

Mitogenome of the Monotypic Genus *Vittina*: Genomic Characterization and Phylogenetic Analysis

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ABSTRACT

Mitochondrial genome data for the family Neritidae are limited. In this study, the complete mitochondrial genome sequence of the aquatic snail *Vittina turrita* (Neritidae), an important species in the aquarium trade, was generated using Illumina HiSeq 2000 high-throughput and Sanger sequencing technologies for analyses of its composition and phylogenetic relationships. The entire mitochondrial genome of *V. turrita* was 15,692 bp (GenBank accession number PP706666), with an AT content of 64.1%. The mitochondrial genome sequence consisted of 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes, and 1 non-coding region. The 13 PCGs used ATN as the start codon and TAN and T as the stop codons. The DHU arms of tRNA-Ser1 and tRNA-Ser2 were missing in 22 tRNA genes. The genome sequence and structure were highly similar to those of other species in the family. Complete mitochondrial sequences of species in the Neritidae family were downloaded from GenBank for a phylogenetic analysis based on 13 PCGs. *V. turrita* and *Vitta usnea* were closely related, and the most recent classification of *Vittina* as a monotypic genus was supported. This study enriches the available sequence information for the family Neritidae and provides data for future phylogenetic analyses, resource conservation, and genetic diversity research.

Article Information

Received 13 September 2024

Revised 19 September 2024

Accepted 23 September 2024

Available online 09 July 2025
(early access)

Authors' Contribution

XZ and BZ designed the study. XZ and SL executed experimental work. XZ and QS analyzed the data. XZ wrote the paper. XZ provided the laboratory equipment. XZ supervised the research.

Key words

Gastropoda, Neritidae, Phylogenetic analysis, Second-generation sequencing

INTRODUCTION

The mitochondrial genomes of terrestrial mollusks are characterized by a small size (13–36 kb), with matrilineal inheritance, rare recombination, a conserved genome organization, and faster rates of evolution than those of the nuclear genome (Avise *et al.*, 2003). They are widely used in systems biology, phylogenetics, systematic geography, and population genetic analyses of different taxonomic groups (White *et al.*, 2011; Gaitán-Espitia *et al.*, 2013; Menegon *et al.*, 2014). Despite controversies regarding the use of the mitochondrial genome in systematic research (Delsuc *et al.*, 2003), mitochondrial DNA remains the most used genetic marker. Next-generation sequencing technology has accelerated research in mitochondrial genomics, and the mitochondrial genomes of many vertebrates and insects have been sequenced and

studied (Boore, 1999; Hahn *et al.*, 2013; Wang *et al.*, 2014). However, research on the mitochondrial genomes of mollusks is scarce (Kurabayashi and Ueshima, 2000; Boore *et al.*, 2004; Grande *et al.*, 2008; Sun *et al.*, 2024a, b, c).

Terrett *et al.* (1996) analyzed the structure of the mitochondrial genome of the terrestrial snail *Cepaea nemoralis*, providing insight into the evolutionary development of the species. Subsequently, mitochondrial genomes for gastropods have been sequenced extensively, including analyses of gene sequences, gene structure, genetic applications, and phylogenetic relationships (Terrett *et al.*, 1996; Grande *et al.*, 2002; Steiner, 2003; Dejong *et al.*, 2004; Knudsen *et al.*, 2006; Kerr, 2013). Ongoing efforts are aimed at expanding whole mitochondrial genome data for gastropod species. Incomplete genome sequencing data and species information limit in-depth explorations of the classification and evolution of gastropods.

Vittina turrita (Gmelin, 1791) snails in the family Neritidae have shells that are oblong-conical, lightly striated, shiny, spire elevated, pointed, and yellow, with oblique, curved, or rippled black stripes. The shells are 25–32 mm in length. The aperture is white and the columellar area is yellow-tinted. This species is found throughout the Indo-Pacific region, including Madagascar, Japan, Indonesia, and the western Pacific Ocean Islands; it has not been found in India and Australia (Hristov, 2020).

