The highly rearranged mitochondrial genomes of three economically important scale insects and the mitochondrial phylogeny of Coccoidea (Hemiptera: Sternorrhyncha)

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Abstract
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**Introduction**

The scale insects (Coccoidea) are well-known sap-sucking hemipterans which are economically important pests causing severe damage to native crops and plants (*Kondo et al.*, 2008). Adult males of Coccoidea are hyperpaurometamorphosis, whereas the adult females are paurometamorphosis and resemble their nymphs (*Gullan & Kosztarab*, 1997). These insects are usually smaller than 5 mm and often appear similar color with their host plants. Most scale insects can produce waxy secretion covering their bodies as a protection armature (*Gullan & Kosztarab*, 1997), which also causes difficulty in using chemical control methods.

When compared with other superfamilies of the monophyletic suborder Sternorrhyncha: Aphidoidea (aphids), Aleyrodoidea (whiteflies) and Psylloidea (jumping plant lice), the superfamily Coccoidea possess a higher biodiversity and morphological variety (*Gullan & Martin*, 2003; *Gullan & Cook*, 2007). Despite of the previous morphological and molecular contributions (*Koteja*, 1974; *von Dohlen & Moran*, 1995; *Gullan & Cook*, 2007; *Cook et al.*, 2007; *Hodgson & Hardy*, 2013), the scale insect systematics especially the family-level classification still remains unresolved.

Morphology of scale insects has apparent limits when used for resolving the higher-level phylogeny of scale insects, which is expected to be improved by the DNA sequence data. Mitochondrial genome (mitogenome) usually contains a typical set of 37 genes: 13 protein-coding genes (PCG), 22 transfer RNA genes (tRNA), two ribosomal RNA genes (rRNA) and a non-coding control region (CR) and has become one of the most popular molecules used in insect phylogenetic studies (*Cameron*, 2014). Recently, Deng *et al.* (2019) and Lu *et al.* (2020) respectively sequenced
the mitogenomes of the two scale insects, *Ceroplastes japonicus* Green, 1921 and *Saissetia coffeae* (Walker, 1852) and investigated the efficiency of using mitogenome data in the phylogeny of Sternorrhyncha. Mitochondrial gene rearrangement and truncation of tRNA genes have been found in the two mitogenomes. To facilitate the resolution of phylogeny and molecular evolution of Coccoidea, we sequenced the complete mitogenomes of *Unaspis yanonensis* (Kuwana, 1923), *Planococcus citri* (Risso, 1813) and *Ceroplastes rubens* Maskell, 1893, which includes the first representatives of Pseudococcidae and Diaspididae. The mitogenomic organizations, gene rearrangements, nucleotide compositions, codon usages of PCGs, secondary structures of tRNA genes and CR were analyzed for the three mitogenomes. In addition, the phylogenetic relationships of four species of Coccoidea were reconstructed to evaluate the validity of the newly obtained molecular data.

**Materials & Methods**

**Sample preparation and DNA extraction**

The specimens of *U. yanonensis*, *P. citri* and *C. rubens* were collected from Chengdu, Sichuan Province of China in October of 2019. The specimens were reliably identified by experts of Sichuan Academy of Agricultural Sciences, and were preserved in 100% ethanol. The total genomic DNA of the three scale insects was isolated using the E.Z.N.A.® Tissue DNA Kit (OMEGA, America) and preserved at −20 °C before the sequencing process.

**Sequencing, assembly and annotation**

The Illumina TruSeq short-insert libraries (insert size = 450 bp) were constructed using 1.0 μg of purified DNA fragments and sequenced by Illumina Hiseq 4000 (Shanghai BIOZERON Co., Ltd). Prior to assembly, raw reads were filtered and high-quality reads were retained and assembled into contigs by SOAPdenovo2.04 (*Luo et al., 2012*). Then the assembled contigs were aligned to the reference mitogenome of *C. japonicus* (GenBank accession number MK847519) using BLAST. The aligned contigs (≥80% similarity and query coverage) were arranged according to the reference mitogenome. Finally, the clean reads were mapped to the assembled draft mitogenome to fix the wrong bases; gaps were filled using GapFiller v2.1.1 (https://sourceforge.net/projects/gapfiller/). The mitogenome sequences of *U. yanonensis*, *P. citri* and *C. rubens* were deposited in GenBank under the accession numbers MT611525, MT611526
and MT677923, respectively.

Most tRNA genes were predicted and depicted by MITOS (Bernt et al., 2013); structures of several tRNA genes of *C. rubens* were predicted manually. PCGs and rRNA genes were identified by homology alignments. Gene boundaries of PCGs were confirmed in ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/). The graphic view of the mitogenomes were computed using CGView Server (http://stothard.afns.ualberta.ca/cgview_server/) (Grant & Stothard 2008). The probable mitochondrial rearrangement scenarios during the evolution of *U. yanonensis*, *P. citri* and *C. rubens* were predicted by the CREx (Common Interval Rearrangement Explorer) online server (Bernt, 2007) using *Drosophila yakuba* as a reference (Clary & Wolstenholme, 1985). Nucleotide composition of each gene and codon usage of PCGs were calculated by MEGA v.6.0 (Tamura et al., 2013). The composition skew analysis was conducted by AT-skew = [A–T]/[A+T] and GC-skew = [G–C]/[G+C] formulas (Perna & Kocher, 1995). The software DnaSP v. 5.10 (Librado & Rozas, 2009) was used to calculate the synonymous substitution rate (Ks) and the nonsynonymous substitution rate (Ka). Presumed secondary structures in the control region were predicted by the online tool Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.advanced.submit.html) and DNAMAN v6.0.3.

