# 鳜鱼优良肉质的生化特征及其遗传控制

# Biochemical Characterization of Muscle Flesh and Genetic Regulation in Chinese Perch

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# 作者简介

**褚武英：**博士，教授，硕士生导师。现任湖南省教育厅“经济动植物品质调控及应用”重点实验室副主任。2006—2008 年在印遇龙院士领衔的中国科学院亚热带农业生态研究所家养动物健康养殖研究中心工作，先后参与了国家 973 项目和中科院知识创新项目等多项研究，在家养动物肉质性状形成的代谢与调控方面做了系统研究。2009 年作为高层次引进人才调入长沙学院，主要从事名贵鱼类优良肉质性状形成机制及其营养调控研究。先后主持国家自然科学基金面上项目 3 项、湖南省自然科学基金项目 2 项、湖南省教育厅重点项目 1 项；在 *Journal of Nutritional Biochemistry*，*Marine Biotechnology* 和 *Current Molecular medicine* 等国内外学术刊物发表学术论文 50 余篇，其中 SCI 论文 30 多篇，单篇最高影响因子 4.88，其学术成果在国内外同行中获得了较高的认可，并被世界水产学会邀请于 2014 年年会作大会报告。已获授权国家发明专利 5 项，参编著作 3 部，获得 2008 年省科学技术三等奖一项。近年来系统地分析了鳜鱼不同品种肌肉营养组成成分，氨基酸和脂肪酸组成模式以及肌纤维分型；阐明了鳜鱼的味道鲜美主要是与其肌肉的高含量鲜味氨基酸（谷氨酸和天冬氨酸等）直接相关；肉质细嫩的分子基础主要与鳜肌纤维密度高、直径小相关。此外，采用基因芯片杂交检测出翘嘴鳜与鲢鱼肌肉组织 375 个表达差异基因，其中 49 个已知功能基因主要是肌肉结构和肌纤维连接等肉质结构性状相关基因。以上研究成果为探索调控和改善鱼类肉品质提供了新思路和新途径。

**张建社：**博士，教授，先后获湖南师范大学生物系生物学学士学位和发育生物学硕士学位，并获加拿大 Dalhousie 大学生物系细胞与分子生物学博士学位，在加拿大多伦多大学动物系和多伦多儿童医院生物医学研究所从事博士后研究工作。2004 年 9 月全职回国工作，现任长沙学院特聘教授，兼任中国科学院亚热带生态研究所研究员、博士生导师，湖南大学生物学院科学院硕士生导师，世界水产学会会员，中国水产学会生物技术专业委员会委员，湖南省水产学会副理事长，湖南省动物营养及生态环境学会副理事长，国内外多家学术期刊编委和审稿人。

一直以来从事鱼类发育生物学研究，近几年以我国名贵鱼类——鳜鱼为研究对象，围绕鳜鱼肉质组成成分、肌肉发生分化过程中的基因表达与调控机制以及

鳜鱼种质资源等进行了较深入和系统的研究。先后完成和承担各类纵向科研课题共10多项，其中包括国家自然科学基金重点项目1项，面上项目2项，先后在*Current Biology*，*JBC*，*PLoS ONE*，*Marine Biotechnology* 和 *Aquaculture Research* 等国内外学术刊物发表论文70多篇，SCI论文40多篇，单篇最高影响因子10.88，单篇最高他引130多次，包括被 *Science*，*Nature* 和 *Cell* 等刊物多次引用，获国家发明专利4项，主编和参编著作各1部。

# 前 言

水产品既是人类食物的重要组成，也是人类生长发育必需的优质蛋白源。自新中国成立后，特别是改革开放以来，我国的水产业得到了迅速发展。如何在保证水产品产量的同时不断提高其肉质是目前消费者最关注的问题之一。因此大力发展名贵鱼类养殖，研究鱼类优良肉质性状特征和分子调控机理，培育优质高产鱼类新品种，对于不断适应社会消费需求，促进我国的水产业优质、高产和稳定健康发展至关重要。

