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三倍体湘云鲫及其亲本线粒体DNA的比较研究

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摘 要:用人工选育的异源四倍体鲫鲤(♂)与白鲫(♀)杂交获得具有明显生长优势的三倍体湘云鲫。采用差速离心和核酸酶处理等方法,从三倍体、白鲫和异源四倍体鲫鲤肝组织中提取线粒体DNA,并用9种限制性内切酶进行单酶酶切分析。经琼脂糖凝胶电泳后计算出各酶切片段的大小,测得三倍体、白鲫和异源四倍体鲫鲤mtDNA的分子大小分别为16.24 kb、16.60 kb和16.20 kb。根据各单倍型间的酶切片段共享度,估算出3个群体间的遗传距离,说明了mtDNA母系遗传的特性。

关键词:白鲫;异源四倍体鲫鲤;三倍体湘云鲫;线粒体DNA;限制性内切酶;遗传距离
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80年代中期,刘筠等[1]在红鲫(*Carassium auratus* red var. ♀)与湘江野鲤(*Cyprinus carpio* ♂)的杂交后代(鲫鲤F_1)中发现部分可育的后代,F_1自交得F_2,到目前为止已繁殖到F_{10},刘少军等[2]采用染色体数目、组型和DNA含量分析,证明鲫鲤F_1～F_2是二倍体,鲫鲤F_3～F_9是异源四倍体。用四倍体鲫鲤(F_3～F_9)(♂)与日本白鲫(*Carassium auratus cuvieri*)(♀)交配得到三倍体湘云鲫。湘云鲫与众多的其它鲫鱼品系相比具有明显的优势,如生长速度快、自身不具有繁殖能力、食性广和抗病力强等,已在全国范围内推广养殖。目前本实验室已对三倍体湘云鲫的染色体数目及组型、性腺发育、形态特征和食性等方面做了大量工作[2,3]。

鱼类线粒体DNA与其它脊椎动物一样,具有分子较小且为闭合环状、遵循母系遗传、基因组结构简单且保守、一级结构进化速度快等特点[4]。80年代后国内在鱼类mtDNA研究方面做了大量工作,但这些研究主要集中于自然界存在的自然进化而来的群体[5～13],以及mtDNA大小的测定、酶切片段及位点的比较和酶切图谱的构建[5～8],对群体间mtDNA比较研究的报道并不多[8～10],而对人工杂交育种获得的多倍体种群mtDNA研究还未见报道。80年代中期,本研究室在鲫鲤杂交后代中选育出异源四倍体,随后形成了一个能稳定繁殖的四倍体种群,本文报道的三倍体湘云鲫,就是以异源四倍体鲫鲤作父本,白鲫为母本,杂交所得的不具有繁殖后代能力的新型高产的养殖鱼类,因此它们之间存在已知的亲缘关系。为探讨湘云鲫的遗传与进化和mtDNA母系遗传特性,本文从分子生物学角度,分析了湘云鲫及其亲本线粒体DNA限制性酶切片段长度的多态性和遗传差异。

1 材料与方法

1.1 材料

实验用异源四倍体鲫鲤和三倍体湘云鲫均取自湖南师范大学四倍体基因库种质资源保护基地,白鲫由湘阴县东湖渔场提供。实验鱼在实验前放置实验室水池内饥饿1～2 d后放血取新鲜肝组织(尽可能除去脂肪组织)。限制性内切酶及其它试剂购自北京华美生物工程公司。

1.2 mtDNA的分离、纯化和酶切

采用差速离心和核酸酶消化法从3种鱼的肝组

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织中提取 mtDNA，具体操作步骤和 mtDNA 酶解条件参照黎双飞等[7]的方法。

1.3 琼脂糖凝胶电泳分析

凝胶质量分数为 0.01～0.12（含溴化乙锭 0.5 mg/L），电泳缓冲液为 TBE，电压 6～8 V/cm，置 4℃条件电泳 3～4 h。以 λDNA/Hind Ⅲ 完全酶解片段作为相对分子质量标准，电泳完毕用凝胶电泳图像分析仪（Gel－Pro Analyzer）摄影，并计算出各酶切片段和 mtDNA 的大小。