**Phylogenetic analysis**

Nucleotide sequences of PCGs derived from four species of Coccoidea, including *U. yanonensis*, *P. citri* and *C. rubens* sequenced in this study, were used in the phylogenetic analysis (Table 1). The species *S. coffeae* was not included in the dataset due to the unannotated and unreliable status of its sequence as noted in Genbank. The two aphids, *Aphis glycines* and *Diuraphis noxia* were used as the outgroups. The 13 PCGs were aligned by MAFFT and concatenated as a combined dataset using SequenceMatrix v1.7.8 (Katoh & Standley, 2013). PartitionFinder v2.1.1 was used to determine the optimal nucleotide substitution models and partitioning schemes by using the Bayesian Information Criterion (BIC) and a greedy search algorithm (Lanfear et al., 2016). Two phylogenetic inferences were conducted with the partition schemes, including Bayesian inferences (BI) and Maximum likelihood (ML) analysis. BI analysis was conducted by MrBayes v3.2.7, with 10 million generations sampling every 1000 generations, running one cold chain and three hot chains with a burn-in of 25% trees (Ronquist & Huelsenbeck, 2003). Stability of the results of BI analysis were examined by Tracer v.1.5. ML analysis was performed by RAxML v8.2.12 with
1000 bootstrap replicates (Stamatakis, 2014). Tree files generated by both BI and ML trees were adjusted and visualized in FigTree v1.4.2.

Results

Mitogenome annotation and nucleotide composition

The complete mitogenomes of *U. yanonensis*, *P. citri* and *C. rubens* were all typical double-strand circular molecules with a length of 15,220 bp, 15,549 bp and 15,387 bp, respectively (Fig. 1), which were similar to other mitogenomes of Coccoidea (Deng et al., 2019; Lu et al., 2020). The standard set of 37 genes (13 PCGs, 22 tRNA genes and two rRNA genes) were all found in the mitogenome of *U. yanonensis* (Table 2), whereas *trnV* was lost in *P. citri* (Table 3); *C. rubens* lacked five tRNA genes, *trnC*, *trnR*, *trnS2*, *trnL1* and *trnV* (Table 4). In *U. yanonensis*, there were nine overlapping nucleotides located in four pairs of neighboring genes (Table 2); while in *P. citri*, there were 36 overlapping nucleotides in nine gene boundaries (Table 3). In *C. rubens*, there were only seven overlapping nucleotides in four gene boundaries (Table 4). The longest overlap was 18-bp long and located between *trnS2* and *ND1* in *P. citri*. There were 227 intergenic nucleotides (IGNs) dispersed in 20 locations for *U. yanonensis*, 126 IGNs in 19 locations for *P. citri* and 478 IGNs in 19 locations for *C. rubens*, indicating a loose structure of the three scale insect mitogenomes.

The whole mitogenomes of *U. yanonensis*, *P. citri* and *C. rubens* were strongly biased toward A and T nucleotides (86.6%, 82.7% and 87.5%, respectively). The *U. yanonensis* mitogenome had negative AT-skew and positive GC-skew, whereas *P. citri* and *C. rubens* exhibited positive AT-skew and negative GC-skew. The A+T contents were also rich in the mitochondrial genes, showing the highest in *trnF* of *U. yanonensis* and *P. citri*, and *trnG* of *C. rubens*.

Gene rearrangement

The mitochondrial genes of *U. yanonensis*, *P. citri* and *C. rubens* were highly rearranged, being different from the two sequenced scale insects, *C. japonicus* and *S. coffeae* (Deng et al., 2019; Lu et al., 2020). When compared with *D. yakuba*, *U. yanonensis* and *P. citri* both showed a conserved gene cluster *trnE-trnF-ND5-trnH-ND4-ND4L-trnT-trnP-ND6-CYTB-trnS2-ND1-trnL1-rRN*L; *C. rubens* had three shorter conserved gene clusters, *COX1-trnL-COX2-trnK-trnD, COX3-trnG-ND3* and *ND5-trnH-ND4-ND4L* (Fig. 2). The mitogenome of *U. yanonensis* exhibited the
rearrangement of three cytochrome c oxidase subunit genes (COX1, COX2, COX3), two NADH dehydrogenase subunit genes (ND2 and ND3) and many tRNA genes. Despite of the multiple tRNA gene rearrangements, the mitogenome of *P. citri* also had a reversal of the ancestral gene cluster COX1- COX2-ATP8-ATP6- COX3- ND3. The mitogenome of *C. rubens* showed fewer rearrangements than *U. yanonensis* and *P. citri*, including two PCGs (ND2 and ATP8) and multiple tRNA genes.

The CREx analysis predicted the alternative scenarios how the three scale insect mitogenomes rearranged from the ancestral type of mitogenome of *D. yakuba* (Fig. 3, 4, 5). The mitochondrial gene order of *U. yanonensis* changed from *D. yakuba* by nine steps of rearrangement events, including the transposition of trnV and rrnS, the subsequent reverse transposition of trnK and trnD, the reversal of trnS1, and additional three reversal events and three tandem duplication and random loss (TDRL) events (Fig. 3). In *P. citri*, the first step is the reversal of trnK, followed by two alternative scenarios: the first one contained two reversal events, one TDRL event and one transposition event; the second one included three reversal events, two TDRL events and one transposition event (Fig. 4). Fewer rearrangement events were predicted in *C. rubens*, including the first step of transposition, the subsequent three reversal events, and final three TDRL events (Fig. 5). Considering the similarly rearranged mitochondrial genes of *C. japonicus* and *S. coffeae*, extensive mitochondrial rearrangement events are expected to occur very frequently in other unsequenced scale insects.

### Protein-coding genes

The 13 PCGs of *U. yanonensis* were similar in size to those of *P. citri*, without truncated or duplicated PCGs (Table 2, Table 3). However, most PCGs of *C. rubens* were shorter than *U. yanonensis* and *P. citri*, especially for ATP8 and ND6 (Fig. 6). Most PCGs of the three mitogenomes utilized the standard ATN start codon (ATA, ATT, ATC and ATG). However, the special start codon TTG was used by COX1 of *U. yanonensis* (Table 2). Twelve PCGs of each mitogenome had the complete termination codon TAN (TAA or TAG), whereas ND6 of *U. yanonensis* and *P. citri* and COX2 of *C. rubens* ended with an incomplete stop codon T. In the previously sequenced scale insect, *C. japonicus*, COX2 also ended with an incomplete T (*Deng et al., 2019*). The relative synonymous codon usage (RSCU) values were calculated for the three mitogenomes (Fig. 7). In *U. yanonensis*, the most frequently used codon was TTA (Leu) whereas
CTG(Leu), TCC(Ser), ACC(Thr), ACG(Thr), GCC(Ala), CAG(Gln), TGC(Cys), CGG(Arg) and AGC(Ser) were not used. In *P. citri*, the mostly used codon was also TTA (Leu), but CTC (Leu), AGC (Ser) and CGC (Arg) were the least. In *C. rubens*, TTA (Leu) was the most frequently used codon.