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0030-9923/2025/0001-0001 \$ 9.00/0



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Although popular in the ornamental aquarium trade, *V. turrita* has not been successfully bred in captivity, and animals are wild-caught, presenting a potential biohazard and depleting natural populations. In this study, the entire mitochondrial genome of *V. turrita* was sequenced, enriching the molecular data for Gastropoda and Neritidae and providing data for the systematic classification of these taxa.

MATERIALS AND METHODS

Sample collection and DNA extraction

V. turrita samples (2024BM) were collected on January 1, 2024, from the Flower, Bird, Fish and Insect Market in Fangcun, Guangzhou, Guangdong Province (23.064612°N, 113.206001°E). After morphological identification, samples were stored in anhydrous ethanol at -20 °C. Sterilized tweezers and scissors were used to obtain a small piece of muscle tissue, which was cut into small pieces. Genomic DNA was extracted according to the manufacturer's instructions. Then, 80 µL of sterilized water was used to dissolve genomic DNA. The DNA quality was assessed using 1% agarose gel electrophoresis, and DNA purity and concentration were determined using an ultra-micro spectrophotometer. DNA barcoding technology was used to ensure the accuracy of identification.

Mitochondrial genome sequencing, assembly, and analysis

Second-generation high-throughput sequencing technology was used to sequence the entire genome of *V. turrita* mitochondria. Quality control was performed by filtering out low-quality reads, sequences with high N-rates, and adaptor sequences. After quality control, the contigs were subjected to sequence alignment using *Vitta usnea* (GenBank No. KU342665) as the reference genome, and paired-end data were input into Genius 9.0.2 for assembly. The ORFfinder online tool was used to determine the boundaries of protein-coding genes (PCGs), and tRNA-scan was used to predict the position and secondary structure of tRNA. Structure and function were annotated using the MITOS online service and manually corrected using Geneious 9.0.2. A gene structure diagram of the assembled sequences was constructed using the OGDRAW online platform. Finally, the complete mitochondrial genome data for *V. turrita* were submitted to the NCBI GenBank database. PhyloSuite v1.2.1 was used to analyze the base composition, codon bias, and usage. The asymmetry of the mitochondrial genome chain (i.e., the deviation in composition between the two chains) was calculated using the following formulas: AT skew = (A-T)/(A+T) and GC skew = (G-C)/(G+C).

Phylogenetic analysis

Based on the 13 PCGs of 26 species from different taxa of the family Neritidae (Table I), a phylogenetic tree was constructed using the maximum likelihood (ML) method, with *Divia briandi* (family Phenacolepadidae) and *Pleuropoma jana* (family Helicinidae) as outgroups. The PCGs were extracted using PhyloSuite v1.2.1 for tandem analyses. An ML tree was constructed using IQ-TREE v1.6.8, and the tree model was determined using ModelFinder. The number of bootstrap replicates was set to 50000. The phylogenetic tree was visualized using iTOL.

Table I. Basic information of 28 mitochondrial genomes used in this study.

Family/ Species	Accession number	Length/ bp	AT (%)
Helicinidae			
<i>Pleuropoma jana</i>	KU342666	15,851	74.6
Neritidae			
<i>Clithon corona</i>	MW694825	15,975	64.8
<i>Clithon lentiginosum</i>	MW694826	15,885	64.8
<i>Clithon oualaniense</i>	MT568501	15,706	65.8
<i>Clithon retropictum</i>	MG190355	15,802	64.9
<i>Clithon sowerbianum</i>	MT230542	15,919	64.5
<i>Clithon squarrosus</i>	MW694827	15,905	65.0
<i>Neripteron violaceum</i>	OL679703	15,618	65.8
<i>Nerita albicilla</i>	MK516738	15,314	64.5
<i>Nerita balteata</i>	MN477253	15,571	63.3
<i>Nerita chamaeleon</i>	MT161611	15,716	65.8
<i>Nerita costata</i>	OM048761	15,604	61.6
<i>Nerita histrio</i>	OM048766	15,538	65.5
<i>Nerita insculpta</i>	OM048762	15,721	61.5
<i>Nerita japonica</i>	MN747116	15,875	65.2
<i>Nerita melanotragus</i>	GU810158	15,261	63.5
<i>Nerita ocellata</i>	OM048763	15,577	63.8
<i>Nerita plicata</i>	OM048764	15,737	61.6
<i>Nerita reticulata</i>	OM048765	15,630	65.3
<i>Nerita undata</i>	MN477254	15,583	63.2
<i>Nerita yoldii</i>	MK395169	15,719	64.7
<i>Neritina iris</i>	MW694828	15,618	64.3
<i>Neritina violacea</i>	MT230543	15,618	65.8
<i>Septaria lineata</i>	MW694829	15,697	65.8
<i>Theodoxus fluviatilis</i>	MT628587	15,667	66.1
<i>Vitta usnea</i>	KU342665	15,574	64.0
<i>Vittina turrita</i>	PP706666	15,692	64.1
Phenacolepadidae			
<i>Divia briandi</i>	MH837541	13,618	61.7