1.4 分析方法

根据 mtDNA 限制性酶切片段共享度的差异，运用 Nei 和 Li 的片段法[14]计算 3 个群体间遗传距离。公式如下：

$$F = 2N_{XY}/(N_X + N_Y)$$

$$P = 1 - \{0.5[(F^2 + 8F)^{0.5} - F]\}^{1/r}$$

其中 N_X、N_Y 分别为群体 X 和 Y 的限制性片段数，N_{XY} 为 2 个群体的片段共享数，r 是限制性内切酶能识别的碱基对数目（在本文中 $r=6$）。根据酶切片段单倍型间的酶切片段共享度（F），求出群体间的遗传距离（P）。

2 实验结果

2.1 9 种限制性内切酶酶切结果

用识别 6 个碱基对的 9 种限制性内切酶对三倍体湘云鲫、白鲫和异源四倍体鲫鲤的 mtDNA 进行消化，9 种内切酶均存在限制性酶切位点。BglⅡ和 XbaⅠ在异源四倍体鲫鲤 mtDNA 上表现出限制性位点的多态性，Ⅰ型结果见图 1－C；Ⅱ型结果见图 1－D，而在白鲫和三倍体湘云鲫 mtDNA 上表现为完全的单态性，EcoRⅠ、BamHⅠ、Hind Ⅲ、XhoⅠ和 SalⅠ在 3 种鱼的 mtDNA 上不存在种群间和种群内的遗传多态性，它们分别具有 4、2、5、1 和 1 个酶切位点，PstⅠ和 KpnⅠ在种群间存在限制性片段长度差异，见图 1，表 1。

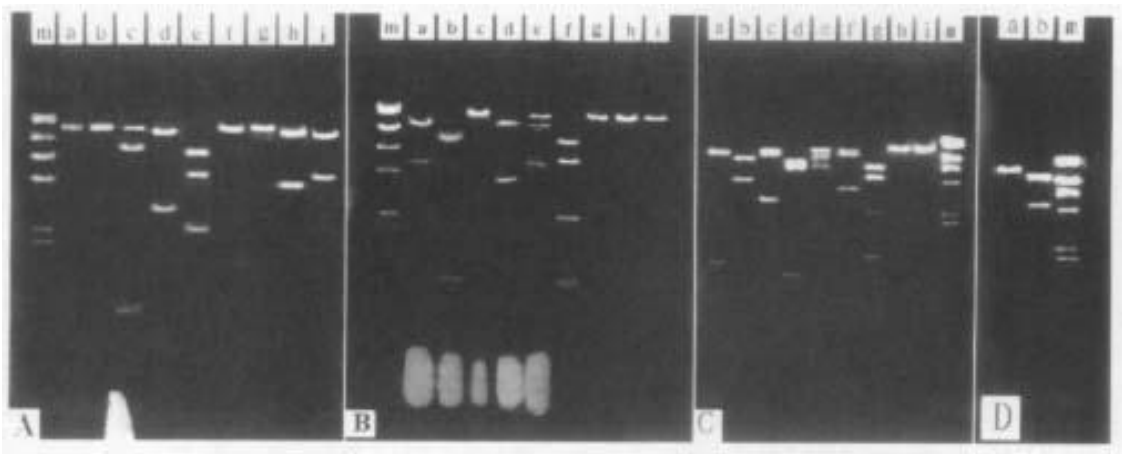

图 1 三倍体湘云鲫、白鲫和异源四倍体鲫鲤 mtDNA 的酶切片段电泳图谱

Fig.1 Electrophoresis patterns of mtDNA from triploids、*C. auratus cuvieri* and allotetraploid hybrids

A：三倍体湘云鲫 Triploid crucian carp；B：白鲫 Japanese crucian carp；C、D：异源四倍体鲫鲤 Alloetriploid hybrids. 后同 The same below.

