A comparative study on the complete mitochondrial genome sequences of 5 species in the genus *Macrotermes*

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Abstract

The genus *Macrotermes* is group of fung-growing termites belonging to the family Macrotermitinae (Isoptera: Termitidae). The complete mitochondrial genome was sequenced and annotated for 2 species, *M. annandalci* and *M. yunnanensis* collected in Yunnan. These are the 4th and 5th whole termite mt genomes reported. Combined with GenBank data sets for *M. Barneyi* which is widely distributed in China, *M. subhyalinus*, and *M. natalensis* from Africa, the evolutionary tree of 5 *Macrotermes* termites were build using Maximum Likelihood (ML) and Bayesian Inference (BI), with *Odontotermes formosanus*, *Reticulitermes speratus* and *Coptotermes formosanus* as out groups. Comparing the different composition structure, coding and a non-coding regions of 5 *Macrotermes* species, the referred mitochondrial genome is important in understanding their radiation and speciation.

1. The obtained sequence length of the whole mitochondrial genome of *M. annandalci* and *M. yunnanensis* were 15375bps and 15965bps respectively.

2. The mitochondrial genome of two Yunnan native *Macrotermes* species contained an extra tandem repeat in the longest non-coding region. The difference between the two genomes promised to serve as a marker for distinguishing those two species.

3. The *genus Macrotermes* originated from *M. subhyalinus* and *M. natalensis* were at the basal position of the genus *Macrotermes*, The Africa species *M. annandalci* and *M. yunnanensis* were inferred to be closely related as a sister group, but *M. Barneyi* more evolutionary than the others.

4. Multiple gene NADH dehydrogenase complex among the 5 species and ATP8 harbored higher mutation rates and Ka/Ks index, suggesting an association with species and environmental adaptability.

5. The metagenomics sequence among 5 species showed high relativity with species distribution and habits.

Keywords: metagenomics; divergence times; *Macrotermes*; *Macrotermes annandalci* (Silvestri); *Macrotermes yunnanensis* Han

Results

Table 1 Primer sequence for PCR amplification of *M. Annandalci* mitogenomics

forward primer	forward primer sequences	reverse primer	reverse primer sequences	amplified region
LYS-C1-F	CACAAG(A)GAT(C)ATTGGAACACT	LYS-C1-R	TGTGTTCTGCTGGTGGT(G)AAG	COX1
LYS-N4-F	TCAACGGAAACCAG(A)TGT(C)AAA	LYS-N4-R	CGTGTTCAGGCTGGTATT(C)TA	ND4
LYS-CB-F	TA(C)AGACACATCTGCCGAGAC	LYS-CB-R	CGTGCT(C)CCGATTCATGTT	CYTB
DL-N2-F	ATGCCCAGA(T)AACTCAACC(T)AA	DL-N2-R	TTCCTGTTAATGAGATT(C)GATGA	ND2
LYS-N1-F	CGAAAGAGGTAAAAATCTC(T)C(T)	LYS-N1-R	GTTATGGTGGGTGTGGCT(C)T	ND1
LYS-CB-S	CTATTCGCATACGCAATCCT	LYS-N1-C	GT(G)GAGTCTGAG(A)CTTGTTTCT	CYTB-ND1
LYS-C1C2-F	TAACATTCTTCCCG(A)CAACA	LYS-C1C2-R	ATTGGTGATGCTCTGTCTTG	COX1-COX2
LYS-N5F	TCCGTTTACACTTCTAAAACAA	LYS-N5R	TTTCTGCTTTAGTTCATTCTTC	ND5
LYS-N4L-S	ACTACGAACCATAGAAACCA	LYS-CB-C	CCTCAAAATGATATTTGTCC	ND4-CYTB
D42-N112S-3S	AAGGTCCAACGCGGACTATC	D42-12SN2-2C	AATGTGGTGGATTTGGCTGG	ND1-ND2
LYS-12S-2S	TAAACGGCGGTATACAAACA	LYS-N2-2C	CACTTTTTAACAGGAGTGGG	12S-ND2
LYS-N1-2S	GAAGACCTCCTAACAAAGAA	LYS-12S-2C	CAGTGTTATTTGTTGTTTGG	ND1-12S
LYS-N5-2S	AAGACCC(T)AACTGTCTCAAAG	LYS-N4-2C	CCCTCTCTTAAT(C)CTTCTTGG	ND5-ND4
LYS-C1-S	TATTCCCAACCCAC(T)ACT(G)AGA	LYS-A6-C	GTGTTCGAATATGTGGTTTG	COX1-ATP6
D42-N1DL-2S	CACACACCAAAGGAGATAGCC	D42-DLN2-3C	GCCATGAATTGGAGGAGACAG	D-loop
LYS-N2-S	AACATTGACAGTCGTATCAG	LYS-C1-C	AAGG(A)GTTAGT(C)GATGGTGGT	ND2-COX1
LYS-C3-S	CTTCGCATCATTCTTCTGAG	LYS-N5-C	CAG(A)TATAAT(C)GGCTTAAGGGT	COX3-ND5
LYS-N4-S	AATAGGCAATCAACGACTTC	LYS-4L-C	TTTGTGGTGTTTGGGTG(T)TT	ND4-ND4L
DL-A6-S	CCA(C)TACATCTTCACC(T)AGAAC	DL-C3-C	GCTTGTAGTGCG(A)GTG(A)AAGT	ATP6-COX3
LYS-12S-F	CAGCCACTTTGTTACGACTT	LYS-12S-R	TTTGCGTGGTTTG(A)C(A)TCTTA(G,T)T	12S