RESULTS

Mitochondrial genome structure and composition

The *V. turrita* mitochondrial genome was 15,692 bp (GenBank accession PP706666) and consisted of 37 genes and a control region (CR), including 13 PCGs, 22 tRNA genes, and two rRNA genes (Fig. 1). Among the 37 mitochondrial genes, the L-chain encoded 22 genes, including six PCGs, 14 tRNA genes, and two rRNA genes, while the H-chain encoded seven PCGs and eight tRNA genes. Six genes overlapped with a total length of 6 bp. Gene intervals with a total length of 156 bp were found at 18 gene junctions, and the longest gene interval was 28 bp, between *COX3* and tRNA-Lys. No overlaps or spaces were observed at the remaining 13 intersections (Table II).

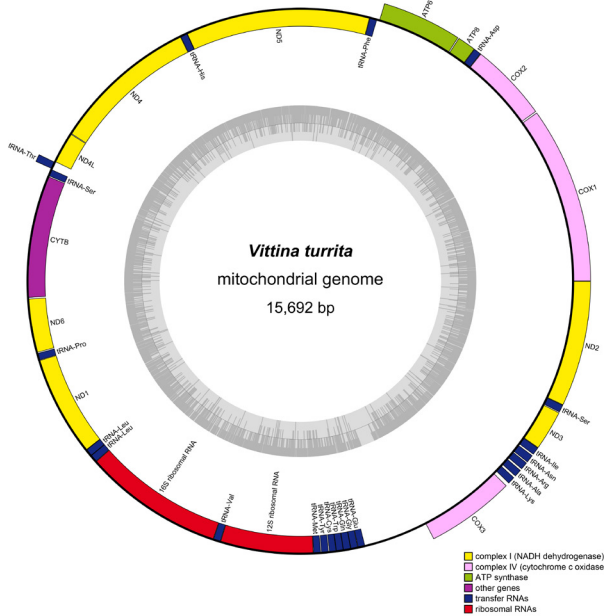


Fig. 1. Mitochondrial genome of *Vittina turrita*.

The basic composition of the 13 PCGs, 22 tRNA genes, and two rRNA genes in the complete mitochondrial genome of *V. turrita* is shown in Table III. The base content of the mitochondrial genome was A = 30.5%, T = 33.6%, C = 15.2%, and G = 20.4%, and the A+T content was 64.1%. The total length of the 13 PCGs was 11274 bp, and the A+T content was 63.2%. The A+T content in the tRNA genes was 62.7%, and that in the rRNA genes was 66.2%, indicating a significant AT bias in the nucleotide composition. In the *V. turrita* mitochondrial genome (Table III), the PCGs of the H-chain and tRNA genes of the H-chain both exhibited T and G biases. The PCGs encoded by the L chain exhibited T and C biases, whereas

the tRNA genes of this chain exhibited A and G biases.

Table II. Composition of *Vittina turrita* mitochondrial genome.