鱼类肌肉组织既是鱼类躯干的结构组织，也是人类重要的可食部分和蛋白源。以经济鱼类为主要养殖对象的水产养殖，从发育分子生物学分析，实质上就是在适宜的生态条件下，采用综合优化养殖技术最大限度地促进鱼类肌细胞的增殖和增长，使鱼体速生快长，达到增加养殖生产效益的目的。因此鱼类肌肉分化生长调控分子机理是鱼类发育生物学、营养学和高效养殖研究领域亟待研究的难点和热点。

鳜鱼(*Siniperea chuatsi*) 属鲈形目，鮨科，鳜属，是我国各主要江河湖泊的名贵食用鱼。其味道鲜美，肉质细嫩，营养价值高，深受广大消费者欢迎。20 世纪 80 年代鳜鱼人工繁殖成功并进行大规模推广养殖，使之成为我国主要名贵养殖鱼类之一。近年来，本课题组在国家自然科学基金项目的支持下，采用现代生物化学和分子生物学综合技术，围绕鳜鱼优良肉质性状的理化特征、遗传控制的基因组学以及肌肉相关功能基因的克隆和表达分析等进行了较系统研究。主要研究成果归纳如下三个方面：

(1)阐明了与翘嘴鳜优质肉质性状相关的功能基因表达谱特征。分别采用高通量的基因芯片技术和 cDNA 文库 - EST 测序技术，首次系统研究了翘嘴鳜肌肉组织转录组学特征，并对筛选和克隆获得的系列翘嘴鳜肉质性状的关键基因结构、功能以及表达特征进行了分析，为进一步研究鳜鱼肌肉分化调控奠定基础。

(2)揭示了影响翘嘴鳜味道鲜美和肉质细嫩的分子基础。与鲢鱼肌肉相比，翘嘴鳜肌肉蛋白整体偏酸，主要与其肌肉鲜味氨基酸(谷氨酸和天冬氨酸等)含量较高有关；基因芯片杂交检测出翘嘴鳜与鲢鱼肌肉 375 个表达差异基因，其中 49 个已知功能基因主要是肌肉结构和肌纤维连接等与肉质结构性状相关基因，主要与翘嘴鳜肌纤维密度高、直径小相关联。

(3)揭示了鳜类种内和种间遗传多样性、遗传变异和亲缘关系。比较分析了 3 种鳜鱼(翘嘴鳜 *S. chuatsi*、大眼鳜 *S. kneri*、斑鳜 *S. scherzeri*)完整线粒体基因组

的结构与组成，初步阐明三种鳜鱼种间差异性和亲缘关系；比较分析了不同水系斑鳜线粒体基因组的差异性，揭示了斑鳜种内遗传多样性；筛选出翘嘴鳜18个多态性微卫星标记，揭示了翘嘴鳜种内遗传多样性和变异性。

上述有关鳜鱼的研究不仅有助于更深一步了解鱼类肌肉胚后生长发育调控机制，而且将为我国鱼类肉质品质改良和促进水产业优质、高效和健康发展提供重要的理论依据。为方便从事鱼类生物学和水产学研究者获取有关鱼类肌肉发育生物学和基因调控的参考资料，我们特将已发表的主要研究论文成果汇集成本书，以飨读者。

多年来，有多位研究生、合作者和学科组教师参与了实验研究，在此一并致谢。衷心感谢中南大学出版社和责任编辑的辛勤努力和支持，是他们的鼓励和帮助才使本书得以出版。衷心感谢国家自然科学基金多年的连续资助，使我们的研究工作顺利进行。本书的出版得到“生物化学与分子生物学”湖南省教育厅“十二五”重点建设学科项目和长沙学院出版基金资助。

