

## Molecular phylogeography of *Reticulitermes* in Yarlung Zangbo Grand Canyon glacial refugia of Tibet

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### Abstract

The genetic diversity of the genus *Reticulitermes* in Tibet was detected, reviewed and analyzed based on morphological methods and molecular evidence using DNA barcoding as well as a partial (658 bp) sequence of the mitochondrial COI gene from 167 samples collected in Tibet. There were 29 representative samples selected to build a phylogenetic tree based on COI sequence. The phylogenetic relationship of *Reticulitermes* was analyzed among Tibet and neighboring areas including Yunnan, Sichuan, Jiangxi, Jiangsu and Fujian. The *Reticulitermes* species were collected at altitude 2356m in Chayu which broke the original collecting record of 2020m. Molecular evidence supported the morphological results, there were 2 species in Tibet, *R. tibetanus* and *R. assamensis*, the former has 3 haplotypes and the latter 5 haplotypes. A total of 8 haplotypes were found but no strong phylogeographic structure was revealed. Intra- and inter-specific genetic distances among the 3 species were 0.090~0.097 and 0.002~0.005, respectively. The two Tibetan *Reticulitermes* species were distributed in both areas of Chayu City and Motuo City. *R. jiangchengensis* was a synonym for *R. assamensis*. Two samples from Chendu, *Reticulitermes* sp.-Chengdu CD1 and *Reticulitermes* sp.-Chengdu2 were considered a sister group with *R. assamensis*. The two *Reticulitermes* species in Tibet have a close relationship with other Chinese continental *Reticulitermes*, indicating that they are not relict species confined to Tibet.

**Key words:** *Reticulitermes*; *R. tibetanus*; *R. assamensis*; Tibet; mtDNA; Revision

### Introduction

Climatic oscillations during the Quaternary could have important effects on the spatial distribution and genetic structure of organisms in the Yarlung Zangbo Grand Canyon (YZGC). However, how the termite species that occur there changed their distribution in response to the Quaternary climatic oscillation remains controversial. Motuo County and Chayu County located in the Linzhi District are the lowest elevation and offer the best termite environment in Tibet with tropic rain forest (Huang, 1985). The termite species in Tibet were first surveyed by Tsai & Huang (1975) who identified *Reticulitermes chayuensis* from their collections but since then there have been 20 species belonging to 3 families reported in Tibet (Huang, *et al.*, 1987; 2006) including 3 species of *Reticulitermes*. We conducted a study of the phylogeography of the *Reticulitermes*, endemic in the canyon using mtDNA molecular markers to resolve phylogenetic relationships at species levels.

### Materials and Methods

**Morphological identification**

Based on the original description and review data (Tsai & Huang,1975; Huang & Han,1985; Huang, *et al.*,2000), specimens were identified to species.

**Molecular identification**

DNA templates were extracted with “TianGen”-kits and kept at -20°C. DNA barcoding COI universe primer pairs (Folmer *et al.*,1994):

LCO1490 (5'-GGT CAACAAATCATAAAGATATTGG-3')

HCO2198(5'-TGATTTTTTGGTCACCCTGAAGTTT A-3')

PCR program: 94°C 1 min, 46°C 1 min, 72°C 2 min, total 35 cycles and keep -4°C.

**Data analysis**

Sequences were edited with DNASTar software, homologous sequence searched via Blast on GenBank, an evolutionary tree was built using MEGA 5.0 software, the genetic distant, polymorphic site and haplotype were analyzed (Ding, 2013).

Table 1 Collecting information, and haplotypes for *Reticulitermes*

Province	sepcies	town	city	haplotype	
Tibet	<i>R. tibetanus</i>	Bangxin	Motuo	X	
		Shama	Motuo	Y	
		Xigonghu	Motuo	Z	
		Xigonghu	Motuo	Z	
		Xigonghu	Motuo	Z	
		Xigonghu	Motuo	Z	
		Xiongjiu	Chayu	Z	
		Xiongjiu	Chayu	Z	
		Zongba	Chayu	Z	
		Ziba	Chayu	Z	
		Xiachayu	Chayu	Z	
		Shams	Chayu	Z	
		<i>R. assamensis</i>	Zala	Chayu	A
	Shama		Chayu	B	
	Dengren		Chayu	C	
	Dengren		Chayu	C	
	Dengren		Chayu	C	
	Miba		Chayu	C	
	Shama		Chayu	C	
	Guba		Chayu	D	
	Migu		Chayu	D	
	Zhuwagen		Chayu	D	
	Yunna	<i>R. jiangchengensis</i>	-	Nuzu	YN1
-			Tengchong	YN2	
Sichuan		<i>R. sp.-Chengdu</i>	-	Chengdu	CD1
			-	Chengdu	CD2
Jiangsu		<i>R. qingjiangensis</i>	-	Nanjing	JS
Fujian		<i>R. leptomandibularis</i>	-	Wuyishan	FJ
Jiangxi		<i>R. leptomandibularis</i>	-	Jiulanshan	JX

**Results and Discussion**

**Morphological identification**

Morphological characters of 29 samples suggested two species groups, *R. tibetanus* and *R. assamensis*.

