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患"白体病"罗氏沼虾腹部肌肉病变的超微结构

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摘　要: 取患"白体病"罗氏沼虾(*Macrobrachium rosenbergii*)腹部的病变组织及其鳃、心肌、肝胰腺、生殖腺等器官和组织, 经光镜和电镜制样观察, 未发现病原体, 表明该病为非病原体引起的疾病。光镜下的病变组织结构与正常组织相比无明显异常。但在电镜下, 病变主要表现为肌肉细胞的线粒体和肌质网的变性、坏死。线粒体肿胀、空泡化, 肌质网增多且形成许多内陷小泡, 表明细胞处于缺氧和钙代谢紊乱状态, 这可能与虾生存的环境和养殖条件有关。根据超微病理与组织学观察结果, 认为"罗氏沼虾白体病"更确切的命名应为"罗氏沼虾肌肉细胞线粒体坏死症"。

关键词: 罗氏沼虾; 白体病; 超微结构
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罗氏沼虾(*Macrobrachium rosenbergii* deMan)主要分布于东南亚各国。近 10 年来, 中国、日本、美国、澳大利亚相继引种, 开展了大规模的养殖[1]。但近年来, 在罗氏沼虾养殖区时常出现幼虾和成虾成批死亡现象。由于病虾表现为机体腹部出现絮状白斑, 故暂称为"白体病"[2]或"白浊病"[2], 该病呈逐年发展蔓延趋势, 成为阻碍罗氏沼虾养殖业发展的一大难题。由于该病病因至今尚未查明[3], 因而查明该病的病因是目前生产上急需解决的问题。本研究对该病病理学进行初步研究, 旨在探明该病的病因, 为生产上的防治提供有价值的资料。

1　材料和方法

1.1　材料

实验用病虾 32 只取自江苏省高邮地区的罗氏沼虾养殖塘, 包括蚤状幼体(体长 7 mm)、仔虾(体长 11～15 mm)和抱卵虾(体长 100～120 mm)。对照组的正常抱卵虾 10 只取自从未发病的苏州吴江地区的罗氏沼虾养殖塘。

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1.2　方法

分别选取"白体病"症状典型的未死仔虾的腹部肌肉组织和抱卵虾的腹部肌肉(病虾取白斑处肌肉)、心肌、肝胰腺、鳃、生殖腺等组织, 由于蚤状幼体在体视显微镜下没有发现絮状白色斑点, 因而对其肌肉采取随机多点取材的方法。光镜取材用福尔马林固定, 石蜡包埋, H-E 染色; 电镜取材用 4% 戊二醛固定, 磷酸缓冲液清洗后再用 1% 四氧化锇固定 2 h, 丙酮梯度脱水, 812 环氧树脂包埋, Reichert-Jung 超薄切片机半薄切片(1～2 μm), 甲苯胺蓝染色, Olympus CH－2 光镜观察。超薄切片(50～80 nm)经醋酸双氧铀、柠檬酸铅双染色, 日立 H600－Ⅱ型透射电镜观察。

2　结果(图版见附页 5)

2.1　病仔虾的外观症状

该病发生在蚤状幼体发育结束后的仔虾时期。从外观上看, 蚤状幼体后期的运动、进食、体色均正常, 虾体用肉眼和体视显微镜观察全身各部位均为透明琼脂色。当蚤状幼体蜕壳发育到仔虾时, 虾体在腹部最后 1～2 节开始出现白色絮状斑点, 以后虾体的白色斑点逐渐增多和扩大并弥散到整个腹部, 严重时可扩张到头胸部, 仔虾在出现体部白斑后, 游

动明显减少，反应活力和应激能力下降，易死亡，但发育个体的大小和进食能力没有明显差异，通常与正常虾混游于水中。

2.2　光镜观察结果

解剖镜观察病变初期和中期病虾的腹部肌肉色泽混浊发白，但其组织切片的光镜观察结果未显示明显异常，此外，组织病理学研究表明正常虾与病变初期和中期病虾的各组织结构相比也无明显差异。