A：a：BglⅡ，b：KpnⅠ，c：EcoRⅠ，d：PstⅠ，e：Hind Ⅲ，f：SalⅠ，g：XhoⅠ，h：BamHⅠ，i：XbaⅠ；

B：a：PstⅠ，b：EcoRⅠ，c：KpnⅠ，d：BamHⅠ，e：XbaⅠ，f：Hind Ⅲ，g：XhoⅠ，h BglⅠⅠ：，i：SalⅠ；

C：a：BglⅡ（Ⅰ型），b：XbaⅠ（Ⅰ型），c：PstⅠ，d：EcoRⅠ，e：KpnⅠ，f：BamHⅠ，g：Hind Ⅲ，h：XhoⅠ，i：SalⅠ；

D：a：BglⅡ（Ⅱ型），b：XbaⅠ（Ⅱ型）；m：λDNA/Hind Ⅲ marker.

2.2 3 个群体间的 mtDNA 遗传距离

在 3 个群体中共检测出 4 种 mtDNA 限制性类型，结果见表 1，估算出 4 种单倍型间的片段共享度和遗传距离（表 2）。从 3 个群体的 P 值比较来看，三倍体湘云鲫与白鲫的遗传距离（0.006 2）最近，与异源四倍体鲫鲤Ⅱ型的遗传距离（0.007 7）次之；三倍体湘云鲫和白鲫与异源四倍体鲫鲤Ⅰ型的遗传距离（分别为 0.019 7，0.027 6）均存在明显差异。

表 1　三倍体湘云鲫、白鲫和异源四倍体鲫鲤 mtDNA 的酶切片段长度

Table 1　Fragment length of mtDNA from triploidy crucian carp、*C. auratus cuvieri* and allotetraploidy hybrids obtained by restriction endonuclease digestion

酶 Enzyme	鱼 Fish	酶切片段长度/kb Length of restriction fragment					合计 Total
Pst Ⅰ	A	13.20	3.05				16.25
	B	11.50	5.10				16.60
	C(Ⅰ,Ⅱ)	12.85	3.30				16.15
EcoR Ⅰ	A	16.30*	7.20	7.20	1.1	0.6**	16.10
	B	7.30	7.30	1.2	0.8**		16.60
	C(Ⅰ,Ⅱ)	7.30	7.30	1.1	0.6**		16.30
Kpn Ⅰ	A	16.30					16.30
	B	16.55					16.55
	C(Ⅰ,Ⅱ)	16.20*	9.60	6.60			16.20
BamH Ⅰ	A	12.50	3.80				16.30
	B	12.70	3.85				16.55
	C(Ⅰ,Ⅱ)	12.50	3.80				16.30
Hind Ⅲ	A	7.10	4.90	2.35	1.2	0.6**	16.15
	B	7.40	5.10	2.30	1.1	0.7**	16.70
	C(Ⅰ,Ⅱ)	6.80	5.05	2.25	1.4	0.6**	16.10
Bgl Ⅱ	A	16.30					16.30
	B	16.55					16.55
	C(Ⅰ)	14.70	1.4				16.10
	C(Ⅱ)	16.20					16.20
Xho Ⅰ	A	16.40					16.40
	B	16.60					16.60
	C(Ⅰ,Ⅱ)	16.20					16.20
Sal Ⅰ	A	16.20					16.20
	B	16.75					16.75
	C(Ⅰ,Ⅱ)	16.15					16.15
Xba Ⅰ	A	11.80	4.50				16.30
	B	16.65*	11.70	4.90			16.60
	C(Ⅰ)	9.20	4.90	2.0			16.30
	C(Ⅱ)	11.70	4.50				16.20
平均长度/kb The average length				A			16.24
				B			16.60
				C(Ⅰ),C(Ⅱ)			16.20

注:A－三倍体湘云鲫 Triploid crucian carp;B－白鲫 *C. auratus cuvieri*;C－异源四倍体鲫鲤 Allotetraploid hybrids, C(Ⅰ),C(Ⅱ)表示多态性 Means polymorphism.

* 示片段为不完全酶切片段;** 示片段为未能检测出的片段。The fragments not being digested completely are marked with "*"; the fragments not being detected are marked with "**".