Gene	Position		Size	Intergen- ic nucleo- tides	Codon		Str- and
	From	To			Start	Stop	
<i>COX1</i>	1	1548	1548	0	ATG	TAA	H
<i>COX2</i>	1560	2249	690	11	ATG	TAA	H
<i>tRNA-Asp</i>	2250	2316	67	0			H
<i>ATP8</i>	2317	2481	165	0	ATG	TAA	H
<i>ATP6</i>	2488	3189	702	6	ATG	TAA	H
<i>tRNA-Phe</i>	3211	3279	69	21			L
<i>ND5</i>	3280	4993	1714	0	ATG	T	L
<i>tRNA-His</i>	4993	5059	67	-1			L
<i>ND4</i>	5060	6416	1357	0	ATA	T	L
<i>ND4L</i>	6419	6712	294	2	ATG	TAA	L
<i>tRNA-Thr</i>	6716	6784	69	3			H
<i>tRNA-Ser</i>	6790	6855	66	5			L
<i>CYTB</i>	6861	7997	1137	5	ATG	TAA	L
<i>ND6</i>	8008	8508	501	10	ATT	TAA	L
<i>tRNA-Pro</i>	8515	8581	67	6			L
<i>ND1</i>	8583	9515	933	1	ATG	TAG	L
<i>tRNA-Leu</i>	9515	9583	69	-1			L
<i>tRNA-Leu2</i>	9583	9653	71	-1			L
<i>16S rRNA</i>	9654	10957	1304	0			L
<i>tRNA-Val</i>	10958	11026	69	0			L
<i>12S rRNA</i>	11027	11889	863	0			L
<i>tRNA-Met</i>	11890	11957	68	0			L
<i>tRNA-Tyr</i>	11962	12030	69	4			L
<i>tRNA-Cys</i>	12035	12100	66	4			L
<i>tRNA-Trp</i>	12100	12166	67	-1			L
<i>tRNA-Gln</i>	12166	12235	70	-1			L
<i>tRNA-Gly</i>	12235	12301	67	-1			L
<i>tRNA-Glu</i>	12302	12368	67	0			L
<i>CR</i>	12369	12959	591	0			H
<i>COX3</i>	12960	13739	780	0	ATG	TAA	H
<i>tRNA-Lys</i>	13768	13835	68	28			H
<i>tRNA-Ala</i>	13851	13919	69	15			H
<i>tRNA-Arg</i>	13933	14002	70	13			H
<i>tRNA-Asn</i>	14010	14082	73	7			H
<i>tRNA-Ile</i>	14094	14163	70	11			H
<i>ND3</i>	14164	14517	354	0	ATG	TAG	H
<i>tRNA-Ser2</i>	14522	14590	69	4			H
<i>ND2</i>	14591	15692	1102	0	ATG	T	H

Table III. Nucleotide composition of the *Vittina turrita* mitochondrial genome.

Regions	Strand	Proportion of nucleotides (%)						AT skew	GC skew	Size (bp)
		A	T	G	C	AT (%)	GC (%)			
Full genome		30.5	33.6	20.4	15.2	64.1	35.6	-0.048	0.147	15,692
PCGs		25.3	37.9	18.5	18.4	63.2	36.9	-0.199	0.003	11,274
PCGs	H	23.2	39.8	22.3	14.7	63.0	37.0	-0.264	0.207	5,340
PCGs	L	27.2	36.2	15.0	21.7	63.4	36.7	-0.142	-0.182	5,934
tRNAs		30.5	32.2	21.9	15.3	62.7	37.2	-0.027	0.177	1,507
tRNAs	H	28.1	33.0	23.8	15.1	61.1	38.9	-0.080	0.222	555
tRNAs	L	31.9	31.8	20.8	15.4	63.7	36.2	0.002	0.148	952
rRNAs		35.7	30.5	17.3	16.6	66.2	33.9	0.078	0.020	2,167

PCGs

The lengths of the 13 PCGs in *V. turrita* ranged from 165 bp (*ATP8*) to 1714 bp (*ND5*), with a total length of 11,274 bp. The AT content was 63.2%, with an AT skew of -0.199 and a GC skew of 0.003. The start codon was ATN, and almost all genes used ATG or ATT as the start codon, with ATG having a higher frequency as the starting codon (Table II). Only a few genes had ATA as the start codon, such as the *ND4* gene. For the termination codon, most genes had TAG or TAA. Partial termination codons with incomplete T codons were found in *ND2*, *ND4*, and *ND5*.

