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Article

# The first complete mitochondrial genome of *Cheyletus malaccensis* (Acari: Cheyletidae): gene rearrangement

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## Abstract

The predatory mite *Cheyletus malaccensis* (Acari: Cheyletidae), commonly occurring in stores of various food commodities, is an important natural enemy of stored product pests. Disentangling the mt genome sequence of *C. malaccensis* at molecular level can decrease uncertainties during morphological identification and is useful in reconstructing the phylogeny of Acariformes group. In this study, the complete mitogenome of *C. malaccensis* was sequenced by the next-generation sequencing. After assembly and annotation, we found the circular 14,732 bp mitogenome of *C. malaccensis*, containing 13 protein coding genes, 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes. Compared with the ancestral mitogenome organization of arthropods, most of tRNA were truncated without D-arm or/and TψC-arm. Rearrangement was found in 12 mitogenome genes. Phylogenetic analyses based on the mitogenome data from other 29 mite species were inferred by Bayesian and maximum likelihood methods, which strongly supported the closer relationship between *C. malaccensis* and Tetranychidae than other mites. The obtained results represent the first complete mitochondrial genome record for Cheyletidae group. It may help improve molecular phylogenetic relationship and population genetics of the Cheyletidae.

**Keywords:** *Cheyletus malaccensis*, complete mitochondrial genome, molecular phylogenetics

## Introduction

The predatory mite *Cheyletus* species, including *Cheyletus malaccensis* Oudemans (Acari: Cheyletidae), are currently widely distributed in food and feed commodity stores in Asia (Ardeshir 2017; Mariana *et al.* 2010), Europe (Stejskal *et al.* 2015) and North America (Sinha & Wallace1973). They feed on various pest phytophagous mites such as *Acarus siro* (Linnaeus), small insects such as book lice (e.g. *Liposcelis spp.*), and stored grain insect eggs and larvae (Cebolla *et al.* 2009; Hubert *et al.* 2006; Lukáš *et al.* 2007). *C. malaccensis* can develop through parthenogenesis or sexual reproduction and can bear a wide range of temperatures. Predatory activity of *C. malaccensis* was found to be compatible with another environmentally friendly pest control methods like diatomaceous earth (Palyvos *et al.* 2006) and antifeedants, such as amylase inhibitors (Hubert *et al.* 2007). These traits make *Cheyletus* species the promising natural enemies that can be widely employed to control stored products pests. However, there are significant differences among predatory and biological ability among various *Cheyletus* species. Therefore, a description of *Cheyletus* spp. at the molecular level is the prerequisite for their population genetic difference and understanding of their biological differences. Molecular understanding of these species may also contribute to accuracy and improvement of studies concerning higher phylogeny of mites and

 

arthropods. However, the currently available molecular data for *C. malaccensis* partially enables only its identification but they do not enable to compare geographical inter-population variability and comprehensive phylogeny analysis.

The mitochondrial (mt) genomes are increasingly used for animal species identification, population genetics and phylogeny inference presently (Chuan *et al.* 2012; Li *et al.* 2015; Nelson *et al.* 2012). It is proofed that the application of the mt genome to phylogenetic analysis has solved many problems ranging from species level to order level (Feng *et al.* 2019). The typical mt genome contains 13 protein-coding genes (PCGs), two ribosome RNA (rRNA) genes and 22 transger RNA (tRNA) genes (Boore 1999; Wolstenholme 1992). For *C. malaccensis*, the *cox1* and *rrnS* genes had been analyzed in 2016 (Yang *et al.* 2016), however, the mt genomes of Cheyletidae have not been sequenced. Species in Cheyletidae are difficult for identification according to morphological features. For example, *Cheyletus fortis* and *C. malaccensis* were formerly considered as two separate species when compared by morphological characteristics. However, recently these two species were confirmed to be the same species by molecular methods (Tian 2011). The species-specific primer to distinguish *C. eruditus* and *C. malaccensis* has also been designed (Wu *et al.* 2016), and has served well for accurate identification of both species in the laboratory practice.

The mt genome was suggested to constitute the base of molecular study regarding *C. malaccensis*. In this study we sequenced the mt genome of *C. malaccensis,* analyzed the data and genome organization, and constructed the phylogenetic tree with limited data. The general goal was to expand our understanding of *C. malaccensis* at the molecular level, and to promote the mitochondrial genome based phylogenetic study of predatory mites.