To evaluate the evolutionary rates of the PCGS, the average ratio of Ka/Ks was calculated for each PCG of the three mitogenomes (Fig. 8). The results showed that *ND4L* had the highest evolutionary rate, followed by *ATP8* and *ND5*, while *COX1* and *CYTB* appeared to be the lowest. The ratios of Ka/Ks were above 1 for most PCGs except for *COX1* and *CYTB*, suggesting that these genes are evolving under positive selection. However, the ratios of Ka/Ks for *COX1* and *CYTB* were below 1, indicating the purifying selection in these genes. The two genes, *COX1* and *CYTB* which with relatively slow evolutionary rates have already been used as efficient phylogenetic markers in insects.

**Transfer RNA genes**

The typical set of 22 tRNA genes were all detected in the mitogenome of *U. yanonensis*, but *trnV* was absent from the mitogenome of *P. citri* (Fig. 9, 10). In *C. rubens*, only 17 tRNA genes were recognized and the three tRNA genes *trnA*, *trnQ* and *trnW* were manually predicted (Fig. 11). Length and A+T content of the tRNA genes were subequal between *U. yanonensis* and *P. citri*, whereas the lengths of tRNA genes of *C. rubens* were generally shorter than *U. yanonensis* and *P. citri*. Individual tRNA gene of the three mitogenomes ranged in size from 49 to 75 bp; the longest tRNA gene was *trnA* in *P. citri* (Table 3); the shortest tRNA gene was *trnY* in *C. rubens* (Table 4). In the mitogenomes of *U. yanonensis* and *P. citri*, most of the tRNA genes could fold into cloverleaf secondary structures, but the dihydouridine (DHU) arms of *trnR* and *trnS1* were consistently lost. In *C. rubens*, most tRNA genes exhibited reduced DHU arms or TΨC arms. Such reductions of DHU arms were also reported in the tRNA genes of *S. coffeae* (*Lu et al., 2020*), suggesting that tRNA gene reduction could be a very common phenomenon in the mitogenomes of scale insects. The anticodons of the tRNA genes were identical among the three scale insects. In the tRNA genes of *U. yanonensis* and *P. citri*, a total of 12 and 19 mismatched base pairs were respectively identified and all of them were G-U pairs. In *C. rubens*, only four mismatched G-U pairs were identified.
Ribosomal RNA genes

There were two rRNA genes identified in each mitogenome. The length and A+T content of each rRNA gene were subequal between *U. yanonensis* and *P. citri*, but the lengths of rRNA genes were much shorter in *C. rubens* (Table 2, Table 3, Table 4). In *U. yanonensis*, the large ribosomal RNA (*rrnL*) gene was 1314 bp with an A+T content of 89.6%; the small ribosomal RNA (*rrnS*) gene was 807 bp with a high A+T content of 90.6%. In *P. citri*, the *rrnL* gene was 1396 bp with an A+T content of 86.9%; the *rrnS* gene was 856 bp with an A+T content of 88.4%. In *C. rubens*, the *rrnL* gene was 1263 bp with a high A+T content of 90.7%; the *rrnS* gene was 587 bp with an A+T content of 87.9%. Locations of the two rRNA genes were similar to *D. yakuba*, being neighbored with the CYTB-ND1 PCG cluster (Fig. 2). Instead of the commonly found *trnV* between the *rrnL* and *rrnS* genes in other insects, the intermediate tRNA gene between the two rRNA genes was *trnA* in *U. yanonensis*, *trnA* and *trnQ* in *P. citri*, and completely absent in *C. rubens*.

Control region

Control region (CR), also known as A+T rich region, was the longest and most variable non-coding area in the three mitogenomes (Fig. 12). The CR of *U. yanonensis* was short with only 260 bp, being located between *trnY* and *ATP8* and with a relatively high A+T content of 81.9% (Table 2). The CR of *P. citri* was much longer than *U. yanonensis* (678 bp), being located between *trnM* and *trnI* and with an A+T content of 84.4% (Table 3). Two putative CRs were found in the mitogenome of *C. rubens*: the 830-bp long CR1 between *rrnS* and *trnF* and the 800-bp long CR2 between *trnF* and *trnM* (Table 4). A+T content of the two CRs was 85.4% and 88.4%, respectively, higher than *U. yanonensis* and *P. citri*. The CR of *C. japonicus* and *S. coffeae* was 507 bp and 1454 bp, respectively, indicating the highly variable length of CRs in scale insects (*Deng et al., 2019; Lu et al., 2020*).

The CR of *U. yanonensis* was composed of 2.9 copies of tandem repeats; the first two copies had a consensus size of 91 bp, whereas the third repeat was 78 bp in length. The CR of *P. citri* contained three types of secondary structures that might function in regulating the replication and transcription of the mitogenome, including 2.3 copies of 110-bp long tandem repeats, one 40-bp long poly-[TA]n stretch, and a 21-bp long stem-loop (SL) structure. The SL structure was initiated by a “TAA” motif and ended with a “GTA” motif. The longer tandem repeats and extra secondary
structures of *P. citri* resulted in the longer CR than that of *U. yanonensis*. The CR1 of *C. rubens* contained 3.6 copies of 33-bp long tandem repeats but had no SL structures. The CR2 of *C. rubens* included 5 copies of 24-bp long tandem repeats and a combined SL structure. The length, nucleotide composition, number and types of structural elements in CRs of the three mitogenomes were found highly variable, which implied that the scale insect mitogenomes were likely to be regulated in different ways during the mitogenomic replication and transcription processes.