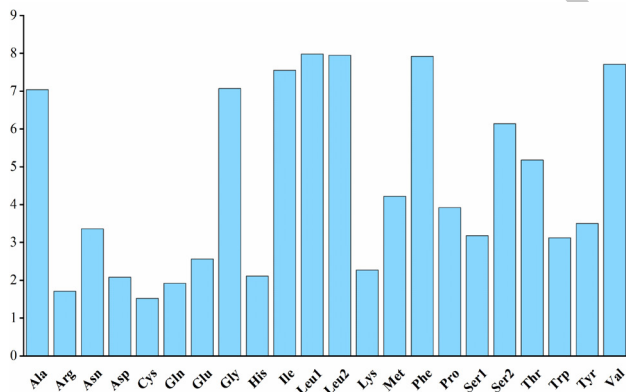


Fig. 2. Frequency of amino acid usage in *Vittina turrita* mitochondrial protein-coding genes (PCGs).

Thirteen PCGs encoded 3758 amino acids. Among the 20 amino acids, leucine (Leu), serine (Ser), phenylalanine (Phe), and valine (Val) were relatively frequent (Fig. 2), accounting for approximately 31.56%. Among these, the most frequent was Leu, accounting for approximately 15.93%. The frequencies of cysteine (Cys) and Arg were relatively low, accounting for only approximately 3.23%. Cys only accounted for approximately 1.52% of all amino

acids. Relative Synonymous Codon Usage (RSCU) eliminates the influence of amino acid composition on codon usage. If there is no preference for the use of a codon, the RSCU value is 1. Values greater than 1 indicate that the codon is used relatively more often, and vice versa. The average frequencies of codon usage for UCU (Ser2), UUA (Leu2), ACU (Thr), CGA (Arg), and GCU (Ala) were relatively high (Fig. 3), with UCU (Ser2) having the highest frequency (2.50) and AGG (Ser1) having the lowest average frequency.

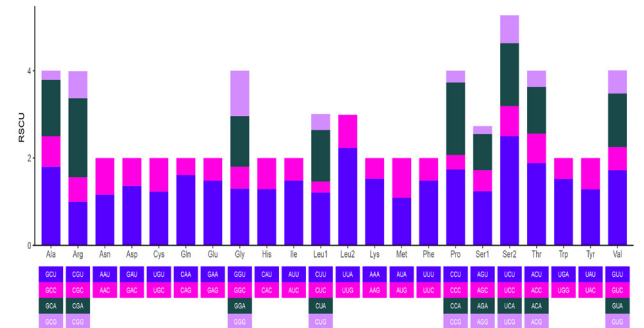


Fig. 3. Same synonymous codon usage of *Vittina turrita* mitochondrial genome.

tRNA genes

V. turrita had 22 tRNA genes, including two large regions, MYCWQGE (tRNA-Met, tRNA-Tyr, tRNA-Cys, tRNA-Trp, tRNA-Gln, tRNA-Gly, and tRNA-Glu) and KARNI (tRNA-Lys, tRNA-Ala, tRNA-Arg, tRNA-Asn, and tRNA-Ile), which are located between the 12S rRNA and *ND3* genes and separated by the *COX3* gene. There were also two small gene fragments, tRNA-Thr/tRNA-Ser and tRNA-Leu/tRNA-Leu2, with the remaining six tRNAs scattered between the PCGs and rRNAs (Fig. 1, Table II). Their length range was 66–73 bp, with the longest being

tRNA-Asn and the shortest being tRNA-Cys and tRNA-Ser. The tRNAs were relatively short, with an average of 68.5 bp. There was a high AT bias, and the base shift was consistent with that of the whole genome and PCGs, showing significant T- and G-specific bias.

