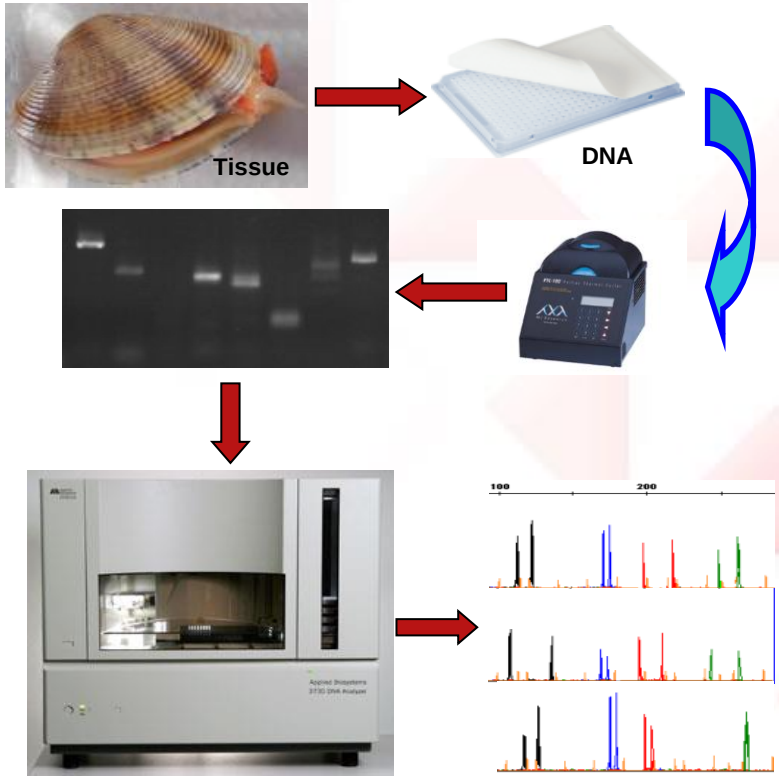
BHA: from Beihai in autumn, N=60;



MICROSATELLITE GENOTYPING

- **DNA extraction from the muscles used improved phenol chloroform method.**
- **Genotyping of microsatellite loci in three populations:**

- An economic method for fluores-cent-labeled PCR fragments was employed. M13(-21)-tailed forward primer.
- Amplified products were detected on an ABI 3730 Genetic Analyzer.
- Allele scoring was performed with GeneMapper v3.5.



STATISTICAL ANALYSIS

- **MICRO-CHECKER 2.2.1** software was used for identifying possible null alleles (1000 randomizations).
- **GENEPOP** was used to identify deviations from Hardy-Weinberg equilibrium (HWE) for each locus and for linkage disequilibrium (LD) between all pairs of loci (exact tests, 1000 iterations), also used to calculate global F_{ST} values between all pairs of populations.
- **ARLEQUIN 3.0** was used to calculate observed and expected heterozygosities, and single-locus F_{ST} and global multilocus F_{ST} values (10 000 permutations).
- **FSTAT 2.9.3** was used to calculate allelic richness (*R*s) and inbreeding coefficient F_{IS} per locus and sample.
- **PopGen32** was used to caculate the Nei's genetic distance between all pairs of populations and construct UPGMA trees.

- All tests were corrected for multiple comparisons by Bonferroni's correction.

POPULATION GENETIC STRUCTURE

- All 20 loci were polymorphism based on overall samples.
- Two loci (***Pam05, 38***) were not amplified in CT, and ***Pam23*** were monomorphic in ZJ and BH; ***Pam33, 37*** were only amplified in less than half individuals, which were excluded from further population genetics analysis.
- High level of genetic diversity (*a*, *Rs*, H_O and H_E) and low inbreeding coefficient (F_{IS}) were observed in population CT. And the medium and low genetic diversity in BH and ZJ (H_O), respectively.
- Three loci (*Pam 14, 20, 36*) deviated from HWE ($P < 0.05$, after Bonferroni correction) in at least 2 populations with heterozygote deficiency, probably due to the presence of null alleles.