**Discussion**

To test the reliability of the three sequenced mitogenomes and investigate the mitochondrial phylogenetic relationships within Coccoidea, nucleotide sequences of available scale insects were obtained from GenBank and used in the phylogenetic analyses (Table 1). The two phylogenetic trees using BI and ML analyses generated identical topological structures for Coccoidea (Fig. 13). The three families of Coccoidea were grouped together, suggesting the probable monophyly of Coccoidea as found in von Dohlen & Moran (1995), which used the small-subunit (18S) ribosomal DNA in the phylogenetic analysis. The monophyly of Coccidae was supported with high values, indicating the efficiency of mitogenome data in grouping members of the same family and partially supporting the correctness of the tree topologies. Pseudococcidae was recovered as the sister group of Diaspididae and the phylogenetic position of their combined clade was supported basal to Coccidae. However, in previous molecular and morphological studies (*Gullan & Cook, 2007; Cook et al., 2007; Hodgson & Hardy 2013*), Pseudococcidae was supported basal to Coccidae and Diaspididae. The insufficient mitogenome data of Coccoidea, and the selection of different taxa and different molecular markers in the phylogenetic analysis were very likely to cause different phylogenetic results especially for the family levels (*Chen et al., 2018*). The new mitogenome data obtained in this study provided a basis for the accurate reconstruction of mitochondrial phylogeny in Coccoidea. The sequencing of more scale insects in future can also provide new data for our understanding of the highly rearranged mitogenomes and evolutionary history of these enigmatic insects. Sufficient representatives and molecular data will furtherer resolve the inner relationship of Coccoidea.
Conclusions

The complete mitochondrial genomes of *U. yanonensis*, *P. citri* and *C. rubens* were sequenced and analyzed. The mitochondrial genes of the three scale insects were highly rearranged and different from other scale insects. The phylogenetic reconstructions with BI and ML methods generated identical phylogenetic topology and supported the inner relationship of Coccoidea as Coccidae + (Pseudococcidae + Diaspididae). More mitogenomes should be obtained in future works to resolve the phylogeny of scale insects.

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References

Bernt M. 2007. CREx: inferring genomic rearrangements using common intervals. *Bioinformatics* 23: 2957–2958.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution* 69:313–319.

Clary DO, Wolstenholme DR. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. *Journal of Molecular Evolution* 22(3):252–271.

Cook LG, Gullan PJ, Trueman HE. 2002. A preliminary phylogeny of the scale insects (Hemiptera: Sternorrhyncha: Coccoidea) based on nuclear small-subunit ribosomal DNA. *Molecular Phylogenetics and Evolution* 25(1):43–52.
Cameron SL. 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annual Review of Entomology* **59**:95–117.

Chen ZT, Zhao MY, Xu C, Du YZ. 2018. Molecular phylogeny of Systellognatha (Plecoptera: Arctoperlaria) inferred from mitochondrial genome sequences. *International Journal of Biological Macromolecules* **111**:542–547.

Deng J, Lu C, Huang X. 2019. The first mitochondrial genome of scale insects (Hemiptera: Coccoidea). *Mitochondrial DNA Part B* **4**(2), 2094–2095.

Green EE. 1921. Observations on British Coccidae: with descriptions of new species. VII. *Entomologist's Monthly Magazine* **57**:257–259.

Gullan PJ, Cook LG. 2007. Phylogeny and higher classification of the scale insects (Hemiptera: Sternorrhyncha: Coccoidea). *Zootaxa* **1668**(1):413–425.

Gullan PJ, Kosztarab M. 1997. Adaptations in scale insects. *Annual Review of Entomology* **42**:23–50.

Gullan PJ, Martin JH. 2003. Sternorrhyncha (jumping plant-lice, whiteflies, aphids and scale insects). In: Resh VH, Cardé RT, eds. *Encyclopedia of Insects*. Amsterdam: Academic Press, 1079–1089.

Hodgson CJ, Hardy NB. 2013. The phylogeny of the superfamily Coccoidea (Hemiptera: Sternorrhyncha) based on the morphology of extant and extinct macropterous males. *Systematic Entomology* **38**(4):794–804.

Kuwana I. 1923. Descriptions and biology of new or little known coccids from Japan. *Department of Agriculture and Commerce, Imperial Plant Quarantine Station Bulletin* **3**:1–67.

Koteja J. 1974. Comparative studies on the labium in the Coccinea (Homoptera). *Zeszyty Naukowe Akademii Rolniczej w Warszawie. Rozprawy Naukowe* **89**:1–162.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**:772–780.

Kondo T, Gullan PJ, Williams DJ. 2008. Coccidology. The study of scale insects (Hemiptera: Sternorrhyncha: Coccoidea). *Ciencia y Tecnología Agropecuaria* **9**(2): 55–61.
Lu C, Huang X, Deng J. 2020. The challenge of Coccidae (Hemiptera: Coccoidea) mitochondrial genomes: The case of Saissetia coffeae with novel truncated tRNAs and gene rearrangements. *International Journal of Biological Macromolecules* 158:854–864.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34:772–773.

Luo RB, Liu BH, Xie YL, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW, Wang J. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *GigaScience* 1:1–18.

Maskell WM. 1893. Further coccid notes: with descriptions of new species from Australia, India, Sandwich Islands, Demerara, and South Pacific. *Transactions and proceedings of the New Zealand Institute* 25(26):201–252.

Perna NT, Kocher TD. 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *Journal of Molecular Evolution* 41:353–358.

Risso A. 1813. Mémoire sur l’histoire naturelle des oranges, bigaradiers, limettiers, cédratiers limoniers ou citroniers, cultivés dans le département des alpes maritimes. *Annales du Muséum National d’Histoire Naturelle* 20:401–431.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and postanalysis of large phylogenies. *Bioinformatics* 30:1312–1313.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729.
von Dohlen CD, Moran NA. 1995. Molecular phylogeny of the Homoptera: a paraphyletic taxon. *Journal of Molecular Evolution* 41(2):211–223.