由于作者水平有限，加之时间仓促，书中错漏定有不少，恳请读者和同行批评指正！

**褚武英　张建社**

**2014年11月于长沙**

# 目 录

# 第一篇　基于线粒体基因组序列的鳜类系统进化分析

鳜类属鲈形目(Perciformes)、鳜亚科(Sinipercinae)的特化淡水名贵鱼类，现在认为鳜类一共有12个种(翘嘴鳜、斑鳜、大眼鳜、长体鳜、暗鳜、波纹鳜、高体鳜、柳州鳜、日本少鳞鳜、中国少鳞鳜、朝鲜少鳞鳜和无斑鳜)。鳜类作为东亚特有的一种淡水经济鱼类，味道鲜美，肉质细嫩，营养价值高，深受广大消费者欢迎，具有十分重要的经济意义和研究价值。近年来，由于环境压力和人为破坏，鳜类野生资源存储量锐减，生物多样性也受到严重威胁。目前国家公布的368个国家级水产种质资源保护区中，以鳜命名的保护区为13处，涉及鳜类保护的保护区为123处，占所有保护区的33.4%。目前关于鳜的遗传基础研究，特别是有关鳜类的线粒体基因组学特征及其物种多样性和适应性研究甚少，且缺乏系统性。线粒体属于母性遗传又靠近呼吸膜内面，缺乏自我修复系统，极易受环境影响，进化速率快，是研究物种起源和进化的有力工具。目前，从线粒体DNA分离纯化、通用引物设计、长距PCR(Long-PCR)、鸟枪法测序、序列拼接、基因预测、RNA二级结构预测、基因组数据提交和Internet公用数据库的建立到基因组进化分析和基因组特征分析，一整套线粒体基因组研究方法已基本确立，线粒体基因组在分子系统进化研究中的使用频率大大提高。因此本实验室经过多年摸索初步确定了鳜类鱼类线粒体基因组克隆的方法；克隆了6种鳜鱼完整线粒体基因组序列并对其结构与组成进行了比较与分析，初步阐明6种鳜鱼种间差异性和亲缘关系；比较分析了不同水系斑鳜线粒体基因组的差异性，揭示了斑鳜种内遗传多样性和对环境适应的分子基础。

# Phylogenetic studies of three sinipercid fishes (Perciformes: Sinipercidae) based on complete mitochondrial DNA sequences

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**Abstract**: The sinipercids are a group of 12 species of freshwater percoid fish endemic to East Asia and their phylogenetic placements have perplexed generations of taxonomists. We cloned and sequenced the complete mitochondrial DNA (mtDNA) of three sinipercid fishes (*Siniperca chuatsi*, *S. kneri*, and *S. scherzeri*) to characterize and compare their mitochondrial genomes. The mitochondrial genomes of *S. chuatsi*, *S. kneri*, and *S. scherzeri* were 16496 bp, 17002 bp, and 16585 bp in length, respectively. The organization of the three mitochondrial genomes is similar to those reported from other fish mitochondrial genomes, which contains 37 genes (13 protein-coding genes, 2 ribosomal RNAs, and 22 transfer RNAs) and a major non-coding control region. Among the 13 protein-coding genes of all the three sinipercid fishes, three reading-frame overlaps were found on the same strand. There is an 81 – bp tandem repeat cluster at the end of CSB – 3 in the *S. scherzeri* control region. The complete mitochondrial genomes of the three sinipercids should be useful for the evolutionary studies of sinipercids and other vertebrate species.

**Keywords**: *Sinipercids*, *sinipercid fish*, *mitochondrial genome*, *phylogenetic analysis*

## Introduction

The sinipercids are a group of freshwater perciform fish endemic in China, Vietnam, Japan, and Korea with a majority of species recorded in China (Zhou et al. 1988). They generally constitute a monophyletic group and have been assigned to a distinct family, Sinipercidae (Chen et al. 1997; Nelson 2006). A total of 12 species in this group were once assigned to only one genus, *Siniperca* (Zheng 1989), or to two genera, *Siniperca* and *Coreoperca* (Liu and Chen 1994; Nelson 2006). *Siniperca chuatsi*, *S. kneri*, and *S. scherzeri* are the three major species in Sinipercinae. Due to overexploitation and environmental stress, Sinipercinae stocks have experienced a drastic decline (Zhao et al. 2007; Ding et al. 2011). Increased demand of *S. scherzeri* production has accelerated an extensive aquaculture of *S. chuatsi* and *S. scherzeri*. These two species have become the most promising farming freshwater fishes in China (Chu et al. 2011). With its small size, high abundance in the cell, maternal inheritance, and rapid evolutionary rate (Curole and Kocher 1999), mitochondrial genome has been considered as a natural marker and being widely applied in population genetic and evolutionary studies (Ballard and Whitlock 2004; Rand 2008). The conserved mitochondrial DNA (mtDNA) has been particularly useful as molecular markers in teleost fish due to the lack of common nuclear markers across a very large number of teleost fish species. In spite of the use of three mitochondrial genes, 16SrRNA, CO1, and CYTB as markers for phylogenetic analysis of sinipercid fishes (Chen et al. 2010), the mitochondrial genomes of sinipercids have not been characterized. The complete genome information of *S. chuatsi*, *S. kneri*, and *S. scherzeri* will provide a broader understanding of interspecific relationships among these fishes.

