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The complete mitogenome of *Callista chinensis* (Bivalvia: Veneridae)

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ABSTRACT

The complete mitochondrial genome of *Callista chinensis* was sequenced via next-generation sequencing. The circular genome was 19,704 bp in length, containing 12 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and a putative control region. The gene order of *nad2* and *nad4l* was reversed when compared with that of other Veneridae species. The phylogenetic analysis indicated that the *C. chinensis* was clustered with *Saxidomus purpurata*. Comparing nucleotide sequences of the partial *cox1* gene from 40 *C. chinensis* individuals displayed high levels of genetic diversity in the analyzed populations. Additionally, demographic history analysis based on neutrality tests and mismatch distributions suggested a recent population expansion in the *C. chinensis*.

Introduction

Callista is a genus of saltwater clams belonging to the family Veneridae. In China, there are two Callista species, one of which is the *Callista chinensis* (Hohen 1802) (Xu and Zhang 2008). The most important distribution area of the *C. chinensis* in China is Pingtan County in Fujian Province. Total production of this species in Pingtan reached more than 20 tons annually in the 1990s (Li et al. 2011). However, due to overexploitation, the natural resources of the *C. chinensis* had decreased dramatically in recent years and the annual production was less than 3 tons. To date, the study about this species was rare and only a few types of research on artificial breeding and reproductive biology had been published (Li et al. 2011).

Mitogenome is a useful tool for population genetic and phylogenetic studies. Currently, the complete mitogenome DNA data have been published for 19 Veneridae species (Bao et al. 2016; Dong et al. 2016; Lv et al. 2018; Hu et al. 2019; Qi et al. 2019). For a better understanding of the evolutionary relationships of *C. chinensis*, we sequenced the complete mitogenome of the species and used this mitogenome to assess the phylogenetic relationships within the family Veneridae. Additionally, we used sequences of the partial *cox1* gene to estimate the genetic variability levels of the population distributed in Pingtan County. This information will provide an important resource for further research on the genetic conservation and molecular evolution of *C. chinensis*.

Materials and methods

Sample collection and preservation

Forty *C.chinensis* specimens were collected from Pingtan County, Fujian Province, China (25°31′28.03″N, 119°47′46.22″E). The muscle samples were stored in absolute ethanol and preserved at −80 °C in the laboratory at Xiamen Ocen Vocational college, Fujian, China (Voucher specimen: XOVC-FJ2020-06-01 – XOVC-FJ2020-06A06-40).

DNA extraction, PCR amplification, and mitogenome sequencing

Total genomic DNA was extracted from the muscle sample using the EasyPure® Genomic DNA Kit according to the manufacturer's instructions (TransGen Biotech Co, Beijing, China). The integrity of the genomic DNA was assessed by 1% agarose gel electrophoresis and the concentration was assessed using NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA). After DNA extraction and quality detection, a sample was used to sequence the complete mitochondrial genome, which was performed on the Illumina Novaseq 6000 platform (Illumina, San Diego, CA, USA) with PE 2 × 150 bp.

Partial sequences of the cox1 gene were amplified using the primer pairs F – 5′-ACTAATCAYAARGATATTGG-3′ and R – 5′-CCAGTAGGAAYAGCAATAAT-3′ modified from Chen et al. (2011). Each PCR reaction was performed using a total volume of 50 μl containing 1 μl (approximately 100 ng) of genomic DNA, 25 μl of 2 × EasyTaq PCR SuperMix (TransGen Biotech, Beijing, China), and 1 μl of 10 μmol/l forward and

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reverse primers. The PCR thermocycler program was as follows: 94 °C for 5 min as initial denaturalization, followed by 35 cycles of 94 °C for 30 s, 50 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR products were purified and sequenced in both directions on an automatic sequencer (ABI PRISM 3730, Boray Biotechnology Co., Ltd., Xiamen, China).