表 2　mtDNA 单倍型间的片段共享度(右上)和遗传距离(左下)

Table 2　The proportion of restriction fragments shared (upper right) and genetic distances (down left) among mtDNA haploidy types

单倍型 Haploid	A	B	C(Ⅰ)	C(Ⅱ)
A		0.895	0.714	0.872
B	0.006 2		0.619	0.821
C(Ⅰ)	0.019 2	0.027 6		0.837
C(Ⅱ)	0.007 7	0.011 2	0.010 0	

3　分析与讨论

3.1　三倍体湘云鲫、白鲫和异源四倍体鲫鲤的 mtDNA 酶切结果

笔者用 Gel－Pro Analyzer 测得三倍体湘云鲫、白鲫和异源四倍体鲫鲤 mtDNA 大小分别为 16.24 kb、16.60 kb 和 16.20 kb, 与有关文献报道的鱼类 mtDNA 分子大小范围在 15.2～19.8 kb 相符合[4], 同时本文报道的白鲫 mtDNA 大小与张四明等[5]报道的结果接近, 从共同使用的 7 种限制性内切酶

(EcoR Ⅰ、Pst Ⅰ、Hind Ⅲ、Sal Ⅰ、Xho Ⅰ、Xba Ⅰ和BamH Ⅰ)酶切结果比较来看,除 Pst Ⅰ 的酶切片段长度有明显差异外(张四明等检测的片段长度分别为11.50、5.00 kb)[5],其它6种酶酶切结果基本一致。酶切结果表明 Bgl Ⅱ 和 Xba Ⅰ 在异源四倍体鲫鲤 mtDNA 上存在种群内限制性酶切位点多态性,但酶切结果Ⅱ型出现的频率比Ⅰ型小很多(大约为1:4)。

3.2 3个群体间 mtDNA 遗传距离分析

从3个群体4种单倍型的片段共享度(F)和遗传距离(P)分析,若单独比较三倍体湘云鲫、白鲫和异源四倍体鲫鲤Ⅰ型之间的遗传距离,发现父本与后代和母本与后代 mtDNA 之间存在明显的遗传差异,即后代 mtDNA 的遗传特性与母本接近;但单独比较三倍体湘云鲫与异源四倍体鲫鲤Ⅱ型的片段共享度和遗传距离时,它们之间存在很小的差异,即后代 mtDNA 的遗传特性与父本和母本均较为接近。异源四倍体鲫鲤 mtDNA 出现酶切片段Ⅰ型和Ⅱ型的多态性,并且2种多态类型间的遗传距离较为明显,其原因可能是由于 mtDNA 的突变率不一致或回复突变的结果;可能暗示湘云鲫和异源四倍体鲫鲤的 mtDNA 之间存在某种关系(父本 mtDNA 对后代 mtDNA 存在明显影响,在无脊椎动物方面有类似报道[15])。作者在对湘云鲫进行随机抽取多个个体的 mtDNA 检测后发现,其 mtDNA 经 Bgl Ⅱ 和 Xba Ⅰ 酶切后并未检测出有与异源四倍体鲫鲤Ⅰ型(约占整个酶切类型的80%)相同的酶切结果,因此可以排除上述提出的第2种可能。所以由上述的分析我们可以得到一个支持 mtDNA 遵循母系遗传理论的很有说服力的证据。目前已有文献报道[8,9],根据群体间或群体内 mtDNA 遗传差异估算单倍型群体间的分离时间,本文虽然计算了3个不同群体4个单倍型间的遗传差异,由于这3种鱼中三倍体湘云鲫和异源四倍体鲫鲤都是在近15年内选育出的杂交种群,群体间存在特殊的亲缘关系,因此不能依据 Brown 等[16]提出的"每百万年 mtDNA 平均碱基突变速率为2%"来估算3个群体内或群体间的分化时间。

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Comparative studies on mitochondrial DNA from triploid crucian carp and its parents (allotetraploid hybrids of crucian carp and common carp, ♂ and *Carassius auratus cuvieri*, ♀)