In this paper, we described the complete sequencing of mitochondrial genomes of *S. chuatsi*, *S. kneri*, and *S. scherzeri*, analyzed the genome organization and gene arrangement of these mitogenomes, and compared them with those of other fish species. These mitochondrial genome sequences will be useful for evolutionary studies as well as for species stock identification of these species.

## Materials and methods

### Sample collection and DNA extraction

*S. chuatsi*, *S. kneri*, and *S. scherzeri* were obtained from the Dongting Lake, Hunan, China. The total genomic DNA was extracted from the skeletal muscle tissues of the fishes as described by Chu et al. (2006).

**Polymerase chain reaction and sequencing of PCR products**

We used 14 sets of primers to amplify contiguous, overlapping segments of the complete mitochondrial genome of three sinipercid fishes as shown in Tab. 1. The primers used to amplify the mitochondrial genome were designed from previously reported partial mitochondrial genome sequences of *S. chuatsi* and complete mitochondrial genome sequence of *Coreoperca kawamebari* (GenBank accession number: AY898953, EF213709, AF47515, EF143387, AF475158, DQ862098, and AP005990). The polymerase chain reaction (PCR) was performed in a Bio-Rad S1000™ thermal cycler, Hercules, California, USA. The amplified DNA fragments were purified via spin columns and sequenced with an ABI 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol.

**Tab. 1 PCR primers used in the analysis of the three sinipercids mitochondrial genomes**

| Primer | Sequence(5′-3′) | $T_m$(℃) |
|---|---|---|
| P-mt1F | TTCTTCGCCTTCCACTTCCTCT | 56-58 |
| P-mt1R | AGGCGGATAGCAGGTGTAAGGT | |
| P-mt2F | AGGACTTGGCGGTGCTTTAGAT | 54-56 |
| P-mt2R | CGTTGTGCCATTCATACAGGTC | |
| P-mt3F | CACCTTACACCTGCTATCCG | 50-52 |
| P-mt3R | ATGTTACGACTTGCCTCCC | |
| P-mt4F | AGACGAGAAGACCCTATGGAGC | 54-56 |
| P-mt4R | TAGATGGGCGATTGAGGAGTAG | |
| P-mt5F | AAGCCTCGCCTGTTTACC | 50-52 |
| P-mt5R | CTTCTTTTCGGTCCTTTCGT | |
| P-mt6F | TACTTTCTTACCCAAGCCACCG | 54-56 |
| P-mt6R | GAGGCAAGAAGGAGAAGGAAGG | |
| P-mt7F | GCAGAACTAAGCCAACCAG | 47-49 |
| P-mt7R | CAGTAGGGATTGCGATGA | |
| P-mt8F | TTCCTTCTCCTTCTTGCCTCTT | 54-56 |
| P-mt8R | CGTGGTCGTGGAAATGAAGG | |
| P-mt9F | TAATGGCACATCCCTCAC | 47-49 |
| P-mt9R | AGGGCTAATAGTCGGTTGT | |
| P-mt10F | AGCCTCCCTTGAATCCTTT | 52-54 |
| P-mt10R | GCTCAGAGGAAGGTGGTTAG | |
| P-mt11F | CATCTGAGCACCATTTCGG | 54-56 |

**Continued**

| Primer | Sequence(5′-3′) | $T_m$(8℃) |
|---|---|---|
| P-mt11R | TGGGTTCGTTCGTAGTTGG | |
| P-mt12F | CCTCTGACTCCCAAAAGCAC | 52-54 |
| P-mt12R | ATAGGGCGACTGGGGTAAAG | |
| P-mt13F | CGTATTCACAACCCTCACAC | 48-50 |
| P-mt13R | AAGTGGTGGGTGGTTAGTTG | |
| P-mt14F | CCATACCTCCTCTGCTCAAACT | 53-55 |
| P-mt14R | TGTGGAGGATGGGGACAACTA | |