## Materials

*Sampling and DNA extraction*

Samples of *C. malaccensis* were collected from Haikou, China, then cultured in Academy of National Food and Strategic Reserves Administration in China. The samples were identified based on their morphology characteristics (Shen 1997). Genomic DNA was extracted from 50 adult mites using TIANamp Micro DNA Kit following the instruction. The UV-Vis Spectrophotometer Q5000 (Q5000, Quawell Technology, Inc. USA) was used to quantify the extracted DNA (23.76ug) for library construction.

*DNA Sequencing and mt genome assembly*

Total DNA of *C. malaccensis* was sent to Berry Genomics Company for library preparation, and finally sequenced on an Illumina sequencer. The insert size was 250bp with 150bp pair-end sequencing.

After getting the raw data, quality assessment was conducted using FastQCv0.11.7 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Trimmomatic v0.36 (Lohse *et al.* 2012) was used to the remove adapter sequences. The mt genome assembly strategy followed Feng *et al.* 2018. Briely, to reconstruct the mt genome, *cox1* gene fragments were chosen as "anchors". We sequenced the *cox1* gene fragments using universal primer pairs LCO1490–HCO2198 (Folmer *et al*. 1994). We applied "map to reference" strategy and mapped all cleaned reads to the "anchor" using Geneious (Kearse *et al*. 2012). Illumina sequence-reads were assembled using the sequence *cox1* as initial references. The contigs were then extended using the assembly parameters: (1) minimum overlap 50 bp, and (2) minimum similarity 99%, until the full circular mt chromosome sequences were obtained.

The PCR amplification of *cox1* gene was in 25 ul containing 12.5ul of 2×Taq Mix (Tiangen, Beijing, China), 1 ul of each primer (10 uM), 1ul genomic DNA and 9.5ul $ddH_2O$. PCR cycling

conditions were: 95°for 3 min, followed by 35 cycles of 94°for 1 min, 53°for 1 min, 72°for 1 min, and finally 72°for 10 min.

*Bioinformatic analyses*

The protein coding genes (PCGs), ribosome RNA (rRNA) genes and transfer RNA (tRNA) genes were identified using MITOS Web Server (http://mitos.bioinf.uni-leipzig.de/index.py) (Bent *et al.*, 2013). PCGs and RNA genes were confirmed by alignment against close species while tRNA genes were confirmed by ARWEN. During tRNA search, we alternatively searched tRNA genes based on the methods by Xue *et al.* (2018). The base composition was analyzed with muscle algothrim in MEGA 7.0 (Kumar *et al.*, 2016). Nucleotide compositional skew was measured using the following formula: AT skew = A-T/A+T and GC skew = G-C/G+C.

*Phylogenetic analysis*

To better understand molecular phylogeny of *C. malaccensis* in Arachnida, a total of 30 Arachnida species were used in phylogenetic analysis, including 29 representative stored mite species (Table S1). All 13 PCGs were aligned in MEGA 7.0. Two rRNA genes were aligned using MAFFT v7.0 online sever (Katoh *et al.* 2019). Then ambiguous positions in the alignment of PCGs and rRNA genes were removed by using Gblocks v0.91b web server (Castresana 2000). The concatenated phylogenetic trees were reconstructed based on the two datasets: 1) dataset "PCGsrRNA", in which there are concatenated 13 protein-coding genes (*atp6*, *atp8*, *cox1*, *cox2*, *cox3*, *cob*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4l*, *nad5*, *nad6*) and two rRNA genes (*rrnL* and *rrnS*); 2) dataset "PCGs", including only 13 protein-coding genes. The Bayesian methods (BI) and maximum likehood (ML) were used to reconstruct the phylogenetic tree. The ML method was performed with PhyML (Guindon *et al.* 2010) (http:/www.atgc-montpellier.fr/phyml/) and the online Smart Model Selection for optimal model selection. General time reversible (GTR) model was finally chosen. We also applied Bayesian method using MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003). The GTR+I+G model was used. The datasets were conducted with two simultaneous runs of 2 million generations, each with one cold and three heated chains. Samples were drawn every 1,000 Markov chain Monte Carlo (MCMC) steps, with the first 25% discarded as burn-in. The stationarity was considered reached and stopped run when the average standard deviation of split frequencies was below 0.01.