Assembly of the complete mitochondrial genome

The quality and quantity of data produced by the Illumina sequencing were measured by FastQC (Andrews 2010). After filtering low-quality reads and reads containing adapters and poly-N regions, the obtained clean reads were applied for reconstructing the mitochondrial genome by NOVOPlasty (Dierckxsens et al. 2016) using *Saxidomus purpurata* mitochondrial genome (GenBank: NC_026728.1) as a reference. The positions of protein-coding genes, ribosomal RNAs (rRNAs), and transfer RNAs (tRNAs) were predicted by the MITOS Web server (http://mitos.bioinf.uni-leipzig.de) (Bernt et al. 2013). The accurate gene boundaries were confirmed using ExPASy online service (https://web.expasy.org/translate/) and ARWEN online service (http://130.235.46.10/ARWEN/) (Laslett and Canbäck 2008).

Phylogenetic analysis

To analyze the phylogenetic placement of *C. chinensis* within the Veneridae family, mitogenomes of 19 Veneridae in-group species and two Solenidae out-group species were retrieved from GenBank. Nucleotide sequences of the complete mitogenome were aligned using MAFFT v.7 with default settings (Katoh et al. 2005). A maximum-likelihood (ML) tree was generated using IQ-TREE v2.0.5 (Minh et al. 2020) with 1000 bootstraps.

Genetic diversity analysis and historical demographic inference

Sequences of the partial *cox1* gene were aligned with the module CLUSTAL W in the program MEGA X using default parameters (Kumar et al. 2018). The genetic diversity indices, including the number of haplotypes (H), number of segregating sites (S), haplotype diversity (h), and nucleotide diversity (π) were calculated with DnaSP 6.0 (Rozas et al. 2017). To determine past demographic changes, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) were calculated using Arlequin 3.5 (Excoffier and Lischer 2010), and the significance of each statistic was tested by generating 10,000 random samples under the hypothesis of selective neutrality and population equilibrium. The mismatch distributions of pairwise differences (Rogers and Harpending 1992; Harpending 1994) between all individual haplotypes were also calculated with 10,000 parametric bootstrap replicates.

Results and discussion

The circular mitochondrial genome of *C. chinensis* is 19,704 bp in length (GenBank accession MT742541), consisting of 12 protein-coding genes (PCGs), 2 ribosomal RNAs (*rrnS* and *rrnL*), 22 transfer RNA genes, and a large non-coding region. All the genes are encoded on the heavy strand. The total nucleotide composition was 28.05% for A, 39.58% for T, 10.83% for C, and 21.55% for G, with the AT content (67.63%) higher than that of CG (32.38%).

The 12 PCGs range from 291 bp (*nad4l*) to 1584 bp (*nad5*) and most use ATG as the start codon and TAA as the stop codon. Four genes (*cox1*, *cox2*, *nad4*, and *nad5*) use ATA as the start codon, while *cox3* starts with GTG. Four genes (*cox1*, *cox3*, *nad4*, and *atp6*) use TAG as the stop codon. In addition to the 12 PCGs, a truncated *atp8* gene encoding for 38 amino acids was found between *rrnL* and *nad4*, which begins with ATG and ends with TAA. The truncated *atp8* genes were also found in other reported mitogenomes of Veneridae (Lv et al. 2018), however, whether the truncated *atp8* gene has a function needs further verification.

The 22 tRNA genes range from 62 to 74 bp, and all of them could be folded into typical cloverleaf secondary structures except for the two *trnS* genes, which lack the DHU arm. The *rrnL* is 1317 bp in length and is located between *cytb* and *atp8*, which is the same as in other Veneridae. The *rrnS* is 993 bp in length and is flanked by *trnT* and *trnM*, just like in *S. purpurata* (Bao et al. 2016), *Cyclina sinensis* (Dong et al. 2016), and three Dosinia species (Lv et al. 2018).

The longest non-coding region is located upstream of the *cox2* and is 2482 bp in length. Near the 3′ end of this non-coding region, a 120 bp unit tandem repeats 9.5 times. Tandem repeat units in the longest non-coding region are commonly found in metazoan mitogenomes including Veneridae, however, their possible function still needs further research.