## Sequence analysis and alignment

We isolated and sequenced three clones for each PCR fragment. The PCR products were sequenced twice. BLASTN programs were used to search nucleotide database using sequenced nucleotide segments (Altschul et al. 1997). All fragments were assembled with DNASTAR (http://www.dnastar.com/). We used tRNAscan-SE 1.23 (http://lowelab.ucsc.edu/tRNAscan-SE), an online program, to detect all tRNAs and to construct cloverleaf structures. In MEGA 4.0, homologous sequences from GenBank were used to aid alignments and to identify certain codon positions and frameshift (Tamura et al. 2007). Finally, the program Sequin (http://www.ncbi.nlm.nih.gov/Sequin/index.html) was used for mitogenome submission. DNA sequences of the complete mitogenome of the three fishes were determined by comparison with previously published sequences to other teleost fishes. The locations of the 13 protein-coding genes and the two rRNA genes were deter mined by alignment and comparison with sequences of other teleost mitochondrial genomes.

## Phylogenetic analysis

Phylogenetic analysis was performed using the mitochondrial genomes of 39 fish species from the representatives of Scombridae, Cichlidae, Terapontidae, Lutjanidae, Sinipercidae, Kyphosidae, Centrarchidae, Pomacanthidae, Oplegnathidae, Sparidae, and Labridae (Tab. 2). From the mitochondrial sequence data, two different datasets were analyzed: (1) concatenated protein-coding nucleotide sequence and (2) each of the protein-coding nucleotide sequence. Each of the datasets was aligned using ClustalX (Thompson et al. 1997) and analyzed by neighbor-joining (N-J) in MEGA 4.0, and bootstrap analysis was performed with 1000 replications.

**Tab. 2 Species used in the present study with GenBank accession numbers and references**

| Classification | Species | Accession number | References |
|---|---|---|---|
| Scombridae | *Thunnus albacares* | NC_014061 | Unpublished |
| Scombridae | *Thunnus maccoyii* | NC_014101 | Unpublished |
| Scombridae | *Thunnus obesus* | NC_014059 | Unpublished |
| Scombridae | *Thunnus orientalis* | GU256524 | Unpublished |
| Scombridae | *Katsuwonus pelamis* | NC_005316 | Unpublished |
| Scombridae | *Scomberomorus cavalla* | DQ536428 | Broughton and Reneau(2006) |
| Scombridae | *Rastrelliger brachysoma* | NC_013485 | Jondeung and Karinthanyakit(2010) |
| Scombridae | *Scomber scombrus* | NC_006398 | Takashima et al. (2006) |
| Scombridae | *Scomber colias* | NC_013724 | Catanese et al. (2010) |
| Scombridae | *Scomber australasicus* | NC_013725 | Catanese et al. (2010) |
| Scombridae | *Scomber japonicus* | NC_013723 | Catanese et al. (2010) |
| Cichlidae | *Red tilapia* | GU477631 | Unpublished |
| Cichlidae | *Oreochromis niloticus* | GU477626 | Unpublished |
| Cichlidae | *Oreochromis mossambicus* | NC_007231 | Unpublished |
| Tetrapturus | *Tetrapturus angustirostris* | NC_012679 | Unpublished |
| Tetrapturus | *Makaira mazara* | NC_012680 | Unpublished |
| Tetrapturus | *Makaira indica* | NC_012675 | Unpublished |
| Tetrapturus | *Tetrapturus audax* | NC_012678 | Unpublished |
| Lutjanidae | *Lutjanus malabaricus* | NC_012736 | Unpublished |
| Lutjanidae | *Lutjanus sebae* | NC_012737 | Unpublished |
| Sinipercidae | *S. chuatsi* | JF972568 | Present study |
| Sinipercidae | *S. kneri* | JN378751 | Present study |
| Sinipercidae | *S. scherzeri* | JN084101 | Present study |
| Kyphosidae | *Girella punctata* | AP011060 | Yagishita et al. (2009) |
| Kyphosidae | *Microcanthus strigatus* | AP006009 | Yagishita et al. (2009) |
| Kyphosidae | *Labracoglossa argentiventris* | AP011062 | Yagishita et al. (2009) |
| Kyphosidae | *Scorpis lineolata* | AP011063 | Yagishita et al. (2009) |
| Kyphosidae | *Kyphosus cinerascens* | AP011061 | Yagishita et al. (2009) |
| Oplegnathidae | *Oplegnathus fasciatus* | AP006010 | Yagishita et al. (2009) |
| Sparidae | *Pagellus bogaraveo* | AB305023 | Ponce et al. (2008) |
| Sparidae | *Parargyrops edita* | EF107158 | Xia et al. (2007) |