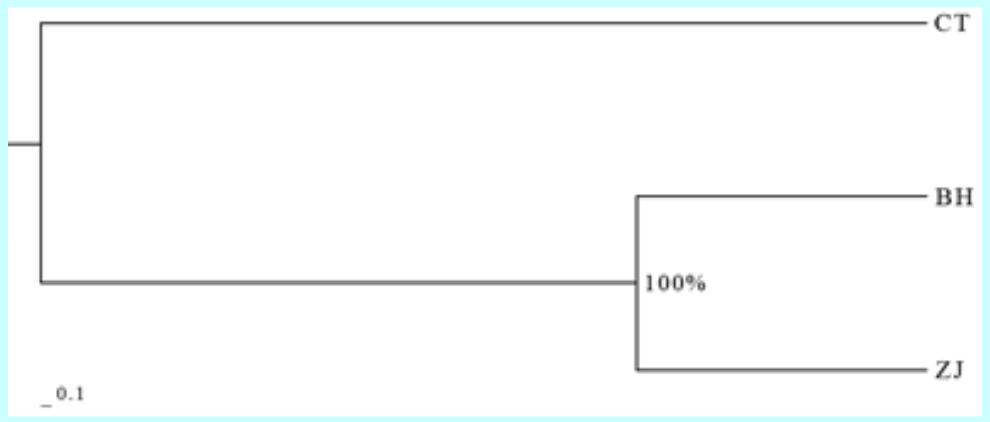
Pop	*a*	*Rs*	H_E	H_O	F_{IS}	Loci deviating HWE
CT	8.8±4.6	8.2±4.1	0.71±0.15	0.70±0.13	0.03	*Pam06,* ***20***, ***36***
BH	8.6±4.2	7.9±3.9	0.70±0.15	0.60±0.17	0.11	*Pam****14***, ***20***, ***36***
ZJ	8.1±4.2	7.5±3.8	0.70±0.15	0.50±0.20	0.10	*Pam08,* ***14***, *16,25,****36***

POPULATION GENETIC STRUCTURE

- Low genetic differentiation was revealed by globe F_{ST} (0.036) and the moderate pairwise F_{ST} (0.052), occured between Vietnamese (CT) and Chinese (BH & ZJ) populations.
- No significant genetic differentiation between population BH and ZJ--- Pairwise F_{ST} (0.002), UPGMA tree also indicated strong gene flow occurred between two Chinese populations (ZJ and BH), but decreasing with distance.

Pop	CT	BH	ZJ
CT		0.051	0.052
BH	0.173		0.002
ZJ	0.187	0.059	

F_{ST}: above diagonal;
Nei's genetic distance: below diagona



MUSCLE COLOR VARIANCE

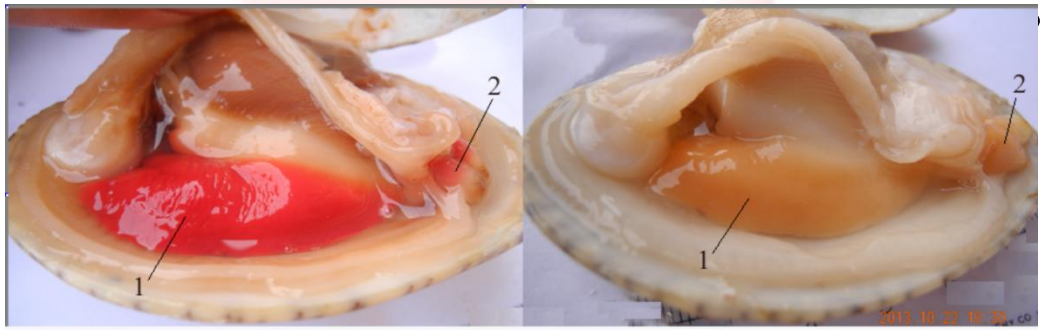


Figure 1 The bright red and the yellowish specimens of *P. amabilis*
Note: 1: foot; 2: inhalant and exhalant siphons

Pop	CT	ZJ	BH*	BHA
Total No.	57	43	60	60
Sample No. with yellow muscle	6	8	11	10
Percentage (%)	10.5	18.6	18.3	16.7

The muscles of the clam's foot and of siphons have a bright red color due to the presence of a new C_{37} skeletal carotenoids, which makes the clam not only beautiful and intriguing, but also healthy.