Within Veneridae, the reported mitogenomes indicated that the gene orders, and especially the location of tRNA genes, were dramatically variable. Lv et al. (2018) had summarized nine different gene orders of 15 Veneridae species from six different genera. The gene order of *C. chinensis* is not completely identical to any one of the previously reported gene orders but is most similar to *S. purpurata* and *C. sinensis*. A distinct gene rearrangement in *C. chinensis* is the transposition of *nad2* with *nad4l-trnI-trnD*. Additionally, *trnW* translocates downstream of *nad2* in *C. chinensis*, which was not found in other Veneridae.

The phylogenetic analysis (Figure 1) revealed well-supported branches. *Callista chinensis* was clustered with *S. purpurata*, indicating a close relationship between the two genera Callista and Saxidomus. This result is consistent with the morphological classifications (Xu and Zhang 2008), in which Callista and Saxidomus were classified into the subfamily Callistinae.

Based on an 800 bp sequence of the *cox1* gene, 35 segregating sites (32 transitions and three transversions) and 33 haplotypes (GenBank: MW367103 – MW367135) were detected among the forty *C. chinensis* specimens. The haplotype diversity was $h = 0.987 \pm 0.010$ and the nucleotide diversity was $\pi = 0.0175 \pm 0.0010$, relatively higher than those reported for other species of marine bivalves (Baker et al. 2008; Ross et al. 2012; Li et al. 2013; Trovant et al. 2015; Zheng et al. 2019; Acosta-Jofré et al. 2020).
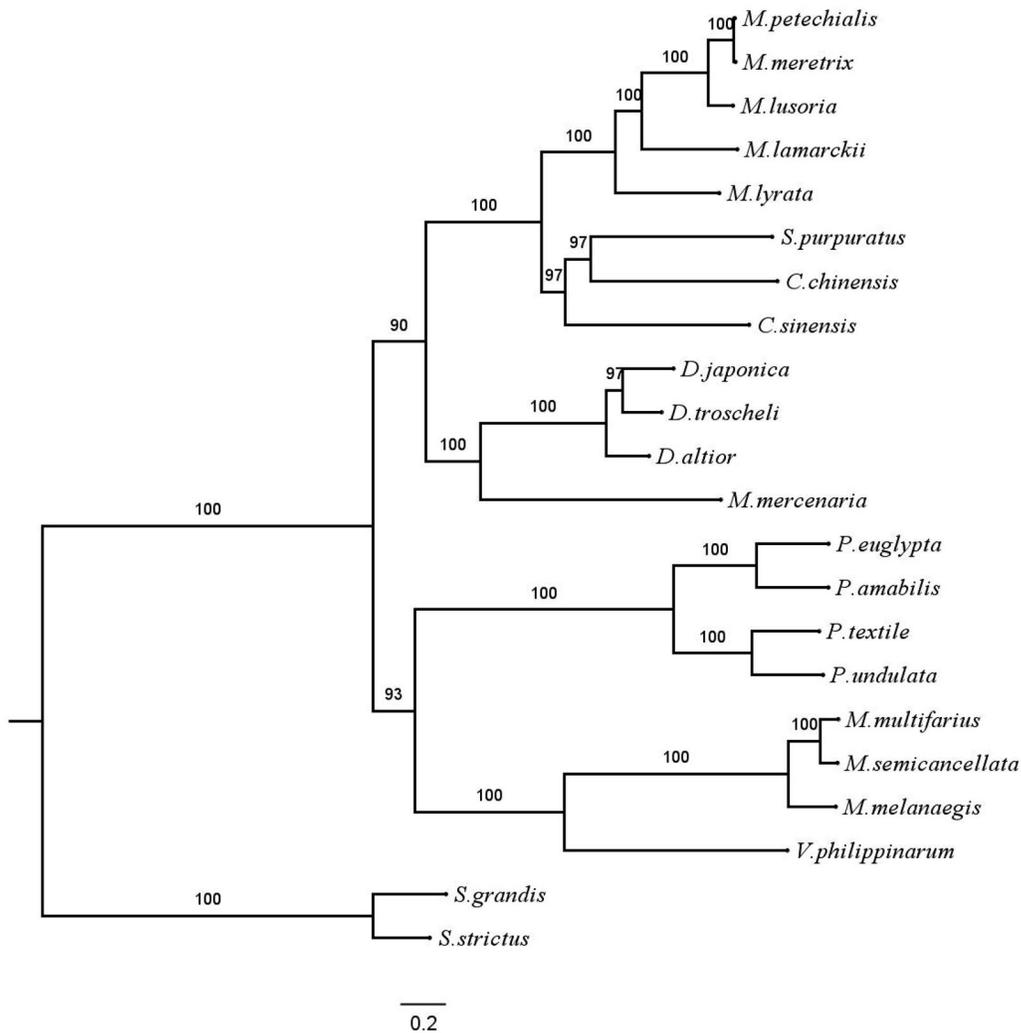
Figure 1. ML phylogenetic analysis for *C. chinensis* and other 19 related species within Veneridae based on the complete mitogenome. *Solen grandis* and *Solen strictus* are designated as outgroup. Numbers on nodes indicate the bootstrap value.
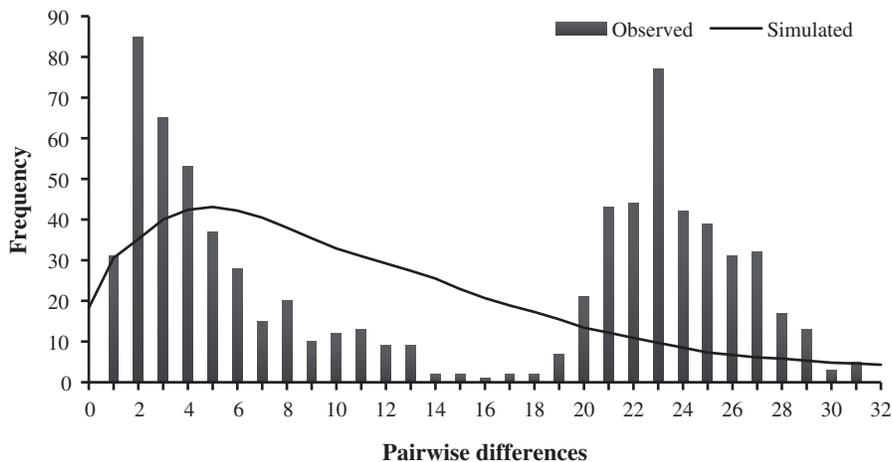
Figure 2. The mismatch distributions under the recent expansion model for the *cox1* haplotypes of *C. chinensis*.