**Continued**

| Classification | Species | Accession number | References |
|---|---|---|---|
| Pomacanthidae | *Centropyge loricula* | AP006006 | Yamanoue et al. (2007) |
| Pomacanthidae | *Chaetodontoplus septentrionalis* | AP006007 | Yamanoue et al. (2007) |
| Chaetodontidae | *Chaetodon auripes* | AP006004 | Yamanoue et al. (2007) |
| Chaetodontidae | *Heniochus diphreutes* | AP006005 | Yamanoue et al. (2007) |
| Scaridae | *Scarus forsteni* | FJ619271 | Unpublished |
| Labridae | *Parajulis poecilepterus* | NC_009459 | Oh et al. (2007) |
| Labridae | *Pteragogus flagellifer* | EF409976 | Unpublished |

## Results and discussion

### Genome organization

*S. chuatsi*, *S. kneri*, and *S. scherzeri* mitochondrial genomes were reported for the first time and their sequences were deposited in the GenBank with accession numbers of JF972568, JN378751, and JN084101, respectively. The overall base compositions of the complete mitogenomes of the three sinipercid fishes are presented in Tab. 3. As observed in other fish species, the C contents are relatively high but the G contents are lowest (Miya et al. 2003; Mabuchi et al. 2007; Oh et al. 2007). The G contents are 16.2% (*S. chuatsi*), 16.2% (*S. kneri*), and 16.5% (*S. scherzeri*), which is relatively low compared with the mitogenomes of other teleost fishes (Oh et al. 2010).

**Tab. 3　Base compositions of the three sinipercid fishes mitochondrial genomes**

| | *S. chuatsi* | | | | *S. kneri* | | | | *S. scherzeri* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | C | G | T | A | C | G | T | A | C | G | T |
| Complete genome Protein | 28.6 | 29.2 | 16.2 | 26.0 | 28.7 | 29.1 | 16.2 | 26.0 | 28.4 | 29.5 | 16.5 | 25.6 |
| First | 25.7 | 27.8 | 25.6 | 20.9 | 25.8 | 27.8 | 25.5 | 20.9 | 25.7 | 27.9 | 25.7 | 20.7 |
| Second | 18.2 | 27.6 | 13.9 | 40.3 | 18.4 | 27.7 | 13.8 | 40.1 | 18.0 | 31.1 | 13.7 | 37.2 |
| Third | 34.3 | 35.6 | 7.1 | 23.0 | 34.3 | 35.6 | 7.1 | 23.0 | 33.3 | 33.3 | 8.2 | 25.2 |
| Total | 26.1 | 30.3 | 15.5 | 28.1 | 26.2 | 30.3 | 15.5 | 28.0 | 25.7 | 30.8 | 15.8 | 27.7 |
| tRNAs | 31 | 24.7 | 20.2 | 24.1 | 31.0 | 24.8 | 20.1 | 24.1 | 30.9 | 25.1 | 20.0 | 24.0 |
| rRNAs | 32.3 | 25.4 | 21.3 | 21 | 32.4 | 25.4 | 21.2 | 21.0 | 32.2 | 25.9 | 21.1 | 20.8 |
| Control region | 33.8 | 20.6 | 15.0 | 30.6 | 33.8 | 20.2 | 15.3 | 30.7 | 34.0 | 20.9 | 15.8 | 29.3 |