However, 10.5% to 18.6%

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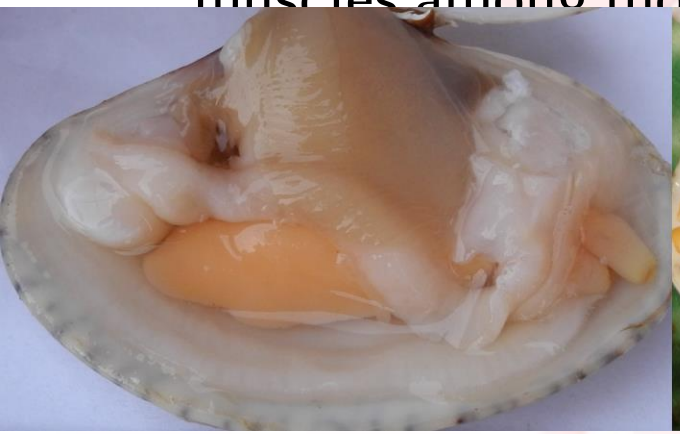
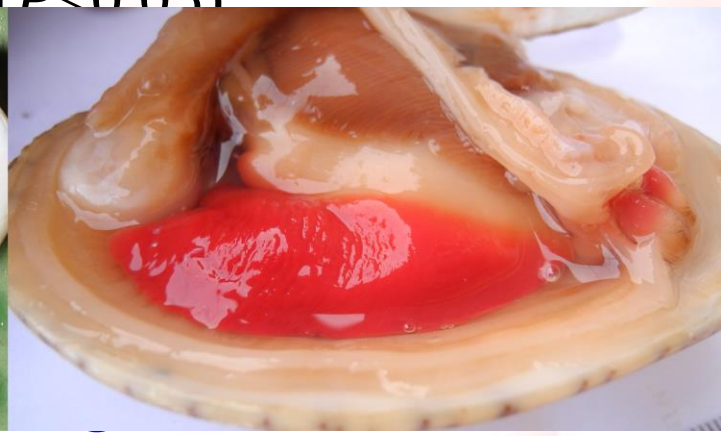
New C_{37} Skeletal Carotenoid from the Clam, *Paphia amabillis*

TAKASHI MAOKA,*,† NAOSHIGE AKIMOTO,§ MI-JIN YIM,# MASASHI HOSOKAWA,# AND KAZUO MIYASHITA#

MUSCLE COLOR VARIANCE

- Chi-Square tests revealed that no significant difference of the sex ratio (near 1:1) and the percentage of individuals with yellowish muscles among those four populations ($P<0.05$):





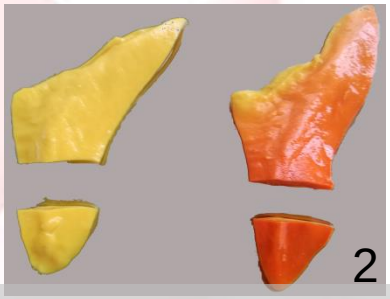
This may be one of the reasons why 8.3% individuals with muscle color between red and yellow in the winter BH population.

Pop	CT	ZJ	BH*	BHA
Total No.	57	43	60	60
No. with yellow muscle	6	8	11	10
Percentage (%)	10.5	18.6	18.3	16.7
♀/♂ with yellow muscle	3 : 5	2 : 6	--#	1:9

*The muscle color of 5 individuals in BH were between red and yellow.

Data not tested.