## Results and Discussion

*Mt genome organization and nucleotide composition*

The length of the mt genome of *C. malaccensis* is 14,732bp (Figure 1), including 13 PCGs, two rRNAs and 22 tRNAs (Table 1). The symmetric nucleotide compositions are A:47.3%, C:14.1%, G:6.8% and T:31.8%. The content of G is small and concentrated in *cox1* (10.5%) and *cox2* (8.7%) genes, which shows that these two genes are more conservative and stable. The AT skew is 0.196, which is similar with most AT skews of the arthropod animals (Dermauw *et al.* 2009; Sun *et al.* 2014a). Four PCG genes (*nad5, nad4, nad4l, nad1*), two rRNA genes and seven tRNA genes (*trnL1, F, P, Q, L2, Y, C*) of *C. malaccensis* were encoded by the minor strand and the other were encoded by the major strand. Two tRNA genes were contained by protein coding gene, *trnH* gene located in *nad5* and *trnV* was in 16S rRNA. In addition, *trnA* was in control region. Compared with the Arthropod ancestral pattern (Palopoli *et al.* 2014), the mt genome of *C. malaccensis* has different arrangements. Rearrangement was found in 12 genes, including 11 tRNA genes (*trnE*, *S1*, *N*, *M*, *Y*, *I*, *L2*, *Q*, *R*, *V*, *L1*) and 12S rRNA gene. Except *trnR* and *trnV* were in different location, *C. malaccensis* has the similar gene rearrangement with *Demodex brevis* and *D. folliculorum* (Palopoli *et al.* 2014), belonging to Cheyletoidea. To fully understand the mitochondrial arrangement of Cheyletoidea, and analyze its characteristics, more species need to be tested.

**TABLE 1** Annotation of the mitochondrial genome of *Cheyletus malaccensis*.

| Gene | Location | Length | Strand | Start codon | Stop codon | Anticodon | Intergenic length |
|---|---|---|---|---|---|---|---|
| *trnL1*(cta) | 1–55 | 55 | N | | | CUA | 0 |
| *rrnS* | 66–706 | 641 | N | | | | 10 |
| *trnF*(tta) | 716–772 | 57 | N | | | UUC | 9 |
| *nad5* | 720–2426 | 1707 | N | ATT | TAG | | -53 |
| *trnH*(cat) | 1765–1815 | 51 | J | | | CAT | / |
| *nad4* | 2474–3742 | 1269 | N | ATG | TAG | | 47 |
| *nad4l* | 4109–4384 | 276 | N | ATA | TAA | | 366 |
| *trnT*(aca) | 4377–4427 | 51 | J | | | ACA | -8 |
| *trnP*(cca) | 4429–4483 | 55 | N | | | CCA | 1 |
| *nad6* | 4487–4894 | 408 | J | ATG | TAA | | 3 |
| *cob* | 4899–6014 | 1116 | J | ATG | TAA | | 4 |
| *trnS2*(tca) | 5978–6024 | 47 | J | | | TCA | -37 |
| *nad1* | 6022–6900 | 879 | N | ATA | TAA | | -3 |
| *rrnL* | 6907–7923 | 1017 | N | | | | 6 |
| *trnV*(gtt) | 7022–7076 | 55 | J | | | GUU | / |
| *trnQ*(caa) | 7881–7935 | 55 | N | | | CAA | -43 |
| *trnL2*(tta) | 7930–7983 | 54 | N | | | UUA | -6 |
| *trnI*(atc) | 7987–8041 | 55 | J | | | AUC | 3 |
| *trnY*(tac) | 8044–8103 | 60 | N | | | UUA | 2 |
| *trnR*(cga) | 8118–8171 | 54 | J | | | CGA | 14 |
| *nad2* | 8163–9137 | 975 | J | ATA | TAA | | -9 |
| *trnW*(tga) | 9136–9202 | 67 | J | | | UGA | -2 |
| *trnM*(atg) | 9202–9266 | 65 | J | | | AUG | -1 |
| *trnN*(aac) | 9275–9335 | 61 | J | | | AAC | 8 |
| *trnS1*(agc) | 9336–9384 | 49 | J | | | AGC | 0 |
| *trnE*(gaa) | 9391–9442 | 52 | J | | | GAA | 6 |
| *trnC*(tgc) | 9470–9529 | 60 | N | | | TGC | 27 |
| *cox1* | 9547–11196 | 1650 | J | ATT | TAA | | 17 |
| cox2 | 11197–11841 | 645 | J | ATG | TAA | | 0 |
| *trnK*(ctt) | 11847–11908 | 62 | J | | | AAG | 5 |
| *trnD*(gac) | 11904–11953 | 50 | J | | | GAC | -5 |
| *atp8* | 11963–12097 | 135 | J | ATA | TAG | | 9 |
| *atp6* | 12094–12756 | 663 | J | ATA | TAA | | -4 |
| *cox3* | 12768–13571 | 804 | J | ATG | TAA | | 11 |
| *trnG*(gga) | 13550–13599 | 50 | J | | | GGA | -22 |
| *nad3* | 13598–13933 | 336 | J | ATA | TAA | | -2 |
| *CR* | 13934–14732 | 336 | J | | | | 0 |
| *trnA*(gcc) | 14309–14367 | 59 | J | | | GCC | / |