Walker F. 1852. *List of the specimens of homopterous insects in the collection of the British Museum, Part IV*. London: British Museum (Natural History).
Figure Captions

Fig. 1. Mitochondrial maps of Unaspis yanonensis, Planococcus citri and Ceroplastes rubens.
A: Unaspis yanonensis; B: Planococcus citri; C: Ceroplastes rubens. Genes outside the map are transcribed clockwise, whereas those inside the map are transcribed counterclockwise. The inside circles show the GC content and the GC skew. GC content and GC skew are plotted as the deviation from the average value of the entire sequence.

Fig. 2. Gene arrangements of Unaspis yanonensis, Planococcus citri and Ceroplastes rubens in comparison with Drosophila yakuba. A: Unaspis yanonensis; B: Planococcus citri; C: Ceroplastes rubens. Conserved gene arrangements are covered in grey areas.

Fig. 3. Reconstruction of mitochondrial gene rearrangement scenarios in the evolution of Unaspis yanonensis. The tRNA genes are represented by the amino acid abbreviations.

Fig. 4. Reconstruction of mitochondrial gene rearrangement scenarios in the evolution of Planococcus citri. The tRNA genes are represented by the amino acid abbreviations.

Fig. 5. Reconstruction of mitochondrial gene rearrangement scenarios in the evolution of Ceroplastes rubens. The tRNA genes are represented by the amino acid abbreviations.

Fig. 6. Comparison of the length for each PCG and rRNA gene in Unaspis yanonensis, Planococcus citri and Ceroplastes rubens.

Fig. 7. Relative synonymous codon usage (RSCU) of PCGs in Unaspis yanonensis, Planococcus citri and Ceroplastes rubens. A: Unaspis yanonensis; B: Planococcus citri; C: Ceroplastes rubens. Full codon families are indicated below the X-axis.
Fig. 8. Average evolutionary rates of PCGs in *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens*. The bar indicates each gene’s Ka/Ks value.

Fig. 9. Secondary structures of tRNA genes in the mitogenome of *Unaspis yanonensis*. A: trnA (Alanine); B: trnN (Asparagine); C: trnD (Aspartic acid); D: trnR (Arginine); E: trnC (Cystine); F: trnQ (Glutamine); G: trnE (Glutamic acid); H: trnG (Glycine); I: trnH (Histidine); J: trnI (Isoleucine); K: trnL1(CUN) (Leucine); L: trnL2(UUR) (Leucine); M: trnK (Lysine); N: trnM (Methionine); O: trnF (Phenylalanine); P: trnP (Proline); Q: trnS1(AGN) (Serine); R: trnS2(UCN) (Serine); S: trnT (Threonine); T: trnW (Tryptophan); U: trnY (Tyrosine); V: trnV (Valine). The tRNA genes are labelled with their corresponding amino acids.

Fig. 10. Secondary structures of tRNA genes in the mitogenome of *Planococcus citri*. A: trnA (Alanine); B: trnN (Asparagine); C: trnD (Aspartic acid); D: trnR (Arginine); E: trnC (Cystine); F: trnQ (Glutamine); G: trnE (Glutamic acid); H: trnG (Glycine); I: trnH (Histidine); J: trnI (Isoleucine); K: trnL1(CUN) (Leucine); L: trnL2(UUR) (Leucine); M: trnK (Lysine); N: trnM (Methionine); O: trnF (Phenylalanine); P: trnP (Proline); Q: trnS1(AGN) (Serine); R: trnS2(UCN) (Serine); S: trnT (Threonine); T: trnW (Tryptophan); U: trnY (Tyrosine). The tRNA genes are labelled with their corresponding amino acids.

Fig. 11. Secondary structures of tRNA genes in the mitogenome of *Ceroplastes rubens*. A: trnA (Alanine); B: trnN (Asparagine); C: trnD (Aspartic acid); D: trnE (Glutamic acid); E: trnQ (Glutamine); F: trnG (Glycine); G: trnH (Histidine); H: trnI (Isoleucine); I: trnL2(UUR) (Leucine); J: trnK (Lysine); K: trnM (Methionine); L: trnF (Phenylalanine); M: trnP (Proline); N: trnS1(AGN) (Serine); O: trnT (Threonine); P: trnW (Tryptophan); Q: trnY (Tyrosine). The tRNA genes are labelled with their corresponding amino acids.
Fig. 12. Predicted structural elements in the control regions of *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens*. Tandem repeat units are indicated by orange boxes. Poly-[TA]n stretch is indicated with purple ellipse. Stem-loop structure is indicated by its shape and base pairs.

Fig. 13. Phylogenetic relationships within Coccoidea inferred by Bayesian inference (left) and maximum likelihood analysis (right). Numbers at the nodes are posterior probabilities (left) and bootstrap values (right). The family names are listed after the species.
Figure 1

Mitochondrial maps of *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens*.

A: *Unaspis yanonensis*; B: *Planococcus citri*; C: *Ceroplastes rubens*. Genes outside the map are transcribed clockwise, whereas those inside the map are transcribed counterclockwise. The inside circles show the GC content and the GC skew. GC content and GC skew are plotted as the deviation from the average value of the entire sequence.
Manuscript to be reviewed

Unaspis yanonensis
15,220 bp

Planococcus citri
15,549 bp

Cerothoas rubens
15,345 bp
Figure 2

Gene arrangements of *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens* in comparison with *Drosophila yakuba*.

A: *Unaspis yanonensis*; B: *Planococcus citri*; C: *Ceroplastes rubens*. Conserved gene arrangements are covered in grey areas.
Figure 3

Reconstruction of mitochondrial gene rearrangement scenarios in the evolution of *Unaspis yanonensis*.

The tRNA genes are represented by the amino acid abbreviations.
**Drosophila yakuba -- Unaspis yanonensis**

- Family diagram for Drosophila yakuba
  - **Scenario:**
    - Transposition
      - Scenarios 1 and 2
    - Reverse transposition
    - Reversal
    - Combination scenarios
- Alternative scenario 1 of 2
  - Reversal
  - Translocation
  - Alternative scenario 2 of 2

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**Notes:**

- PeerJ reviewing PDF | (2020:07:50601:1:2:NEW 11 Aug 2020)
- Manuscript to be reviewed
Figure 4

Reconstruction of mitochondrial gene rearrangement scenarios in the evolution of *Planococcus citri*.