Talbe 2 Primer sequence for PCR amplification of *M. Yunnanensis* mitogenomics

forward primer	forward primer sequences	reverse primer	reverse primer sequences	amplified region
LYS-N1-F	CGAAAGAGGTAAAAATCTC(T)C(T)	LYS-N1-R	GTTATGGTGGGTGTGGCT(C)T	ND1
LYS-C1-S	TATTCCCAACCCAC(T)ACT(G)AGA	LYS-A6-C	GTGTTCGAATATGTGGTTTG	COX1-ATP6
LYS-N2-S	AACATTGACAGTCGTATCAG	LYS-C1-C	AAGG(A)GTTAGT(C)GATGGTGGT	ND2-COX1
LYS-C1C2-F	TAACATTCTTCCCG(A)CAACA	LYS-C1C2-R	ATTGGTGATGCTCTGTCTTG	COX1-COX2
LYS-12S-F	CAGCCACTTTGTTACGACTT	LYS-12S-R	TTTGCGTGGTTTG(A)C(A)TCTTA(G,T)T	12S
LYS-CB-S	CTATTCGCATACGCAATCCT	LYS-N1-C	GT(G)GAGTCTGAG(A)CTTGTTTCT	CYTB-ND1
D50-C3-2S	ACTAGGATCAACATGACCGC	D50-N5-2C	TGGCTTAAGGGTATTTTTGG	COX3-ND5
D50-N5-2S	GAATAACACCACCAGCACAC	D50-N4-2C	CTCCCCCCTCTCTTAATCTT	ND5-ND4
D50-N4-2S	ACAAAAACACCACCAACCAA	D50-4L-2C	TTGGATGCAAGTCGTGTTAT	ND4-ND4L
LYS-N1-2S	GAAGACCTCCTAACAAAGAA	LYS-12S-2C	CAGTGTTATTTGTTGTTTGG	ND1-12S
LYS-12S-2S	TAAACGGCGGTATACAAACA	LYS-N2-2C	CACTTTTTAACAGGAGTGGG	12S-ND2
LYS-N4-F	TCAACGGAAACCAG(A)TGT(C)AAA	LYS-N4-R	CGTGTTCAGGCTGGTATT(C)TA	ND4
DL-N2-F	ATGCCCAGA(T)AACTCAACC(T)AA	DL-N2-R	TTCCTGTTAATGAGATT(C)GATGA	ND2
DL-A6-S	CCA(C)TACATCTTCACC(T)AGAAC	DL-C3-C	GCTTGTAGTGCG(A)GTG(A)AAGT	ATP6-COX3
LYS-CB-F	TA(C)AGACACATCTGCCGAGAC	LYS-CB-R	CGTGCT(C)CCGATTCATGTT	CYTB
LYS-N5F	TCCGTTTACACTTCTAAAACAA	LYS-N5R	TTTCTGCTTTAGTTCATTCTTC	ND5
LYS-N4L-S	ACTACGAACCATAGAAACCA	LYS-CB-C	CCTCAAAATGATATTTGTCC	ND4-CYTB
LYS-C1-FN	CACAAGGACATTGGAACACT	LYS-C1-RN	GTGTGGGTTGGGAATAGAAT	COX1