The structure of the mitochondrial genomes of the three sinipercid fishes are very similar to that of other teleost species (Miya and Nishida 2000; Saitoh et al. 2000; Yue et al. 2006). They all contain 13 protein-coding genes, 2rRNA genes, and 22 tRNA genes, as well as the putative control region. Most genes are encoded on the H-strand, except for the Nad6 and eight tRNA genes (tRNA-Pro, tRNA-Glu, tRNA-Ser$^{AGY(C)}$, tRNA-Tyr, tRNA-Cys, tRNA-Asn, tRNA-Ala, tRNA-Gln) encoded on the L-strand (Tab. 4). The non-coding control region located between the tRNA-Pro and tRNA-Phe genes contains the heavy strand origin of replication (OH). A smaller control region containing the putative light strand origin of replication is found between tRNA-Asn and tRNA-Cys genes, the same order as reported in the other fish species (Oh et al. 2010).

**Protein-coding genes**

Among the 13 protein-coding genes of the three sinipercid fishes, overlaps of three reading frames are found on the same strand: ATP8 and ATP6 overlap by 10 nucleotides, and ND4 and ND4L overlap by 7 nucleotides, and ND5 and ND6 overlap by 3 nucleotides (Tab. 4). The overlap of the ATPase genes appears to be common in most vertebrate mitochondrial genome, and its size in fish (7 – 10 bp) is smaller than that in mammals (40 – 46 bp) (Broughton et al. 2001). Except for CO1, which began with GTG as an initiation codon, all proteins started with an ATG codon. However, the termination codons of the 13 protein-coding genes are varied (Tab. 4). Among the 13 protein-coding genes of the three sinipercid fishes, four genes (ND1, ATP8, ND4L, ND5) ended with TAA, CO1 with AGG, ND6 stopped with TAG, CO3 has an incomplete stop codon (TA), and four genes (CO2, ND3, ND4, CYTB) ended with T. The ND2 gene ended with AGG in *S. chuatsi* and *S. kneri* while it ended with T in *S. scherzeri*. The termination codons of *S. chuatsi* and *S. kneri* were TAA while ended with incomplete stop codon (TA) in ATP6 gene in *S. scherzeri* (Tab. 4). The incomplete termination codons, which are often found within the mitochondrial genomes of teleost fishes, are completed via posttranscriptional polyadenylation (Ojala et al. 1981; Ishiguro et al. 2001; Miya et al. 2003).

The base composition of the 13 mitochondrial protein-coding genes of the three sinipercid fishes is presented in Tab. 3. In the protein-coding genes, the proportion of G at the first position of the codons has no obvious bias, but was relatively low at the second and third positions. Especially at the third position, G was found with only 7.1%, 7.1%, and 8.2% of the codons in *S. chuatsi*, *S. kneri*, and *S. scherzeri*, respectively, which is in agreement with previous reports (Saitoh et al. 2000; Mabuchi et al. 2007; Oh et al. 2007).