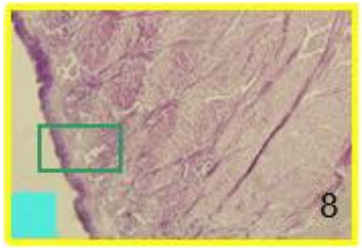
HISTOLOGICAL DIFFERENCES

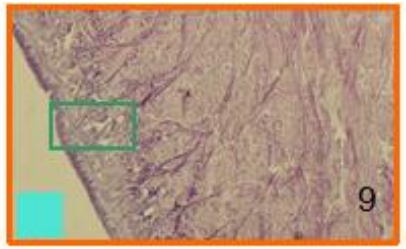


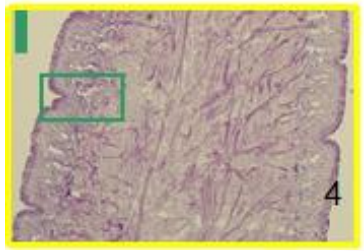
side view and cross-sectional location

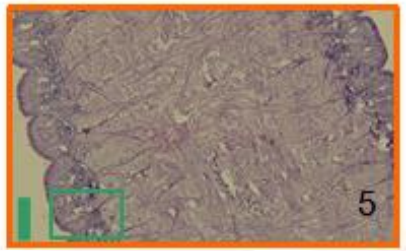
Anterior

Posterior







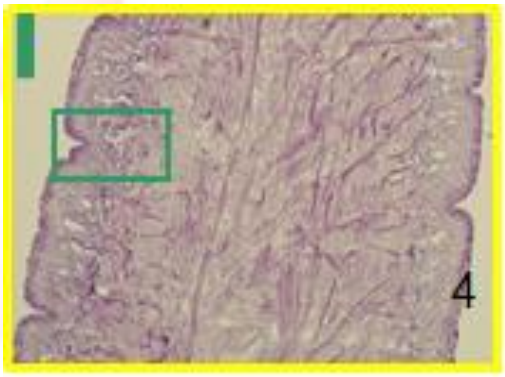


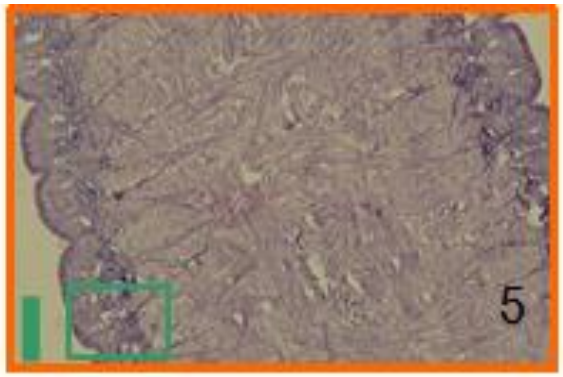
• The transection of foot (Fig. 3) was a rhomboid, anterior thick and short while posterior slender. The subcutaneously connective tissue and lateral muscle (↓) displayed especially deep red color after Bouin 's liquid fixation.

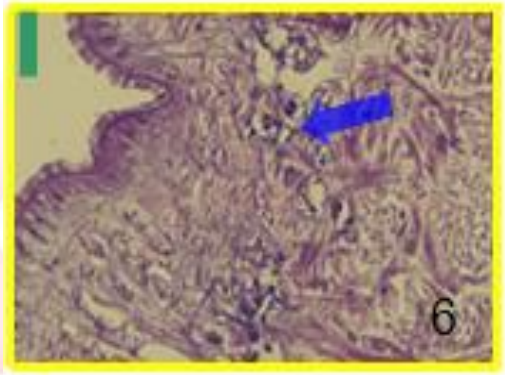
• Histological sections revealed that anterior epidermis was flat (Fig. 8, 9), while the posterior was corrugated (Fig. 4, 5).

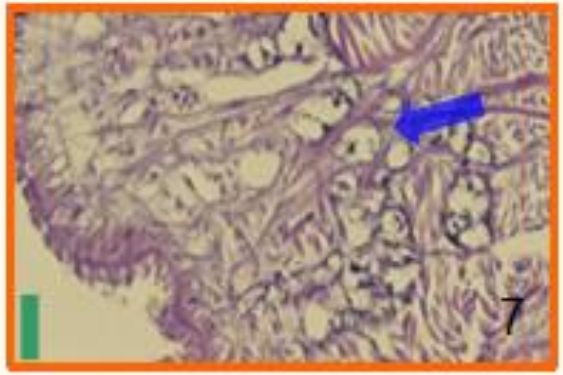
HISTOLOGICAL DIFFERENCES

The shape and the number of fat cells in the subcutaneously connective tissue were significantly different between the yellow and the red foot.