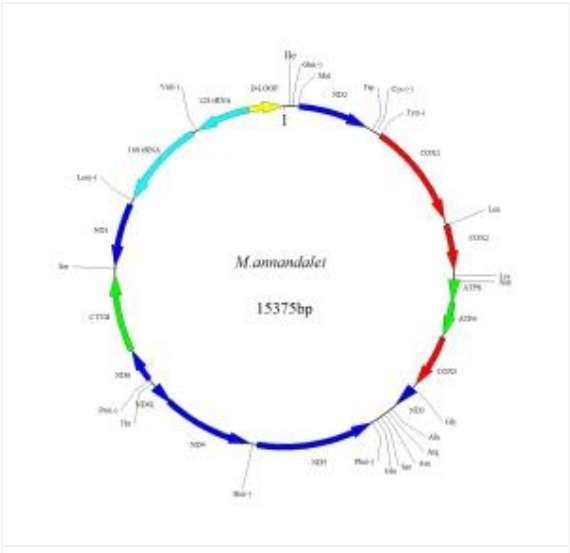

Fig.1 Gene map of the *M. annandalci*

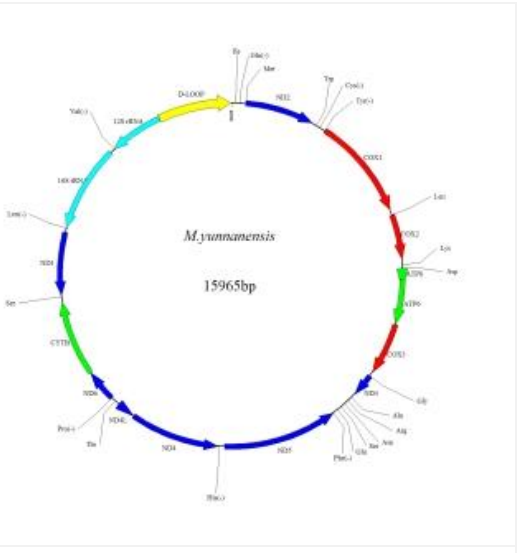

Fig.2 Gene map of the *M. yunnanensis*

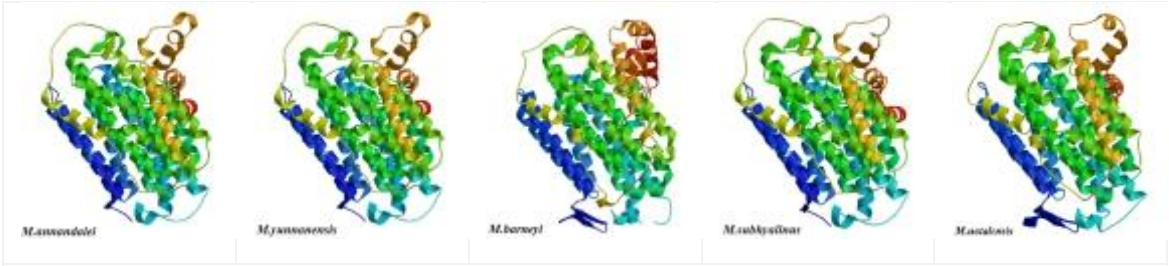

Fig. 3 Simulative tertiary structure of ND5

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