2.3　电镜观察结果

通过电镜观察，在病虾的腹部肌肉、心肌、肝胰腺、鳃、生殖腺等组织中未发现病原体。病虾与正常虾的心肌、肝胰腺、鳃、生殖腺等组织无明显差异。但是在不同时期病虾的腹部肌肉中均发现肌肉细胞的线粒体坏死和肌质网变性现象。所以该病病虾的主要病理学特征表现在肌肉组织和细胞上。

2.3.1　正常虾的肌肉细胞结构　正常虾肌细胞排列整齐，肌细胞膜完整平滑，肌细胞中的线粒体内、外膜清晰可见，其内的嵴很丰富，为密集的网状排列，线粒体内基质着色均一。肌质网表面光滑没有凹陷，不含小泡。肌细胞胞质中肌浆分布较为均匀（图版Ⅰ－1）。

2.3.2　病虾的肌肉细胞结构

（1）抱卵虾　高邮养殖地点抱卵虾的肌细胞中没有发现像蚤状幼体肌细胞线粒体中的高电子密度颗粒，也没有发现线粒体基质中出现密度分布不均匀较高电子密度的团块，但线粒体基质空化现象普遍存在（图版Ⅰ－2）。

（2）蚤状幼体　蚤状幼体的腹部用肉眼和光镜观察均视为正常。电镜观察时发现肌细胞中线粒体的嵴为浓密的网状（管状），其基质中出现密度分布不均匀高电子密度的颗粒（图版Ⅰ－3）。肌细胞中大部分基质形态结构正常，细胞质核糖体均匀分布在细胞基质中，没有发生空化或凝聚现象，但在局部区域肌细胞肌质网外的基质中出现散在分布的高电子密度颗粒。肌丝、肌膜及其他结构基本正常。

（3）仔虾　发病初期仔虾腹部肌细胞的线粒体形态出现异常，体积肿胀膨大，线粒体基质中出现密度分布不均匀较高电子密度的团块（图版Ⅰ－4）。发病中期溶酶体分解变性的细胞器，残余小体产生，线粒体的数量明显减少（图版Ⅰ－5）。肌质网出现内陷小泡，肌浆空化（图版Ⅰ－6）。在病变后期，肌细胞出现水肿，残余小体增多，肌丝肌节萎缩损伤，细胞核结构基本正常。

3　讨论

3.1　罗氏沼虾"白体病"的病理学特点及病因分析

3.1.1　病虾肌肉细胞的超微病理学特征　超微病理学观察显示，患病前期、中期病虾的主要病理学特征是肌细胞线粒体和肌质网的病变，而这 2 种细胞器在肌肉收缩运动时起着重要的作用。线粒体是肌细胞活动代谢的能量供应场所[4－5]。通常状态下肌肉细胞中的线粒体数目很多，线粒体内嵴很发达，大量的线粒体聚集在肌丝周围，为肌肉细胞收缩运动提供大量的能源[6－7]。线粒体损伤破坏必然影响肌细胞的正常功能。

肌质网则与钙离子释放和肌纤维收缩密切相关，它有主动运输 Ca^{2+} 的作用，而这种主动运输系统能提高 Ca^{2+} 的浓度，并通过这种作用调节肌丝周围肌浆中 Ca^{2+} 的浓度。当使肌肉收缩的神经信号到达肌肉时，肌原纤维周围的肌质网的囊泡就释放出 Ca^{2+}，Ca^{2+} 弥散到肌浆中，于是肌原纤维开始收缩。正常情况下神经冲动传导结束后，肌质网的通透性降低，肌质网膜上的 Ca^{2+} 激活 ATP 酶（钙泵）与肌浆中的 Ca^{2+} 结合，利用水解 ATP 释放的能量，通过主动运输方式把 Ca^{2+} 回送到肌质网中[4－5]。病虾肌细胞内线粒体的损伤必然阻碍肌质网 Ca^{2+} 回收。从比较形态上可以看出正常的肌质网膜结构圆润光滑（图版Ⅰ－1），发病的肌质网的小囊泡表面出现内皱和凹陷吸泡现象（图版Ⅰ－6）。这可能是由于线粒体功能衰退和肌质网功能异常使得钙离子回收能力下降，肌细胞中肌浆的 Ca^{2+} 浓度增加和肌质网内 Ca^{2+} 浓度减少而造成的，这也许是导致肌肉组织白化的原因之一。