The Tajima's *D* neutrality test was positive but not significant ($D = 2.429$, $p > 0.05$), while the Fu's *Fs* was significantly negative ($Fs = -13.223$, $p < 0.01$). As Fu's *Fs* statistic is more powerful for detecting deviation from neutrality when testing for population expansion (Fu 1997), these results suggested a recent population expansion of the *C. chinensis*. Further, the

mismatch distribution analysis was bimodal (Figure 2), however, the *SSD* and *Rag* indices ($\tau = 1.000$; $SSD = 0.0245$, $p = 0.1307$; $Rag = 0.0132$, $p = 0.2863$) showed nonsignificant values, so the null hypothesis of demographic expansion was not rejected. Recent demographic expansions have been found for a variety of bivalves and the most proposed

common explanation is the effect of climatic changes after the late Pleistocene (Baker et al. 2008; Zheng et al. 2019; Acosta-Jofré et al. 2020). However, the exact time and factors of *C. chinensis* population expansion need further study.

In conclusion, we sequenced and characterized the complete mitochondrial genome of *C. chinensis* and confirmed the close relationship between the genera Callista and Saxidomus within Veneridae. High haplotype diversity and high nucleotide diversity detected within the population of *C. chinensis* suggest that overexploitation in recent years has not had a significant impact on the genetic diversity of *C. chinensis* for the time being. Further research on more populations and continuous monitoring of genetic diversity would aid the protection of *C. chinensis* resources.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov, reference number MT742541. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA689005, SRR13340502, and SAMN17193283, respectively.

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