**Tab. 4　Summary of the mitochondrial genomes of the three sinipercid fishes**

| | Codon | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *S. chuatsi* | | | *S. kneri* | | | *S. scherzeri* | | |
| Feature | Start | Stop | Position | Start | Stop | Position | Start | Stop | Position |
| tRNA – Phe | | | 1 – 68 | | | 1 – 68 | | | 81 – 148 |
| 12SrRNA | | | 69 – 1014 | | | 69 – 1014 | | | 149 – 1093 |
| tRNA – Val | | | 1015 – 1086 | | | 1015 – 1086 | | | 1094 – 1165 |
| 16SrRNA | | | 1087 – 2778 | | | 1087 – 2778 | | | 1168 – 2856 |
| tRNA – $Leu^{UUA(R)}$ | | | 2779 – 2852 | | | 2779 – 2852 | | | 2857 – 2930 |
| ND1 | ATG | TAA | 2853 – 3827 | ATG | TAA | 2853 – 3827 | ATG | TAA | 2931 – 3905 |
| tRNA – Ile | | | 3832 – 3902 | | | 3832 – 3902 | | | 3910 – 3980 |
| tRNA – *Gln* | | | 3902 – 3972 | | | 3902 – 3972 | | | 3980 – 4050 |
| tRNA – Met | | | 3973 – 4041 | | | 3972 – 4040 | | | 4050 – 4118 |
| ND2 | ATG | AGG | 4042 – 5091 | ATG | AGG | 4041 – 5090 | ATG | T – | 4119 – 5163 |
| tRNA – Trp | | | 5089 – 5159 | | | 5088 – 5158 | | | 5165 – 5235 |
| tRNA – *Ala* | | | 5161 – 5229 | | | 5228 – 5160 | | | 5237 – 5305 |
| tRNA – *Asn* | | | 5231 – 5303 | | | 5302 – 5230 | | | 5307 – 5379 |
| tRNA – *Cys* | | | 5341 – 5407 | | | 5406 – 5340 | | | 5417 – 5482 |
| tRNA – *Tyr* | | | 5408 – 5477 | | | 5476 – 5407 | | | 5483 – 5552 |
| CO1 | GTG | AGG | 5479 – 7038 | GTG | AGG | 5478 – 7037 | GTG | AGG | 5554 – 7113 |
| tRNA – $Ser^{UAN(A)}$ | | | 7030 – 7100 | | | 7099 – 7029 | | | 7105 – 7175 |
| tRNA – Asp | | | 7104 – 7175 | | | 7103 – 7174 | | | 7179 – 7254 |
| CO2 | ATG | T – | 7184 – 7874 | ATG | T – | 7183 – 7873 | | | |
| tRNA – Lys | | | 7875 – 7948 | | | 7874 – 7947 | | | 7950 – 8023 |
| ATP8 | ATG | TAA | 7950 – 8117 | ATG | TAA | 7949 – 8116 | ATG | TAA | 8025 – 8192 |
| ATP6 | ATG | TAA | 8108 – 8791 | ATG | TAA | 8107 – 8790 | ATG | TA – | 8183 – 8865 |
| CO3 | ATG | TA – | 8791 – 9575 | ATG | TA | 8790 – 9575 | ATG | TA – | 8866 – 9650 |
| tRNA – Gly | | | 9576 – 9647 | | | 9575 – 9646 | | | 9651 – 9722 |
| ND3 | ATG | T – | 9648 – 9996 | ATG | T – | 9647 – 9996 | ATG | T – | 9723 – 10071 |
| tRNA – Arg | | | 9997 – 10065 | | | 9996 – 10064 | | | 10072 – 10140 |
| ND4L | ATG | TAA | 10066 – 10362 | ATG | TAA | 10065 – 10361 | ATG | TAA | 10141 – 10437 |
| ND4 | ATG | T – | 10356 – 11736 | ATG | T – | 10355 – 11735 | ATG | T – | 10431 – 11811 |
| tRNA-His | | | 11737 – 11805 | | | 11736 – 11804 | | | 11812 – 11880 |
| tRNA-$Ser^{AGY(C)}$ | | | 11806 – 11875 | | | 11805 – 11872 | | | 11881 – 11948 |
| tRNA – $Leu^{CUA(N)}$ | | | 11878 – 11950 | | | 11877 – 11949 | | | 11953 – 12025 |
| ND5 | ATG | TAA | 11951 – 13789 | ATG | TAA | 11950 – 13788 | ATG | TAA | 12026 – 13864 |
| ND6 | ATG | TAG | 13786 – 14307 | ATG | TAG | 13785 – 14306 | ATG | TAG | 13861 – 14382 |
| tRNA – *Glu* | | | 14308 – 14376 | | | 14375 – 14307 | | | 14383 – 14451 |
| CYTB | ATG | T – | 14381 – 15521 | ATG | T – | 14886 – 16026 | ATG | T – | 14456 – 15596 |
| tRNA – Thr | | | 15522 – 15593 | | | 16027 – 16098 | | | 15597 – 15668 |
| tRNA – *Pro* | | | 15593 – 15662 | | | 16167 – 16098 | | | 15668 – 15737 |
| D – Loop | | | 15663 – 16496 | | | 16172 – 17002 | | | 15758 – 16579 |

Italic text Underlining indicates a gene encoded on the L-strand