除此以外，肌浆中还出现了残余小体，残余小体是初级溶酶体消化细胞内衰老的细胞器及内溶物的最终残存物质（图版Ⅰ－5）。胞浆中的线粒体、肌质网损伤后被溶酶体吞噬消化形成残余小体[8]。

3.1.2　罗氏沼虾"白体病"致病机理初步分析　有报道认为罗氏沼虾"白体病"是细菌感染所致[3]，但无详细研究结果和照片。我们对白体病的罗氏沼虾幼体进行的组织学和超微结构的观察显示未发现任何寄生虫、细菌、病毒之类的病原微生物。这表明该病与病原微生物无关，这一结果与一些学者在对虾病害中发现的肌肉坏死病相一致[9－10]。在对虾病害研究中有人也曾发现对虾腹部肌肉变白、浑浊的症状，研究结果表明该病为肌肉坏死病，是一种环境

因素疾病[11]。罗氏沼虾"白体病"与对虾的这一疾病非常相似,可能也是一种环境不良条件下造成的营养性疾病。我们从抱卵虾、蚤状幼体、仔虾腹部肌细胞中都可以发现线粒体和肌质网的病变(图版Ⅰ-2~6),因而我们初步分析该病的发生可能与环境及养殖条件有关,如池塘缺氧、水温波动较大、养殖密度过大、水质受化学物质污染等[12-13]。当然,也不排除病虾在机体机能衰弱时会继发感染其他病原体的可能性,但这种感染可能是二次感染,并不是"白体病"的第1致病因素。

3.2 罗氏沼虾"白体病"的命名

目前水生动物疾病的命名不很规范,常以其症状来定,这是不科学的,而且容易造成病害诊断上的混乱。因为同一症状的疾病可能是由不同原因引起的。如虾的腹部发白、浑浊可由病毒[9,14]、细菌[15]、酵母[16]、微孢子虫[17]等病原体引起,虽都称为"白体病",但病因不同。我们所观察的罗氏沼虾"白体病"情况和以上报道的虾"白体病"也明显不同,是一种非病原体致病的疾病,而且发现无论幼体病虾还是成体病虾,其腹部肌肉细胞的线粒体和肌质网都有异常现象,这可能是导致该病发生的主要原因,这与对虾的肌肉坏死病很相似[11],所以称该病为"罗氏沼虾肌肉细胞线粒体坏死症"更为确切。

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Ultrastructural observation on muscle tissues of *Macrobrachium rosenbergii* with white-body disease

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Abstract: The light microscopy and electron microscopy were used to observe the pathological changes in *Macrobrachium rosenbergii* suffering from white-body disease. The pathogen were not found in the pathological area-abdominal muscle or other nonpathological areas such as gill, heart, hepaticopancreas and gonad. The results indicate that the disease is not caused by any pathogen. The histopathological observation shows that there is no obvious difference between normal and diseased tissues in the early and middle periods of the disease. But the ultrapathological observation shows obvious pathological process in the mitochondria and sarcoplasmic reticulum in abdominal muscle in those periods. The phenomenon of tumescent and disruptive mitochondria sometime with dense electronic particles and increased sarcoplasmic reticulum with many small bubbles indicate that the muscle cells are in the conditions of oxygen deficit and Ca^{2+} metabolic disorder, which maybe due to the deterioration of environment and cultural conditions. Based on the ultrastructural results, the white-body disease of *M. rosenbergii* should be named as 'Muscle Cell Mitochondria Necrosis'.