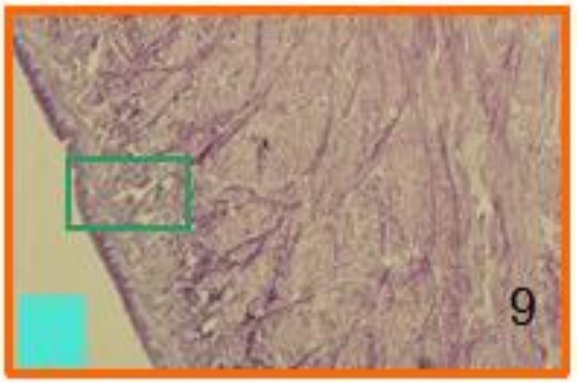


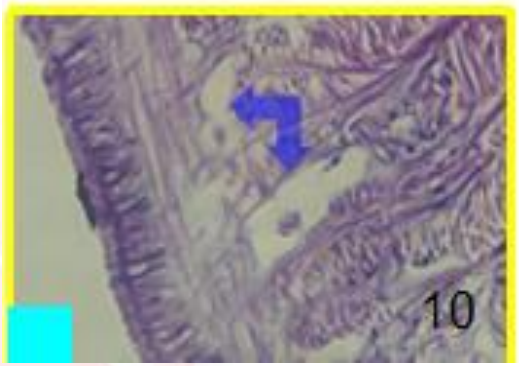


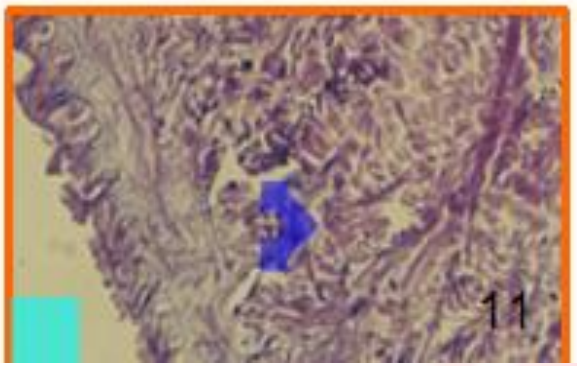
In the red foot, the subcutaneous fat cells of the posterior are smaller, round in shape (Fig. 7), and the density is much higher than that of yellowish foot (Fig. 6).

HISTOLOGICAL DIFFERENCES

- In the anterior, the subcutaneous fat cells are large and irregular in shape, more compact and finer in the red foot (Fig. 11) than that in the yellow one (Fig. 10).
- In general the amount and the density of fat cells are higher in the red foot than that in the yellow foot.







- That suggested that carotenoids may be more concentrated in the subcutaneous fat cells of the *P. amabilis'* foot.

MUSCLE COLOR, AND ITS GENETIC MECHANISM

- Little is known about the reason causing such color differentiation in *P. amabilis*. Carotenoids in shellfishes are usually from the algae they eat, for the animals themselves cannot synthesize carotenoids. But we know little about the genetic and regulatory mechanism of the enrichment of carotenoids in *P. amabilis* muscles yet.

- The allele number and frequencies of red and yellow muscles from three natural populations were compared. No specific markers or alleles were found in the yellow or red-muscle individuals---the quantity of carotenoids in the scallop muscles probably associated with the methylation level of the 5' regulatory regin of actin gene in *Patinopeton yessoensis*, with golden adductor muscle which was selected by genetic breeding.

CONCLUSION

- The impressive bright red color of *Paphia amabilis* not only tease people's appetite and mood, its color variance also attracted scientists′ interest.
- It is necessary to develop the aquaculture industry of this nutritious and intriguing clam. The significant genetic differentiation between the Chinese and Vietnamese populations implied the probability to develop particular strains for the benefit of aquaculture.
- In addition, this clam could also be an ideal animal for studying the genetic and metabolic mechanism of carotenoids accumulation.

ACKNOWLEDGEMENT

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