**Note:** N/J indicates that the gene was encoded by the minor/major strand, / indicates that tRNA gene was contained by protein coding gene or CR.

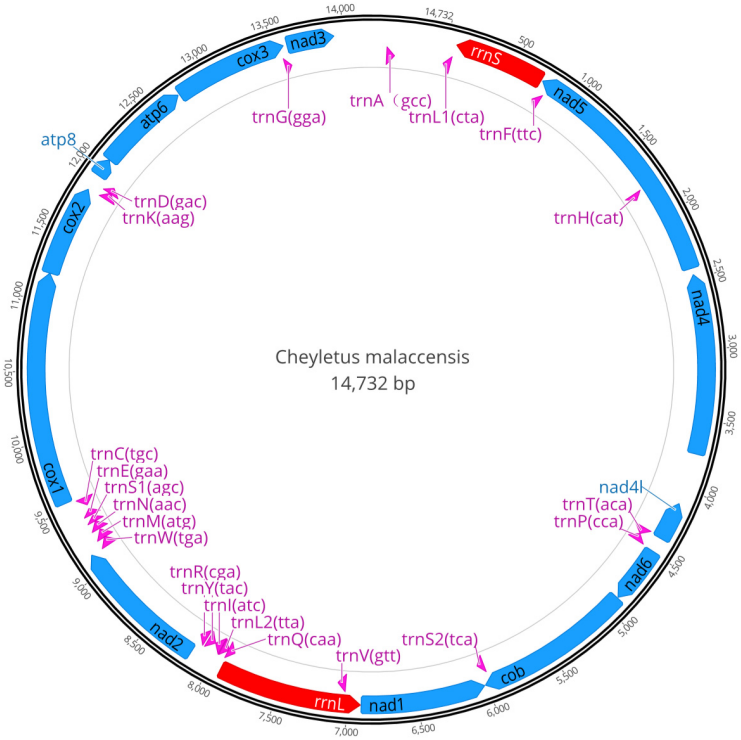


**FIGURE 1.** The mitochondrial genome arrangements of *Cheyletus malaccensis*.
**Note:** the blue is PCGs, the red is rRNA genes, the purple is tRNA genes.

The total length of intergenic sequences in *C. malaccensis* was 548 bp, including two intergenic sequences longer than 30 bp, 366 bp (between *nad4* gene and *nad4l* gene) and 47 bp (between *nad4* gene and *nad5* gene). The total length of *C. malaccensis* overlapping regions was 195 bp with three overlapping regions longer than 30 bp: 53 bp (between *trnF* gene and *nad5* gene), 43 bp (between *trnQ* gene and *trnV* gene) and 37 bp (between *trnS2* gene and *cob* gene), respectively.

*Protein coding genes*

The mt genome of *C. malaccensis* has three kinds of start codon (ATT, ATA, ATG) and two kinds of stop codon (TAA, TAG). ATT is the start codon of *cox1* and *nad5*, ATA is the start codon of *atp8, atp6, nad3, nad4l, nad1* and *nad2*. ATG is the start codon for other genes. TAG is the stop codon of *atp8, nad5* and *nad4*, while the other genes end with TAA.