Key words: *Macrobrachium rosenbergii*; white-body disease; ultrastructure

图版Ⅰ说明　(图版Ⅰ见附页 5)

1. 苏州来源的正常抱卵虾腹部肌肉组织超微结构，示其内线粒体(Mi)结构完整清晰，肌质网(↑)分布均匀，肌丝(F)清晰，×8 000。
2. 高邮来源的异常抱卵虾腹部肌肉组织超微结构，示其内线粒体(Mi)肿胀且空泡化，肌质网(↑)增多，肌丝(F)结构无明显异常，×8 000。
3. 蚤状幼体期的发病虾腹部肌肉组织超微结构，示其内线粒体形态异常的线粒体肿胀膨大，其基质中出现密度分布不均匀高电子密度的颗粒(↑)，肌细胞的肌质网外，基质中出现散在分布的高电子密度颗粒(△)，×12 000。
4. 病仔虾腹部肌肉组织超微结构，示线粒体(Mi)肿胀其内有高电子密度的染色区(↑)，×12 000。
5. 病仔虾腹部肌肉组织超微结构，示其结构异常的肌质网(↑)和残余小体(▲)，×12 000。
6. 病幼虾腹部肌肉的组织超微结构，示肿胀、破裂的线粒体(Mi)和出现许多内陷小泡的肌质网(↑)，×17 000。

Caption for Plate Ⅰ　(For Plate Ⅰ see attached page 5)

1. The ultrastructure of abdominal muscle of the normal oviferous shrimp from Suzhou district, showing its mitochondria (Mi), sarcoplasmic reticulum (↑) and filament (F) with normal and distinct structure, ×8 000.
2. The ultrastructure of abdominal muscle of the diseased oviferous shrimp from Gaoyou district, showing its vacuolar degeneration mitochondria(Mi), sarcoplasmic reticulum (↑) and filament (F) with normal and distinct structure, ×8 000.
3. The ultrastructure of abdominal muscle of the diseased shrimp in its flea-like period, showing its abnormal structure of mitochondria (Mi) with increscent volume and many electron dense particles (↑), which are also in the sarcoplasmic reticulum (△), ×12 000.
4. The ultrastructure of abdominal muscle of the diseased shrimp in its young period, showing its tumescent mitochondria (Mi) with many electron dense particles (↑), ×12 000.
5. The ultrastructure of abdominal muscle of the diseased shrimp in its young period, showing its sarcoplasmic reticulum with abnormal structure(↑) and residual body (▲), ×12 000.
6. The ultrastructure of abdominal muscle of the diseased shrimp in its young period, showing its tumescent and disruptive mitochondria (Mi) and sarcoplasmic reticulum with many small bubbles (↑), ×17 000.

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Sequencing of ribosomal internal transcribed spacer regions and mitochondrial gene fragments in *Crassostrea gigas*

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Abstract: The ribosomal internal transcribed spacer regions (ITS-1 and ITS-2) and mitochondrial gene fragments (16S rDNA and COI) of *Crassostrea gigas* were amplified via PCR, and the PCR products were ligated into T-vectors, cloned and sequenced. The nucleotide sequences of 543, 791, 530 and 700 bp from ITS-1, ITS-2, partial 16S rRNA gene and partial COI gene were obtained, respectively. The contents of A, T, G and C were 23.57%, 20.07%, 29.47% and 26.89% in ITS-1, 27.43%, 19.22%, 27.05% and 26.30% in ITS-2, 29.25%, 29.25% 23.02% and 18.49% in 16S rDNA, and 22.71%, 39.43%, 20.43% and 17.43% in COI in turn. The primers of ITS-1 and ITS-2 proved to be very universal in a variety of mollusk species. The potential uses of these four sequences for species identification and relevant research of several closely related oyster species were discussed.