The tRNA genes are represented by the amino acid abbreviations.
Figure 5

Reconstruction of mitochondrial gene rearrangement scenarios in the evolution of *Ceroplastes rubens*.

The tRNA genes are represented by the amino acid abbreviations.
Figure 6

Comparison of the length for each PCG and rRNA gene in *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens*.
Figure 7

Relative synonymous codon usage (RSCU) of PCGs in *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens*.

A: *Unaspis yanonensis*; B: *Planococcus citri*; C: *Ceroplastes rubens*. Full codon families are indicated below the X-axis.
Figure 8

Average evolutionary rates of PCGs in *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens*.

The bar indicates each gene’s Ka/Ks value.
Figure 9

Secondary structures of tRNA genes in the mitogenome of *Unaspis yanonensis*.

A: trnA (Alanine); B: trnN (Asparagine); C: trnD (Aspartic acid); D: trnR (Arginine); E: trnC (Cystine); F: trnQ (Glutamine); G: trnE (Glutamic acid); H: trnG (Glycine); I: trnH (Histidine); J: trnI (Isoleucine); K: trnL1(CUN) (Leucine); L: trnL2(UUR) (Leucine); M: trnK (Lysine); N: trnM (Methionine); O: trnF (Phenylalanine); P: trnP (Proline); Q: trnS1(AGN) (Serine); R: trnS2(UCN) (Serine); S: trnT (Threonine); T: trnW (Tryptophan); U: trnY (Tyrosine); V: trnV (Valine). The tRNA genes are labelled with their corresponding amino acids.
| A | B | C | D | E |
|---|---|---|---|---|
| Alanine (A) | Asparagine (N) | Aspartic (D) | Arginine (R) | Cysteine (C) |
| F | G | H | I | J |
| Glutamine (Q) | Glutamic (E) | Glycine (G) | Histidine (H) | Isoleucine (I) |
| K | L | M | N | O |
| Leucine (L1, CUN) | Leucine (L2, UUR) | Lysine (K) | Methionine (M) | Phenylalanine (F) |
| P | Q | R | S | T |
| Proline (P) | Serine (S1, AGN) | Serine (S2, UCN) | Threonine (T) | Tryptophane (W) |
| U | V |
| Tyrosine (Y) | Valine (V) |

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Diagram labels:
- Discriminator nucleotide
- Amino acid acceptor (AA) arm
- Variable (V) loop
- Antidrome (AC) arm
- Dihydouridine (DHU) arm
Figure 10

Secondary structures of tRNA genes in the mitogenome of *Planococcus citri*.

A: trnA (Alanine); B: trnN (Asparagine); C: trnD (Aspartic acid); D: trnR (Arginine); E: trnC (Cystine); F: trnQ (Glutamine); G: trnE (Glutamic acid); H: trnG (Glycine); I: trnH (Histidine); J: trnI (Isoleucine); K: trnL1(CUN) (Leucine); L: trnL2(UUR) (Leucine); M: trnK (Lysine); N: trnM (Methionine); O: trnF (Phenylalanine); P: trnP (Proline); Q: trnS1(AGN) (Serine); R: trnS2(UCN) (Serine); S: trnT (Threonine); T: trnW (Tryptophan); U: trnY (Tyrosine). The tRNA genes are labelled with their corresponding amino acids.
Figure 11

Secondary structures of tRNA genes in the mitogenome of *Ceroplastes rubens*.

A: trnA (Alanine); B: trnN (Asparagine); C: trnD (Aspartic acid); D: trnE (Glutamic acid); E: trnQ (Glutamine); F: trnG (Glycine); G: trnH (Histidine); H: trnI (Isoleucine); I: trnL2(UUR) (Leucine); J: trnK (Lysine); K: trnM (Methionine); L: trnF (Phenylalanine); M: trnP (Proline); N: trnS1(AGN) (Serine); O: trnT (Threonine); P: trnW (Tryptophan); Q: trnY (Tyrosine). The tRNA genes are labelled with their corresponding amino acids.
Figure 12

Predicted structural elements in the control regions of *Unaspis yanonensis*, *Planococcus citri* and *Ceroplastes rubens*.

Tandem repeat units are indicated by orange boxes. Poly-[TA]n stretch is indicated with purple ellipse. Stem-loop structure is indicated by its shape and base pairs.
A. Tandem repeats in *Unaspis yanonensis*

- 91 bp
- 91 bp
- 78 bp

B. Tandem repeats in *Planococcus citri*

- 110 bp
- 110 bp
- 35 bp
- Poly-TA
- 40 bp
- Stem-loop
- 21 bp

C. Tandem repeats in *Ceroplastes rubens CR1*

- 33 bp
- 33 bp
- 33 bp
- 22 bp

D. Tandem repeats in *Ceroplastes rubens CR2*

- 24 bp
- 24 bp
- 24 bp
- 24 bp
- 24 bp
Figure 13

Phylogenetic relationships within Coccoidea inferred by Bayesian inference (left) and maximum likelihood analysis (right).

Numbers at the nodes are posterior probabilities (left) and bootstrap values (right). The family names are listed after the species.
Table 1 (on next page)

Species of Hemiptera used in this study.
**Table 1:**

Species of Hemiptera used in this study.

| Superfamily | Family      | Species              | Accession number |
|-------------|-------------|----------------------|------------------|
| Coccoidea   | Coccidae    | *Ceroplastes japonicus* | MK847519         |
|             |             | *Ceroplastes rubens*  | MT677923         |
|             | Diaspididae | *Unaspis yanonensis*  | MT611525         |
|             | Pseudococcida | *Planococcus citri*  | MT611526         |
| Aphidoidea  | Aphididae   | *Aphis glycines*      | MK111111         |
|             |             | *Diuraphis noxia*     | KF636758         |
Table 2 (on next page)