*The tRNA and rRNA genes*

Twenty-two tRNA genes were found in the mt genome of *C. malaccensis*. All present tRNA genes were extremely truncated, ranging in size from 47 bp to 67 bp. Four tRNA (*trnM*, *W*, *N* and *K*) have complete cloverleaf secondary structures, most of predicted tRNA genes are folded into the atypical cloverleaf secondary structures with the absence of either D-arm or TψC-arm (Figure 2). As reported in some species in Arachnida, which also found the tRNA lost one arm either the D-arm or TψC-arm such as *Panonychus citri* (Yuan *et al.* 2010), *D. brevis* and *D. folliculorum* (Palopoli *et al.* 2014), *Caloglyphus berlesei* (Sun *et al*. 2014b) and *Tyrophagus longior* (Yang & Li 2015). It seems that lose arms is a normal situation in Arachnida and it does not affect mitochondrial protein synthesis.

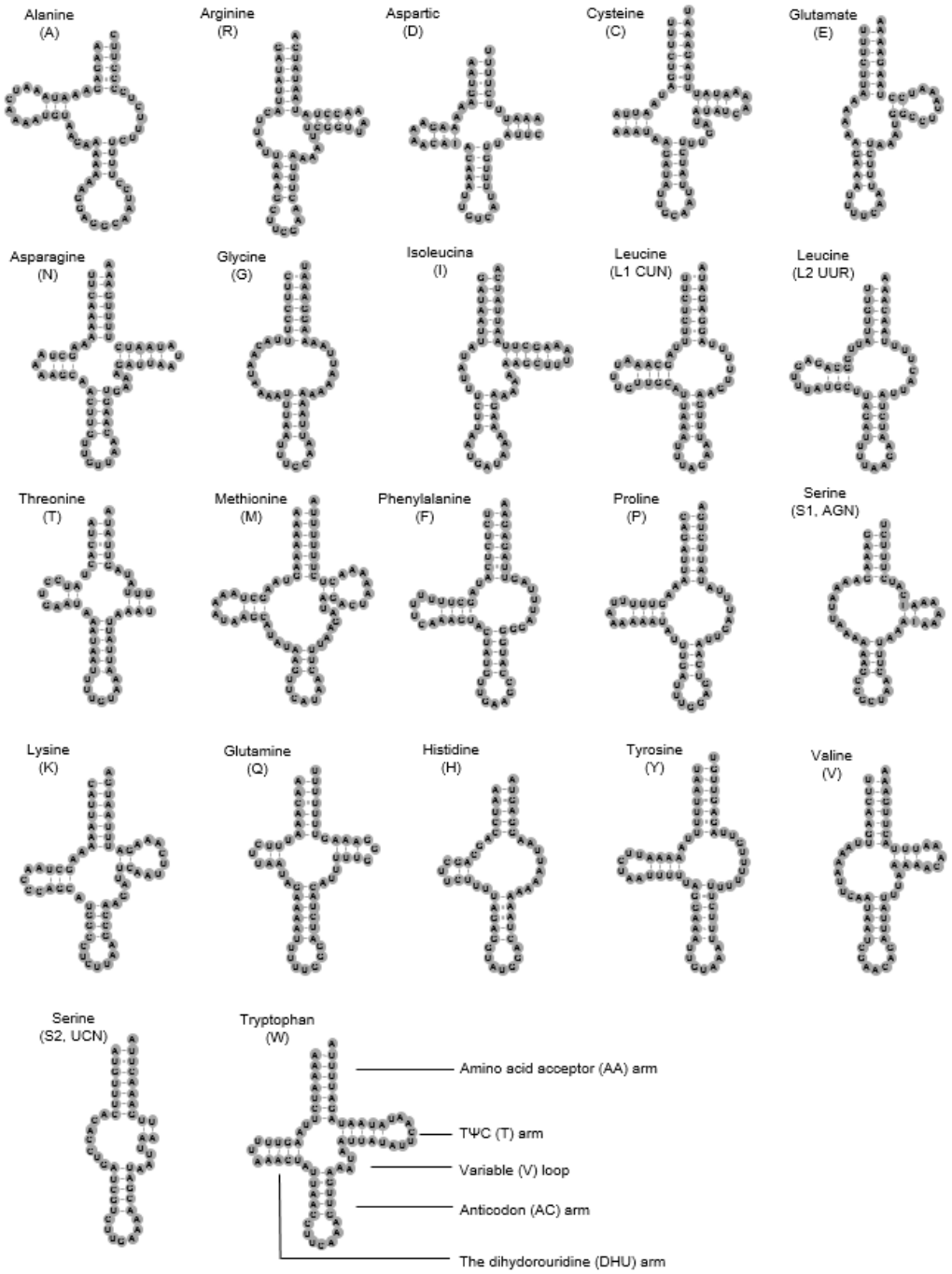


**FIGURE 2.** The 22 tRNAs of *Cheyletus malaccensis*

For *C. malaccensis*, rearrangements of tRNA genes occur much more than other genes. The truncated tRNA genes observed in *C. malaccensis* would seem to require the evolution of extensive tRNA editing capabilities. The molecular machinery necessary for these unusual tRNAs to function might provide an explanation for *C. malaccensis* adaptability and becoming the dominant predator mite in grain depot.