Key words: *Crassostrea gigas*; ITS-1; ITS-2; COI; 16S rDNA; sequencing

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For a long time it has been believed that there are five oyster species with commercial importance along the northern coast of China, which are Zhe oyster (*Crassostrea plicatula*), Pacific oyster (*C. gigas*), Suminoe oyster (*C. ariakensis*), Dalianwan oyster (*C. talienwhanensis*) and Monk-hat oyster (*C. cucullata*)[1-2]. Because of morphological variation of shells, there are argument and confusions on the identification and their species status except Suminoe oyster. Some people believed they were different species[1], but some others argue that Dalianwan oyster is a variety of *C. gigas*; monk-hat is synonymous with Zhe oyster, even Pacific oyster is also synonymous with Zhe oyster[3-4]. By analysing allozyme variation in Zhe oyster *C. plicatula* and Pacific oyster, we supported that they may be synonymous with each other; Liu[5] checked several Crassostrea species with RAPD technique and concluded that Dalianwan oyster, Zhe oyster and Pacific oyster are sib species with each other. In view of the above, it is necessary to figure out the problems with more sensible or powerful DNA techniques for the management, conservation and reasonable uses of oyster stock resource in the northern coast of China. Past efforts to investigate and identify differences among populations and species for oysters in northern coast of China using allozyme frequency data[6] and RAPD technique[5,7] have provided useful information, but it is far beyond enough.

The genes or gene fragments of interest could be amplified by PCR using universal primers which targeted highly conserved regions. Ribosomal internal transcribed spacer regions (ITS-1 and ITS-2) and mitochondrial 16S rRNA and COI gene fragments are among these popular candidates because of their rela-

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tively higher variability and easy amplification[8-9]. In this study, the ITS-1 and ITS-2 regions and mitochondrial 16S rDNA and COI gene fragments in Pacific oyster are amplified and sequenced for the further work on species identification, phylogenetic research and structuring populations of these species.

1 Materials and Methods

The sample oysters were collected from Qingdao markets. The total DNAs are extracted from mantle tissue using DNA extraction Kit (Pure Gene, Gentra). ITS-1 and ITS-2 regions are amplified using two pairs of universal primers ITS-1A/1B and ITS-2A / 2B (courtesy of Dr. Patrick Gaffney, University of Delaware, USA), respectively. The primer sequences are:

ITS-1A/1B: GGTTCTGTAGGTGAACCTGC/CTGCGTTCTTCATCGACCC;

ITS-2A/2B: GGGTCGATGAAGAACGCAG/GCTCTTCCCGCTTCACTCG.

16S rRNA and COI gene fragments are amplified using two pairs of universal primers 16sar-L/16sbr-H[11] and COIL 1490/COIH 2198[12] in turn:

16sar-L/16sbr-H: GCCTGTTTATCAAAAAC - AT/CCGGTCTGAACTCAGATCACGT;

COIL1490/COIH2198: GGTCAACAAATCATAAAGATATTGG/TAAACTTCAGGGTGACCAAAAAATCA.

Amplification of the products are performed using PTC-100 thermal cycler (MJ Research, USA); 25 μL of amplification reaction solution contains: 2.0 mmol/L $MgCl_2$; 200 μmol/L of each dNTP; 0.2 μmol/L of each primer; 1 μL template DNA; 1 unit Taq polymerase (Sangon, Shanghai) with supplied buffer. For all amplifications, hot start PCR is initiated by addition of polymerase and primers followed an initial 2 min denaturation at 94 ℃. The PCR cycling parameters are: 35 cycles of 94 ℃/45 sec, 48 ℃ (COI), 50 ℃ (ITS-1 and 16S) or 52 ℃ (ITS-2)/1 min and 72 ℃/ 1 min followed by 5 min final extension at 72 ℃. PCR products are purified using UNIQ-5 Column PCR Products Purification Kit (Sangon, Shanghai), ligated into pMD18-T Vector by following instruction of Takara DNA Ligation Kit ver.2 and used to transform competent JM109 *E. coli* cells using standard protocols. Recombinant colonies are identified by blue-white screen. Inserts of the correct size are detected via restriction enzyme digestion (*Eco*RI and *Hin*d III). Vector DNA containing the desired inserts is further purified using Pharmacia EasyPrep Kit, and the sequencing is performed for both strands of every sample on ABI PRISM 377XL DNA Sequencer using ABI PRISM BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Perkin Elmer).

2 Results and Discussion

Clear PCR products of ITS-1, ITS-2, 16S and COI are amplified, and the sequencing indicated that their sizes are 543, 791, 530 and 700 bp, respectively (Figs 1 and 2). Their base composition proportion of A, T, G and C are showed in Table 1.