tRNA-Ser and tRNA-Ser2 could not fold into typical secondary structures and their dihydrouracil (DHU) arm was missing, forming only one ring (Fig. 4). Except for these two tRNAs, all other tRNA genes had a typical clover structure (Rich and Rajbhandary, 1976). Some tRNA genes do not comply with the principle of complementary base pairing and abnormal cycling. G-U base pairs have been found in the secondary structures of tRNA genes, and these mismatched base pairs form unstable hydrogen bonds in the secondary structure. Most of the remaining base pairs comply with the principle of complementary base pairing, namely A-T pairing and G-C pairing, which play important roles in maintaining the stability of this tRNA secondary structure.

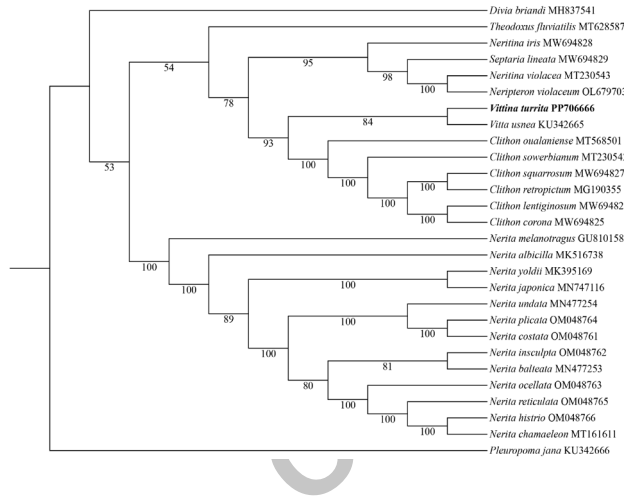


Fig. 4. Secondary structure diagram of 22 tRNA genes in *Vittina turrita*.

rRNA genes and control region

V. turrita contained two types of rRNAs: 16S rRNA and 12S rRNA. These were located between the tRNA-Leu2 and tRNA-Met genes and were separated by the tRNA-Val gene. The total length of rRNA was 2,167 bp, the length of the 16S rRNA was 1,304 bp, and the length of 12S rRNA was 963 bp (Table II). There was a high AT bias in the base composition (i.e., 66.2%). In addition, rRNA exhibited an opposite shift to the entire genome, with an AT shift of 0.078, indicating that most of the base content in rRNA was biased towards A.

The CR was the largest non-coding region. The *V. turrita* mitochondrial CR was located between the tRNA-

Glu and *COX3*, and this region exhibited a high AT bias. The CR region had high variability and a length of 591 bp. In addition to the CR, relatively large regions non-coding regions were found between *COX3* and tRNA-Lys, with an interval of approximately 28 bp, and between *ATP6* and tRNA-Phe, with an interval of approximately 21 bp (Table II).

Phylogenetic analysis

A phylogenetic tree (Fig. 5) showed that species in the family Neritidae clustered into a monophyletic group. *Clithon* and *Nerita* were well-supported monophyletic groups. The *Neritina* clade included *Septaria lineata*, indicating that the monophyly of *Neritina* should be evaluated using additional mitochondrial genome data. Further analyses are required to determine the location of *Theodoxus fluviatilis*. *V. turrita* was closely related to *Vitta usnea* and distantly related to the *Neritina* genus. These results support the taxonomic status of *Vittina* as a monotypic genus.