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Abstract: The triploid crucian carp, with obvious advantage in growth, was obtained by crossing allotetraploid hybrids(♂) from offsprings($F_3 \sim F_9$)of red crucian carp (*Carassius auratus red var.*)(♂) × common carp (*Cyprinus carpio* L.) (♀), and *C. auratus cuvieri* (♀). The mitochonodrial DNA (mtDNA) of liver were isolated from the triploids, Japanese crucian carp (*C. auratus cuvieri*) and allotetraploid hybrids by means of density gradient centrifugation, DNase I and RNase digestion, and were digested by 9 kinds of restriction endonucleases. By analysis of agrose gel electrophoresis, the sizes of mtDNA fragments were estimated that the mtDNA sizes of triploids, *C. auratuscuvieri* and allotetraploid hybrids were 16.24×10^3, 16.60×10^3 and 16.30×10^3 bp, respectively, and the genetic distances among mtDNA haploidy types in the 3 populations were calculated according to the numbers of common fragments that they had the matrilinear inheritence character of mtDNA.

Key words: *Carassius auratus cuvieri*; allotetraploid crucian carp; triploid crucian carp; mitochondrial DNA (mtDNA); restriction endonuclease; genetic distance

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Sequence study and potential uses of ribosomal DNA internal transcribed spacers in scallop *Chlamys farreri*

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Abstract: The ribosomal internal transcribed spacer regions ITS－1 and ITS－2, extracted from mantle tissue of wild *Chlamys farreri*, were amplified via PCR using relevant primers. The PCR products were ligated into T－vector, then cloned and sequenced. Two nucleotide sequences were got with very suitable sizes of 340 bp and 510 bp for studies on genetic variation and phylogeny. The contents of A, T, G and C were 32.06%, 20.59%, 22.35% and 25.00% in ITS－1 and 30.00%, 21.37%, 24.12% and 24.51% in ITS－2. The primers of ITS－1 and ITS－2 proved to be very universal in a large variety of mollusc species. The potential uses of the 2 sequences for genetic variation studies and phylogenetic research in scallop species were discussed too.

Key words: *Chlamys farreri*; ITS－1; ITS－2; nucleotide sequence

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In recent years, aquaculture practices to *Chlamys farreri* have suffered high mortality before harvest season in the northern coast of China. Introduction of broodstock from other *C. farreri*-distributing areas such as Korea and the People's Republic of Korea is believed one of the resolutions for the problem. However, some work needs to be done of molecular genetic analysis for the reasons of stock or broodstock management and discrimination of future culture practices. As far as molecular genetic techniques available at present, mtDNA gene[4,5] or nuclear gene analysis is one of the suitable approaches.

Some efforts to investigate and identify differences among populations using allozyme frequency data have been proved very useful[1], but so far, almost nothing on DNA level has been done. DNA polymorphisms are useful tools for ecological, genetic and evolutionary studies of both terrestrial and marine organisms, which can be applied to from identification of species and analysis of population structure delineation to monitoring of genetic change in wild or domesticated populations[2]. Sequencing is one of these tools which is employed more frequently than before in recent years. The base substitutions, deletions and insertions can be detected by the sequencing, and then the comparison between different species, populations or individuals can be made by base differences in corresponding sequences.

The genes or gene fragments of interest can be

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amplified by PCR using universal primers targeting highly conserved regions. Ribosomal internal transcribed spacer regions ITS－1 and ITS－2 are two popular candidates for their relatively higher variability and easy amplification. In this study *C. farreri*'s ITS－1 and ITS－2 regions were amplified and sequenced so that a further study on this population structure and phylogeny can be conducted.