Table 1 Base composition of ITS-1, ITS-2, 16S and COI sequences in Pacific oyster

表1 太平洋牡蛎 ITS-1、ITS-2、16S 和 COI 序列的基本组成 %

	A	T	G	C	A+T	Total/bp
ITS-1	23.57	20.07	29.47	26.89	43.64	543
ITS-2	27.43	19.22	27.05	26.30	46.65	791
16S	29.25	29.25	23.02	18.49	58.50	530
COI	22.71	39.43	20.43	17.43	62.14	700

We have tried to amplify the ITS-1 and ITS-2 in other mollusk such as oysters (*C. sikamea*), scallops (*Patinopecten yessoensis*, *Argopecten irradians* [*unpul. data*] and *Chlamys farreri*[12]), mussels (*Dreissena polymorpha*, Quagga mussel *Dreissena* sp., *Ischadium recurvum*, Mytilus), surf clam (*Spisula*), horseshoe crab (*Limulus*), shipworm (*Bankia setacea*, *Escarpia laminata*) and abalone (*Haliotis rufescens*) etc (unpul. data). The amplifications were successful in most of these species after optimizing, although the product size varied from species to species(unpubl. data). It is indicated that

the targeted sequence is highly conserved, so the primers are very universal in mollusk that they can be used in species identification and further phylogenetic reconstruction.

The internal transcribed spacer regions of ribosomal RNA genes are known to evolve faster than the coding region, therefore, they are variable at subspecific and population levels with various species. King (unpubl. data) used ITS-1 sequence to examine phylogeography of bivalve species Lasmigona subviridis, indicating it was a good fragment for the work. The similar work in rodent and Xenopus species also indicats that ITS regions are suitable to be employed in species identification and phylogenetic analysis[13-14]. Therefore it is believed that these two fragments can be good candidates used in the studies of genetic variation and phylogenetic research in mollusk.

ITS-1 GenBank Accesion No: AF280609

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  1 GGTTTCTGTA GGTGAACCTG CGGAAGGATC ATTAACAAAA CAAAATCGGG GCTCGTTGGC AGAGCGACCG AGAGGCTAAC
 81 GCAAAAACAC AGCCGAGGGT TGTTGTTAAA GTCGGATCGA CCTCGAACGC CGAGCGACCA GGTCGCTAGG CCGAGGGATC
161 CAGGCGAACT CGCTGGGGGG GTTTATTTCC TCTCAGCGCC TTGGGCCGTC GAAGCCTTCC TGCTCCGTCG CCTTAAGTAC
241 AGACGAGCTC GACCGCGACC CCGGGCTTCC GGAACGGTGG GTCGCTAAAA CAAAACAAAA AGCACTCGGC ATCGTCGGGG
321 TGGAAACCTC GACGTTATAA GCACCCGAGT ATGTACTTTC TCACGTATAC CAACAGATTT CTTACACAAA ACCCGGGAAG
401 GCGGAGTCGG CAAGGCTCTT GCCTTTTTTC GGGGGTCGGC CGGCGTACCT CGTTGCGCGC CGCGACCGAC CGAATTGGCA
481 TCATACTATT TTGACAACTC TAAGTGGTGG ATCACTCGGC TCGGGGGTCG ATGAAGAACG CAG
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ITS-2 GenBank Accesion No: AF280610