Mitochondrial genome structure of *Unaspis yanonensis*. 
| Gene               | Position (bp) | Size (bp) | Direction | Intergenic nucleotides | Anti- or start/stop codons | A+T% |
|-------------------|---------------|-----------|-----------|-------------------------|------------------------------|------|
| Control region    | 1–78          | 260       | +         | 78                      | –                            | 81.9 |
| ATP8              | 261–428       | 168       | +         | 0                       | ATT/TAA                      | 93.5 |
| ATP6              | 429–1121      | 693       | +         | 0                       | ATT/TAA                      | 86.0 |
| trnL2 (UUR)       | 1127–1193     | 67        | +         | 5                       | TAA                          | 85.1 |
| trnMet (M)        | 1196–1260     | 65        | +         | 2                       | CAT                          | 89.2 |
| trnVal (V)        | 1261–1326     | 66        | +         | 0                       | TAC                          | 90.9 |
| rrnS              | 1327–2133     | 807       | +         | 0                       | –                            | 90.6 |
| trnAla (A)        | 2134–2202     | 69        | +         | 0                       | TGC                          | 72.5 |
| trnLeu1 (CUN)     | 2203–3516     | 1314      | +         | 0                       | –                            | 89.6 |
| trnSer2 (UCN)     | 3517–3581     | 65        | +         | 0                       | TGA                          | 84.5 |
| CYTB              | 4587–5759     | 1173      | –         | 2                       | ATA/TAA                      | 81.8 |
| ND6               | 5760–6300     | 541       | –         | 0                       | ATG/T–                       | 93.0 |
| trnPro (P)        | 6306–6373     | 68        | +         | 5                       | TGG                          | 91.2 |
| trnThr (T)        | 6374–6437     | 64        | –         | 0                       | TGT                          | 95.3 |
| ND4L              | 6441–6728     | 288       | +         | 3                       | ATT/TAA                      | 89.9 |
| ND4               | 6731–8062     | 1332      | +         | 2                       | ATT/TAA                      | 87.8 |
| trnHis (H)        | 8062–8121     | 60        | +         | –1                      | GTG                          | 93.3 |
| ND5               | 8131–9810     | 1680      | +         | 9                       | ATA/TAA                      | 88.2 |
| trnPhe (F)        | 9818–9882     | 65        | +         | 7                       | GAA                          | 95.4 |
| trnGlu (E)        | 9890–9957     | 68        | –         | 7                       | TTC                          | 95.6 |
| COXI              | 9959–11515    | 1557      | +         | 1                       | TTG/TAA                      | 78.4 |
| COX3              | 11554–12288   | 735       | +         | 38                      | ATT/TAA                      | 82.3 |
| trnGln (Q)        | 12339–12407   | 69        | –         | 50                      | TTG                          | 89.9 |
| trnGly (G)        | 12417–12479   | 63        | +         | 9                       | TCC                          | 88.9 |
| ND3               | 12480–12830   | 351       | +         | 0                       | ATT/TAA                      | 88.3 |
| trnArg (R)        | 12831–12883   | 53        | +         | 0                       | TCG                          | 86.8 |
| trnCys (C)        | 12885–12954   | 70        | +         | 1                       | GCA                          | 94.3 |
| trnSer1 (AGN)     | 12956–13014   | 59        | +         | 1                       | GCT                          | 89.8 |
| trnAsn (N)        | 13013–13080   | 68        | –         | –2                      | GTT                          | 83.8 |
| ND2               | 13082–14107   | 1026      | +         | 1                       | ATT/TAA                      | 92.1 |
| COX2              | 14104–14793   | 690       | –         | –4                      | ATT/TAA                      | 83.6 |
| trnIle (I)        | 14795–14861   | 67        | –         | 1                       | GAT                          | 83.6 |
| trnTrp (W)        | 14862–14928   | 67        | +         | 0                       | TCA                          | 94.0 |
| trnLys (K)        | 14929–14997   | 69        | +         | 0                       | CTT                          | 91.3 |
| trnAsp (D)        | 15001–15069   | 69        | –         | 3                       | GTC                          | 92.8 |
| trnTyr (Y)        | 15074–15142   | 69        | –         | 4                       | GTA                          | 85.5 |
Table 3 (on next page)

Mitochondrial genome structure of *Planococcus citri*.
| Gene           | Position (bp) | Size (bp) | Direction | Intergenic nucleotides | Anti- or start/stop codons | A+T% |
|----------------|---------------|-----------|-----------|------------------------|----------------------------|------|
| trnIle (I)     | 1–70          | 70        | –         | 0                      | GAT                        | 84.3 |
| ND2            | 76–1089       | 1014      | +         | 5                      | ATT/TAA                    | 87.4 |
| trnTrp (W)     | 1088–1156     | 69        | +         | –2                     | TCA                        | 89.9 |
| trnTyr (Y)     | 1167–1232     | 66        | –         | 10                     | GTA                        | 84.8 |
| trnAsn (N)     | 1232–1295     | 64        | +         | –1                     | GTT                        | 84.4 |
| trnSer1 (AGN)  | 1295–1359     | 65        | +         | –1                     | GCT                        | 80.0 |
| trnCys (C)     | 1368–1432     | 65        | +         | 8                      | GCA                        | 92.3 |
| trnArg (R)     | 1434–1497     | 64        | –         | 1                      | TCG                        | 79.7 |
| ND3            | 1504–1854     | 351       | –         | 6                      | ATT/TAA                    | 84.3 |
| trnGly (G)     | 1855–1918     | 64        | –         | 0                      | TCC                        | 92.2 |
| COX3           | 1928–2716     | 789       | –         | 9                      | ATG/TAA                    | 76.6 |
| ATP6           | 2721–3395     | 675       | –         | 4                      | ATG/TAA                    | 80.1 |
| ATP8           | 3389–3550     | 162       | –         | –7                     | ATT/TAA                    | 85.8 |
| trnAsp (D)     | 3551–3616     | 66        | –         | 0                      | GTC                        | 90.9 |
| trnLys (K)     | 3629–3695     | 67        | +         | 12                     | CTT                        | 86.6 |
| COX2           | 3700–4380     | 681       | –         | 4                      | ATT/TAA                    | 78.6 |
| trnLeu2 (UUR)  | 4384–4451     | 68        | –         | 3                      | TAA                        | 85.3 |
| COXI           | 4460–5989     | 1530      | –         | 8                      | ATA/TAA                    | 74.4 |
| trnGlu (E)     | 5991–6057     | 67        | +         | 1                      | TTC                        | 94.0 |
| trnPhe (F)     | 6057–6124     | 68        | –         | –1                     | GAA                        | 94.1 |
| ND5            | 6130–7803     | 1674      | –         | 5                      | ATT/TAA                    | 84.3 |
| trnHis (H)     | 7822–7886     | 65        | –         | 18                     | GTG                        | 84.6 |
| ND4            | 7889–9199     | 1311      | –         | 2                      | ATA/TAA                    | 83.5 |
| ND4L           | 9220–9507     | 288       | –         | 20                     | ATT/TAA                    | 86.8 |
| trnThr (T)     | 9510–9575     | 66        | +         | 2                      | TGT                        | 90.9 |
| trnPro (P)     | 9575–9641     | 67        | –         | –1                     | TGG                        | 83.6 |
| ND6            | 9645–10212    | 568       | +         | 3                      | ATG/T–                     | 86.6 |
| CYTB           | 10210–11349   | 1140      | +         | –3                     | ATT/TAA                    | 77.3 |
| trnSer2 (UCN)  | 11348–11412   | 65        | +         | –2                     | TGA                        | 81.5 |
| ND1            | 11395–12333   | 939       | –         | –18                    | ATA/TAA                    | 80.2 |
| trnLeu1 (CUN)  | 12334–12402   | 69        | –         | 0                      | TAG                        | 87.0 |
| rrnL           | 12403–13798   | 1396      | –         | 0                      | –                          | 86.9 |
| trnAla (A)     | 13799–13873   | 75        | –         | 0                      | TGC                        | 84.0 |
| trnGln (Q)     | 13879–13946   | 68        | +         | 5                      | TTG                        | 91.2 |
| rrnS           | 13947–14802   | 856       | –         | 0                      | –                          | 88.4 |
| trnMet (M)     | 14803–14871   | 69        | –         | 0                      | CAT                        | 84.1 |
| Control region | 14872–15549   | 678       | +         | 0                      | –                          | 84.4 |
Table 4 (on next page)