1 Materials and Methods

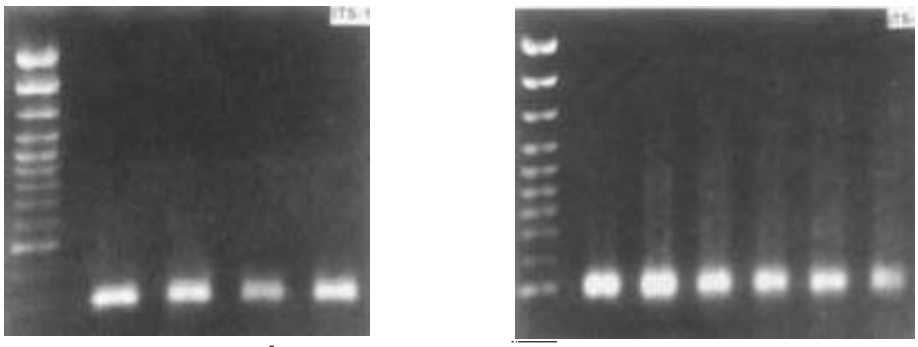
Genomic DNAs were extracted from mantle tissue of wild *C. farreri* collected in Hongdao County, Shandong Province, using DNA extraction Kit (Pure Gene, Gentra USA). ITS－1 and ITS－2 regions were amplified using two pairs of universal primers: ITS－1A/1B and ITS－2A/2B (Courtesy of Dr. Patrick Gaffney, University of Delaware, USA) respectively. The primer sequences are:

ITS－1A/1B: 5′－GGTTCTGTAGGTGAACCTGC－3′
5′－CTGCGTTCTTCATCGACCC－3′
ITS－2A/2B: 5′－GGGTCGATGAAGAACGCAG－3′
5′－GCTCTTCCCGCTTCACTCG－3′

Amplification of both products was performed on PTC－100 (MJ Research, U.S.A.) Thermal Cycle; 25 μl of amplification reaction containing: 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, Canada) and buffer. For all amplifications, hot start PCR was initiated by addition of polymerase and primers following an initial 2 min's denaturation at 94℃. The PCR cycling parameters were: 35 cycles/[45 s at 94℃, 1 min at 50℃ (ITS－1) or 52℃ (ITS－2), and 1 min at 72℃] followed by 5 min's final extension at 72 ℃. The PCR products were verified by electrophoresing 5 μl of amplified products in 1.5% agarose gel containing 1×TBE at 5 V/cm, and then purified by UNIQ－5 Column PCR Products Purification Kit (Sangon, Canada) and ligated into pMD18－T Vector (Takara, Japan) according to the instruction of Takara DNA Ligation Kit ver.2, and then was used to transform competent JM109 *Escherichia coli* cells using standard protocols[3]. The recombinant colonies were identified by blue－white screening. The inserts with correct sizes were detected via restriction enzyme digestion (EcoRI and Hind III). The Vector DNA containing desired insert was further purified using Pharmacia EasyPrep Kit, and the sequencing was performed on ABI PRISM 377×L DNA Sequencer using ABI PRISM BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Perkin Elmer, USA).

2 Results and Discussion

Clear fragments of ITS－1 and ITS－2 were got at the sizes of 340 bp and 510 bp respectively (Fig.1). The composition proportion of A, T, G and C are 32.06%, 20.59%, 22.35% and 25.00% in ITS－1 and 30.00%, 21.37%, 24.12% and 24.51% in ITS－2 separately. Some efforts have been made to amplify the 2 fragments in other molluscs, such as oyster, mussel, scallop and abalone, etc.



Marker: 100 bp DNA ladder (100～2 000 bp). Arrows indicate 500 bp

Fig.1 PCR products of ITS－1 (left) and ITS－2 (right) of *Chlamys farreri*

The amplifications were successful in most of them after optimization, although the product sizes varied from species to species (unpub. data). It indicates that the targeted sequences are highly conserved and the sizes of amplified products vary in different groups, so the primers are very universal in mollusc that they can be used in further phylogenetic reconstruction.