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  1 GGGTCGATGA AGAACGCAGC CAGCTGCGTG AATTAATGTG AATTGCAGGA CACATTGAAC ATCGACATCT TGAACGCACA
 81 TGGCGGCCTC GGGTAACTCC CGAGGCCACG TCTGTCTGAG GGTCGGTGAA ACTATCAATC GACTAAAATT TCATTATTCT
161 AGTCGCCTTG GGCCGTCGAA GCCTCTCACG GCTCCGTCGC CTTAAATGCA GACCGATGAC CAAAGAAACG GCTCTATTGG
241 TGACTCGGTG CCTCGGCGGG ACGAAACACG TCTCGACTTC CACTCTTTTC GCTAAACTCT TATCCCGAAC GGCTGCTCGG
321 TCGGACGGCG AAGGAGGACG CGGGGTGCGA GGGCGCAGAA AAAGACGGCA ACGCGGTCTG GCCACATACG CGAAGCTCCG
401 GCTATAGCGG CGGGTCTCGA CCATCAAAAG CGTGCCCGAA AGTGATGCAA ACTCGCAACA AACTTCCCCG GACTCTCCAT
481 CGCTTTGGCC TTGCCTGGCC TTAGTACGGA TAACAAAGGC GGTTAAAGAA GGAGATCGTT TCGCTGCAGA GTCCTGCCAT
561 TCAAAAAGGG GCTGAAGAGT CAACAGGGGC TCAAGGGCAT CGCGAGGGAT CCGCGTTTTA AGAAAAAAAG CGCGGACCTC
641 GATAAAACCA AAAACGGGAC AACCAATTTT TTTTCAATCA CTCCGACCTC AGATCAGGCG AGACTACGCC CTGAACTTAA
721 GCATATCACT AAGGGCAGGA AAAGAAACTA ACTAGGATTC CCCTAGTAAC GGCGAGTGAA GCGGGAAGAG C
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Fig.1 Nucleotide sequences of ITS-1 and ITS-2 in Pacific oyster *C. gigas*

图1 太平洋牡蛎ITS-1和ITS-2核酸序列

Oysters are among the most common, extensively-studied and morphologically-variable of all marine invertebrate taxa. However, the knowledge of oyster phylogeny and systematics is seldom known. There had been over 100 recorded species of oysters until 1970s, but two-thirds of them were synonymous with each other according to Harry's estimation[15]. In China, Zhe oyster (*C. plicatula*), Pacific oyster (*C. gigas*), Suminoe oyster (*C. rivularis*), Dalianwan oyster (*C. talienwhanensis*) and Monkhat oyster (*C. cucullata*) have been five major economic species in the northern coast. Because they (except Suminoe oyster) all occur along the coast with greatly variable morphology, it is difficult to distinguish them, which made much trouble in their broodstock management and aquaculture practice. The common concerns in mollusk aquaculture in the northern coast of China is that if they are genetically distinct species, subspecies or different populations. Similar situations also happened in several closely related oysters: *C. virginica*, *C. gigas*, *C. sikamea* and *C. ariakensis* in the United States[9, 16].

Banks et al[16] discriminated closely related oyster species *C. gigas* and *C. sikamea* via PCR/

RFLPs of mitochondrial 16S rRNA gene. O'Foighil[9] succeeded in distinguishing *C. virginica* from two closely related oysters *C. gigas* and *C. ariakensis*, and *C. gigas* from *C. ariakensis* by employing sequencing and mitochondrial RFLPs of partial 16S rRNA gene (443 bp). Sequence data revealed that the latter two species showed higher levels of similarity to each other (95%) than to *C. virginica* (84%-86%). Besides, COI and ITS-1 also had been used to diagnose *C. gigas* vs. *C. ariakensis* via PCR/RFLP (Gaffney, unpul. data). O'Foighil[17] made a comparison of 579 nucleotide fragments of COI among Portuguese oysterrr *C. angulata* and several Japanese oysters and indicated that Portuguese oyster haplotypes clustered robustly within a clade of Asian origin for Portuguesee oyster. As for nuclear marker, in *Mytilus*, a couple of species can be identified by means of DNA markers based on the glue gene responsible for the polyphenolic protein.

Molecular genetic markers have become indispensable tools in the study of species identification as well as wild populations, and this is more important for marine organisms where direct observation of behavior, breeding structure and migration patterns are more difficult than those for most terrestrial organisms[18]. The conclusion is that ITS-1, ITS- 2, 16S rRNA and COI gene fragments would be four nice candidates of species identification and related research for Zhe oyster (*C. plicatula*), Pacific oyster (*C. gigas*), Suminoe oyster (*C. rivularis*), Dalianwan oyster (*C. talienwhanensis*) and Monkhat oyster (*C. cucullata*).