*Phylogeny*

Leveraging two datasets (PCG123rRNA and PCG) as well as two methods (BI and ML), we reconstructed the phylogeny of Acariformes which had been reported on NCBI (Figure 3). We uncovered for the first time the mt genome belonging to Cheyletidae, which provided a good opportunity to resolve its phylogenetic position. Cheyletidae had the closest relationship to Tetranychidae (PCGsrRNA Bayesian posterior probability / PCGs Bayesian posterior probability / PCGsrRNA ML bootstrap value/ PCGs ML bootstrap value = 1/1/89/87) and they formed a clade with sister relationship with Demodicidae (support values = 1/1/96/94). The phylogenetic relationship among the three families would be clear with the implement of our mt genome data. Phylogenetic relationships among these families could be more clear when supplementing enough molecular information of Acariformes.

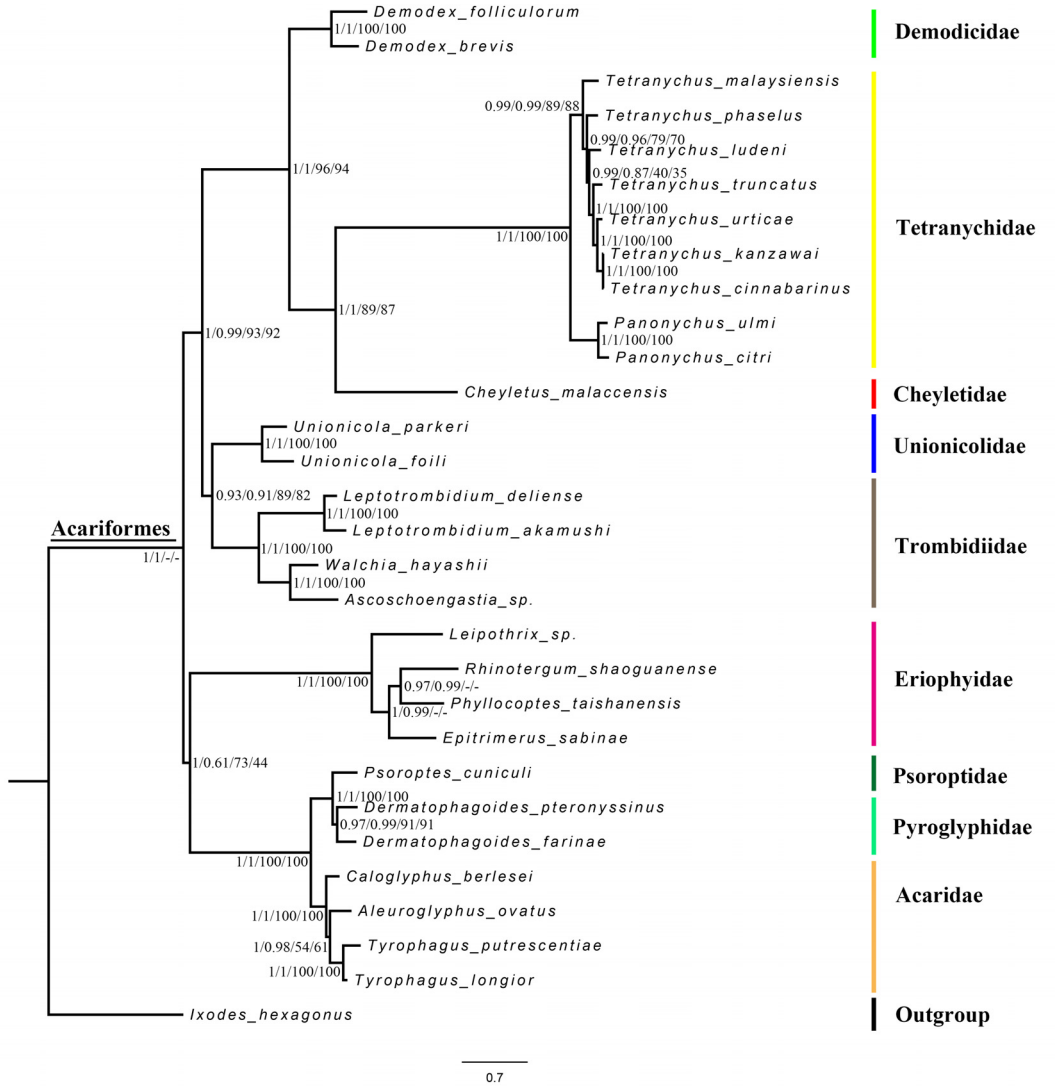


**FIGURE 3.** Phylogenetic tree of 30 species from Acariformes.
**Note:** The values in each node represented posterior probabilities of Bayesian methods and bootstrap value from maximum likehood method: PCGsrRNA Bayesian/ PCGs Bayesian/ PCGsrRNA ML/ PCGs ML.

## Summary

This study reported the complete mitochondrial genome of *C. malaccensis*, presenting its structure and sequence. According to our knowledge, it is the first description of the complete mitochondrial genome recorded for Cheyletidae. The size of *C. malaccensis* mitogenome is 14,732 bp. Most of tRNA were truncated without D-arm or/and TψC-arm. Rearrangement was found in 12 mitogenome genes. Furthermore, the phylogeny of Acariformes inferred with all 29 reported mt genomes, prove that *C. malaccensis* is more close to Tetranychidae than other mites. This study enriched the database of mt genome of Acariformes. We hope that the obtained results and the discovered unresolved questions will stimulate further studies regarding molecular phylogenetic relationship and population genetics using mitochondrial gene fragments.