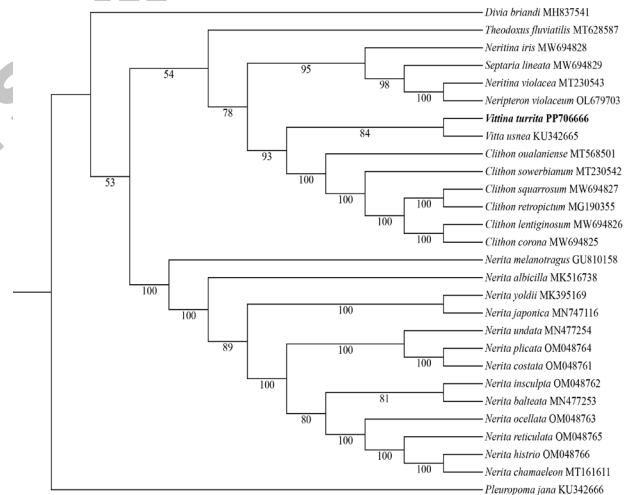


Fig. 5. Maximum likelihood tree constructed based on mitochondrial genome PCGs.

DISCUSSION

The mitochondrial genomes of mollusks typically exhibit significant AT and GC biases (Perna and Kocher, 1995). In the *V. turrita* mitochondrial genome, the PCGs of the H-chain and tRNA genes of the H-chain exhibited T and G biases, whereas the PCG encoded by the L-chain exhibited T and C biases and the tRNA genes of this chain exhibited A and G biases. A prominent feature of the mitochondrial genome of metazoans is the asymmetry in base composition between the two chains, usually with

the L-chain biased towards A and C and the H-chain biased towards T and G (Perna and Kocher, 1995). The AT and GC skew of the *V. turrita* mitochondrial genome differed from the strand skew of mitochondrial DNA in other mollusks. The termination codons of most genes were TAG or TAA. Incomplete T codons were observed in *ND2*, *ND4*, and *ND5*; this incomplete termination codon is usually supplemented during transcription to obtain the complete termination codon T(AA) (Ojala *et al.*, 1981).

Other than tRNA-Ser and tRNA-Ser2, which did not fold into typical secondary structures and were lacking a DHU arm (Fig. 4), the tRNA genes had a typical clover structure (Rich and Rajbhandary, 1976). Overall, the anti-codon arm and DHU arm of the tRNA secondary structure are relatively conserved regions, while the TΨC arm and circular region exhibit high levels of variation, including point mutations, insertions, and deletions (Wang *et al.*, 2018). G-U base pairs were found in the secondary structure of the genes, and these mismatched base pairs formed unstable hydrogen bonds in the secondary structure of tRNA. Mismatched base pairs in the secondary structure of tRNA may be caused by mutations or base mismatches during mitochondrial gene copying. Because the mitochondrial genome is a naked snail DNA strand with no externally protected histones, it is susceptible to mutations caused by external environmental factors. In addition, there is no repair mechanism for nuclear genes during mitochondrial DNA replication, which increases the probability of mismatches during mitochondrial DNA replication. Most mismatches have been found in the amino acid arm (AA), DHU ring, and anticodon arm, as observed in many insects (Awise *et al.*, 2003; Bae *et al.*, 2004; He *et al.*, 2005).

The CR of the entire mitochondrial genome contains short gene intervals that may serve as splice-recognition sites during transcription (He *et al.*, 2005). The CR was the largest non-coding region in *V. turrita* and was located between the tRNA-Glu and *COX3* genes. This area typically exhibits a high AT bias (Meng *et al.*, 2013). It is also necessary for mitochondrial genome replication and transcription initiation (Fernández-Silva *et al.*, 2003). A phylogenetic analysis showed that *V. turrita* and *Vitta usnea* are closely related and supported the classification of *V. turrita* as a monotypic genus.

DECLARATIONS

Acknowledgements

We kindly acknowledge anonymous reviewers for their fruitful and critical comments. We would like to thank Editage (www.editage.com) for their editing support.

Funding

Funded by Natural Science Research Project of Anhui Educational Committee, P.R. China (No. 2023AH051456).

Ethical approval

All specimens in this study were collected in accordance with the relevant Chinese laws. The collection and sampling of the specimens were reviewed and approved by the Animal Ethics Committee of Nanjing Forestry University. All the experiments were conducted with respect to animal welfare and care.

Data availability

Data presented in this study are openly available in the NCBI repository with accession numbers: PP706666

Statement of conflict of interest

The authors have declared no conflict of interests.

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