Mitochondrial genome structure of *Ceroplastes rubens*.
Table 4:
Mitochondrial genome structure of *Ceroplastes rubens*.

| Gene          | Position (bp) | Size (bp) | Direction | Intergenic nucleotides | Anti- or start/stop codons | A+T% |
|---------------|---------------|-----------|-----------|------------------------|-----------------------------|------|
| COX1          | 1–1527        | 1527      | +         | 42                     | ATA/TAA                     | 80.4 |
| trnLeu2 (UUR) | 1532–1600     | 69        | +         | 4                      | TAA                         | 88.4 |
| COX2          | 1601–2261     | 661       | +         | 0                      | ATA/T–                       | 83.4 |
| trnLys (K)    | 2262–2328     | 67        | +         | 0                      | CTT                         | 83.6 |
| trnAsp (D)    | 2325–2383     | 59        | +         | –4                     | GTC                         | 93.2 |
| ATP6          | 2411–3091     | 681       | –         | 27                     | ATA/TAA                     | 89.7 |
| COX3          | 3118–3891     | 774       | +         | 26                     | ATA/TAA                     | 86.3 |
| trnGly (G)    | 3894–3950     | 57        | +         | 2                      | TCC                         | 94.7 |
| ND3           | 3951–4286     | 336       | +         | 0                      | ATA/TAA                     | 90.8 |
| trnAla (A)    | 4291–4350     | 60        | –         | 4                      | TGC                         | 91.7 |
| trnAsn (N)    | 4370–4424     | 55        | +         | 83                     | GTT                         | 87.3 |
| trnSer1 (AGN) | 4424–4469     | 46        | +         | –1                     | GCT                         | 80.4 |
| trnGlu (E)    | 4469–4522     | 54        | +         | –1                     | TTC                         | 94.4 |
| trnTrp (W)    | 4527–4577     | 51        | +         | 4                      | TCA                         | 94.1 |
| ND5           | 4579–6189     | 1611      | –         | 56                     | ATT/TAA                     | 88.3 |
| trnHis (H)    | 6264–6320     | 57        | –         | 74                     | GTG                         | 89.5 |
| ND4           | 6325–7605     | 1281      | –         | 4                      | ATA/TAA                     | 89.4 |
| ND4L          | 7619–7963     | 345       | –         | 13                     | ATT/TAG                      | 92.2 |
| ND6           | 7980–8375     | 396       | +         | 16                     | ATA/TAA                     | 89.6 |
| trnPro (P)    | 8375–8433     | 59        | –         | –1                     | TGG                         | 89.8 |
| ATP8          | 8435–8524     | 90        | –         | 1                      | ATA/TAA                     | 90.0 |
| trnIle (I)    | 8546–8612     | 67        | +         | 21                     | GAT                         | 86.6 |
| ND2           | 8613–9551     | 939       | +         | 0                      | ATT/TAA                     | 91.5 |
| trnTyr (Y)    | 9558–9606     | 49        | –         | 6                      | GTA                         | 87.8 |
| trnThr (T)    | 9608–9659     | 52        | +         | 1                      | TGT                         | 90.4 |
| CYTB          | 9660–10736    | 1077      | +         | 0                      | ATC/TAA                     | 85.0 |
| trnGln (Q)    | 10745–10796   | 52        | –         | 8                      | TTG                         | 92.3 |
| ND1           | 10823–11728   | 906       | –         | 86                     | ATT/TAG                     | 86.5 |
| rrlL          | 11729–12991   | 1263      | –         | 0                      | –                           | 90.7 |
| rnsS          | 12992–13578   | 587       | –         | 0                      | –                           | 87.9 |
| Control region 1 | 13579–14408  | 830       | +         | 0                      | –                           | 85.4 |
| trnPhe (F)    | 14409–14476   | 68        | –         | 0                      | GAA                         | 79.4 |
| Control region 2 | 14477–15276   | 800       | +         | 0                      | –                           | 88.4 |
| trnMet (M)    | 15277–15345   | 69        | +         | 0                      | CAT                         | 82.6 |