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*Author contributions statement*

YW, YC and ZHL conceived the ideas for the study; YW collected the samples; YML performed the experiments; SQF, LYX and YML analyzed the data; YML, SQF, YC, ZHL, VS, RA and YW composed the manuscript. All authors have read and approved the final manuscript.

Additional information

**Accession code:** The sequencing data have been deposited in the NCBI with the accession code MT273119

**Competing financial interests:** The authors declare no competing financial interests.

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**TABLE S1.** Mitochondrial genomes of mites (from NCBI) used in the phylogenetic tree

| GenBank accession code | Species | Length |
|---|---|---|
| NC034150 | *Rhinotergum shaoguanense* | 13646 |
| NC029209 | *Phyllocoptes taishanensis* | 13475 |
| NC028725 | *Tyrophagus longior* | 13271 |
| NC026102 | *Demodex folliculorum* | 14150 |
| NC026101 | *Demodex brevis* | 14211 |
| NC026079 | *Tyrophagus putrescentiae* | 13288 |
| NC024679 | *Tetranychus phaselus* | 13084 |
| NC024678 | *Tetranychus malaysiensis* | 13049 |
| NC024637 | *Caloglyphus berlesei* | 14273 |
| NC012571 | *Panonychus ulmi* | 13115 |
| NC007600 | *Leptotrombidium deliense* | 13731 |
| KX027362 | *Leipothrix sp.* | 14216 |
| KR604966 | *Epitrimerus sabinae* | 13531 |
| KM111296 | *Tetranychus truncatus* | 13089 |
| KJ957822 | *Psoroptes cuniculi* | 14247 |
| KJ729018 | *Tetranychus ludeni* | 13064 |
| KJ729017 | *Tetranychus kanzawai* | 13091 |
| KJ571488 | *Aleuroglyphus ovatus* | 14305 |
| HQ386015 | *Unionicola parkeri* | 14734 |
| HM753535 | *Tetranychus cinnabarinus* | 13092 |
| HM189212 | *Panonychus citri* | 13077 |
| GQ465336 | *Dermatophagoides farinae* | 14266 |
| EU884425 | *Dermatophagoides pteronyssinus* | 14203 |
| EU856396 | *Unionicola foili* | 14738 |
| EU345430 | *Tetranychus urticae* | 13103 |
| AB300501 | *Ascoschoengastia sp.* | 16067 |
| AB300500 | *Walchia hayashii* | 14857 |
| AB194045 | *Leptotrombidium akamushi* | 13698 |
| KR259803 | *Hypsosinga pygmaea* | 14193 |