ITS-1 (GenBank accession No:AF245687)

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1    GGTTTCTGTA GGTGAACCTG CGGAAGGATC ATTACCGAAC TCCTAAAAAA CACCGGGTTA
61   CGCCCGCGTG TGAAAAACAC AAAATCGGAC AAACAAAAAC AGCCGAAGGA GATCAGGGCC
121  AGACTCTGCC TCCTAGCTCG GCAAAAAAAC AAACTGTGAC AACGCAAAAC TTGCAAACCT
181  TTTCGGGGTA CCTGGCATCG GTTTAATGAG CAACGGCCGC CCCGACATTA TATTTTCACG
241  TAACAATTTG TAACCAAAAA AATTGTTGTC TCTAACGAGA TGATCTCATT ACCACCCTAA
301  GCGGTGGATC ACTCGGCTCG TGGGTCGATG AAGAACGCAG
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ITS-2 (GenBank accession No:AF245688)

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1    GGGTCGATGA AGAACGCAGC CAGGTGCGTG AATTAATGTG AATTGCAGGA CACATTGAAC
61   ATCGATATCT TGAACGCACA TTGCGGCTCC GGGTCACTCC CGGAGCCACG CCTGTCTGAG
121  GGTCGGCAAA ACATCTATCG CAACCTGCAT GGCACAGCAG CATTGCGCCT TGGACCGTCT
181  CGTCTCCCCG GCGAGCGGTC TTAAATGAGG AATCAGAGTC TCCAATCGAA CCAAAAACAA
241  ACCAGTACGG AACAAATTAC AAGAGACGCA GTTCGATTAA AACACAAAAT GCGCTCTCGG
301  TTCCGTGAAA CTGTAGGTTC GCCTTTTACA TGTGAAAAAA AAGGTATGAT GGTGTAAATT
361  TCTCTGGTCG ACACAACACT TTGTGTTTGT CTTTTCACAC TCCGACCTCA GATCAGACGA
421  GACTACCCGC TGAATTTAAG CATATCACTA AGCGGAGGAA AAGAAACTAA CAAGGATTCC
481  CCCAGTAACG GCGAGTGAAG CGGGAAGAGC
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Fig. 2 The nucleotide sequences of Ribosomal Internal Transcribed Spacer 1 and 2 of *Chlamys farreri*

The internal transcribed spacer regions of ribosomal RNA genes are known to evolve faster than the coding region, therefore, they are very variable on the subspecific and population levels in various species[6, 7]. Mizukami[7] reported that in *Porphyra yezoensis* (algae) the nucleotide sequences of 371 bp ITS-1 regions were considerably different from each other among individual thalli collected from two different coastal sites in Japan. This indicated that the nucleotide sequences in ITS-1 regions vary considerably on the intraspecific level of this species. King (Aquatic Ecology Lab, Leetown Science Center, Biological Resources Division. unpub. data) using ITS-1 sequence to examine the phylogeography of bivalve species *Lasmigona subviridis*, indicated it was a good fragment for the work. The similar work in rodent and *Xenopus* species also indicated that ITS regions were suitable to be employed in species identification and phylogenetic analysis[8,9]. In scallops, Kenchington (unpub. data) sequenced ITS-1 and ITS-2 of several scallop species along the Atlantic Ocean and reconstructed molecular evolutionary tree. Therefore, it is believed that these two fragments are good candidates to be used in the studies of genetic variation and phylogenetic research in scallops.

The two sequences have been accepted by GenBank, accession No: AF245687 and AF245688.

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栉孔扇贝核糖体 DNA 转录间隔子序列研究及其潜在应用

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摘　要：以相应引物 PCR 扩增栉孔扇贝(*Chlamys farreri*)核基因组的核糖体 DNA 两个转录间隔子(ITS－1 和 ITS－2)，PCR 产物经 T 载体连接后进行克隆、测序，分别得到了 340 bp 和 510 bp 的碱基序列，序列大小非常适合遗传变异及分子系统学研究。其 A、T、G、C 含量在 ITS－1 分别为 32.06%，20.59%，22.35% 和 25.00%，在 ITS－2 分别为 30.00%，21.37% 24.12% 和 24.51%。这两个变异性较大的序列在扇贝种群中应用潜力很大，可广泛用于种内群体间遗传变异研究、种质鉴别及系统学研究。

关键词：栉孔扇贝；ITS－1；ITS－2；基因序列