16S rDNA GenBank Accesion No: AF280611

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  1 CCGGTCTGAA CTCAGATCAC GTAGGGTATT AAAGGTCGAA CAGACCTACT TTCAAAGCCT CTACGCCTTG AAGCTATTAT
 81 CCCTGATTCA ACATCGAGGT GCCAATCCCT TTAGCCAATA CGAACTCTAC TAAAGGATTA GCCTGTTATC CCCGGCGTAA
161 CTTCTCCTAT GATCGAATAT AATCGGGTCA AATCCGGAAA GAGTTAGTTA TTCAGAAAGG TTAGACTTGC TTTCTAGGCG
241 CCCCACCTAA AAATCTTTTA CTCCTGAATA AAGTTAATTT TCAAAGTTGC ACGGGGTCTT TTTGTCTAAC TTTTAAATGA
321 AGGTATCTTC ACCTTCAGTA CAATTTCAAT AAAAAATTTA GAGACAGTCA AACCCTCGTT AAACCATTCA TGCAGGCCCA
401 CAATCAAAAG GCAAGGAATT TCGCTACCTT AGCACCCTCA CGCTAGGGCG GCCGTTTACA GTAATATTTC GCACTGGGCG
481 GGTATTGCTA AAAGTCTTTA TCTTCTAGTG ATGTTTTTGA TAAACAGGCG
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COI GenBank Accesion No: AF280608

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  1 TAAACTTCAG GGTGACCAAA AAATCAAAAC AAATGCTGAA ATAAGACAGG GTCCCCCCCT CCGACAGGGT CAAAAAAAGA
 81 GGTATTAAAA TGACGATCAG TCAAAAGTAT AGTAAGACCT CCAGCTAACA CTGGGAGAGT AGTCAAAAGC AAGAATGAAG
161 TAACCTTAAT AGATCAAGGG AATAGTGCTA GTAAATGGCC CCCAACAGAT CGCATATTTC TAATCGTTAC TATGAAATTA
241 ATTGACCTGA AAATAGAGCT AATACCAGCA AGGTGAAGGC TTAGAATTGC AAGGTCTATA CAAACTCCAT GATAAGAGTA
321 AGTTGATAAA GGAGGGTAAA TTGTTCACCC TGCCCCAACT CCATTTTCTA CAATGTTAGA CATAAGCATA AGATAAAGAG
401 ACCCTGGCAA AACTCAAAAT CTAAATGCAT TTAATCGAGG AAATTGCATG TCTGCTACTA GAAGCATCAA AGGGATAAGC
481 CAGTTACCAA ACCCCCCAAT TATTACAGGT ATAACAAAGA AAAAAATCAT AACCAACGCA TGCCTAGTTA CAACTGCATT
561 ATAAGTCACG GGGTCTAAAA ACTTAGCTCC AGGGTTATAA AGTCTCCAAC GAATAAGAGA CCTAAACCTA GTTCCCGCAA
641 GAACAGCTCA AAATCCAAAT ACTATATAAA ACCTTCCAAT ATCTTTATGA TTTGTTGACC
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Fig. 2 Nucleotide sequences of partial 16S rRNA and COI genes in Pacific oyster *C. gigas*

图 2 太平洋牡蛎线粒体基因核酸序列

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太平洋牡蛎核糖体 DNA 转录间隔子和线粒体基因片段序列测定

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摘要: 以相应引物经 PCR 扩增了太平洋牡蛎(*Crassostrea gigas*)的核糖体转录间区域(ITS-1 和 ITS-2)及线粒体 16S rDNA 和 COI 基因片段。PCR 产物经 T-载体连接后进行克隆和测序, 分别得到长度为 543、791、530 和 700 bp 的核苷酸序列。4 个 DNA 片段的 A、T、G 和 C 碱基含量分别为 23.57%、20.07%、29.47% 和 26.89%(ITS-1), 27.43%、19.22%、27.05% 和 26.30%(ITS-2), 29.25%、29.25%、23.02% 和 18.49%(16S rDNA), 22.71%、39.43%、20.43% 和 17.43%(COI)。实验证明 ITS-1 和 ITS-2 引物在贝类中通用性良好。文中同时讨论了 4 个序列在我国几种牡蛎的种类鉴别及相关研究的应用潜力。

关键词: 太平洋牡蛎; ITS-1; ITS-2; COI; 16S rDNA; 基因序列