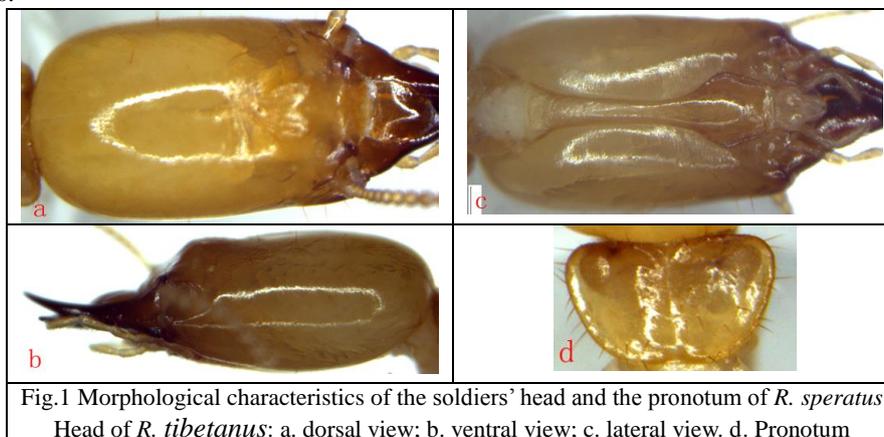


Fig.1 Morphological characteristics of the soldiers' head and the pronotum of *R. speratus*  
 Head of *R. tibetanus*: a. dorsal view; b. ventral view; c. lateral view. d. Pronotum

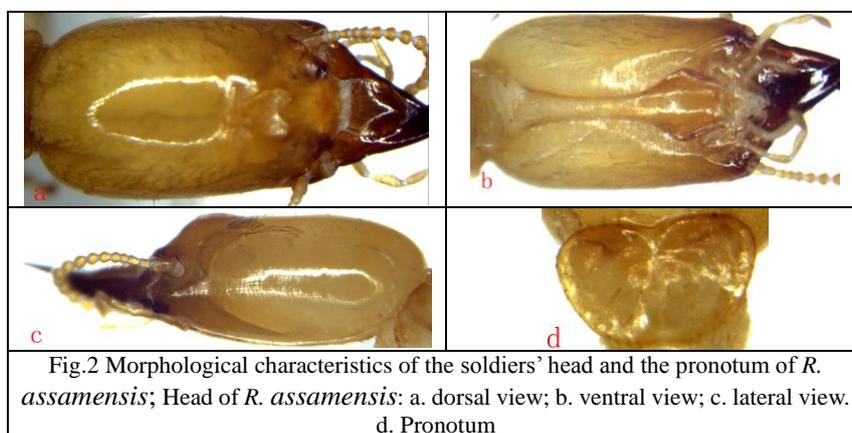


Fig.2 Morphological characteristics of the soldiers' head and the pronotum of *R. assamensis*; Head of *R. assamensis*: a. dorsal view; b. ventral view; c. lateral view. d. Pronotum

**Molecular identification**

A standard DNA barcode was obtained using a 658bp COI fragment and the fragment averaged G+C 42.0%, and A+T content 58.0%. *R. tibetanus* had 3 haplotypes, *R. assamensis* had 5 haplotypes.

Table 2 Haplotype variation at 5 nucleotide sites among *R. assamensis*

Haplotype	39	40	518	556	640
D	T	C	G	A	T
A	.	.	.	G	.
B	A	.	A	.	.
E	.	A	.	.	.
C	.	.	.	.	C

Table 3 Genetic distance pairwise-species and within-species of *Reticulitermes* in Tibet

No.	Specie	1	2	3	4	5	6	7	8
1	<i>R. tibetanus</i> X	—							

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2	<i>R. tibetanus</i> Y	0.003	—
3	<i>R. tibetanus</i> Z	0.002	0.002 —
4	<i>R. assamensis</i> A	0.093	0.095 0.093 —
5	<i>R. assamensis</i> B	0.095	0.097 0.095 0.005 —
6	<i>R. assamensis</i> C	0.090	0.092 0.090 0.003 0.005 —
7	<i>R. assamensis</i> D	0.091	0.093 0.092 0.002 0.003 0.002 —
8	<i>R. assamensis</i> E	0.093	0.095 0.093 0.003 0.005 0.003 0.002 —

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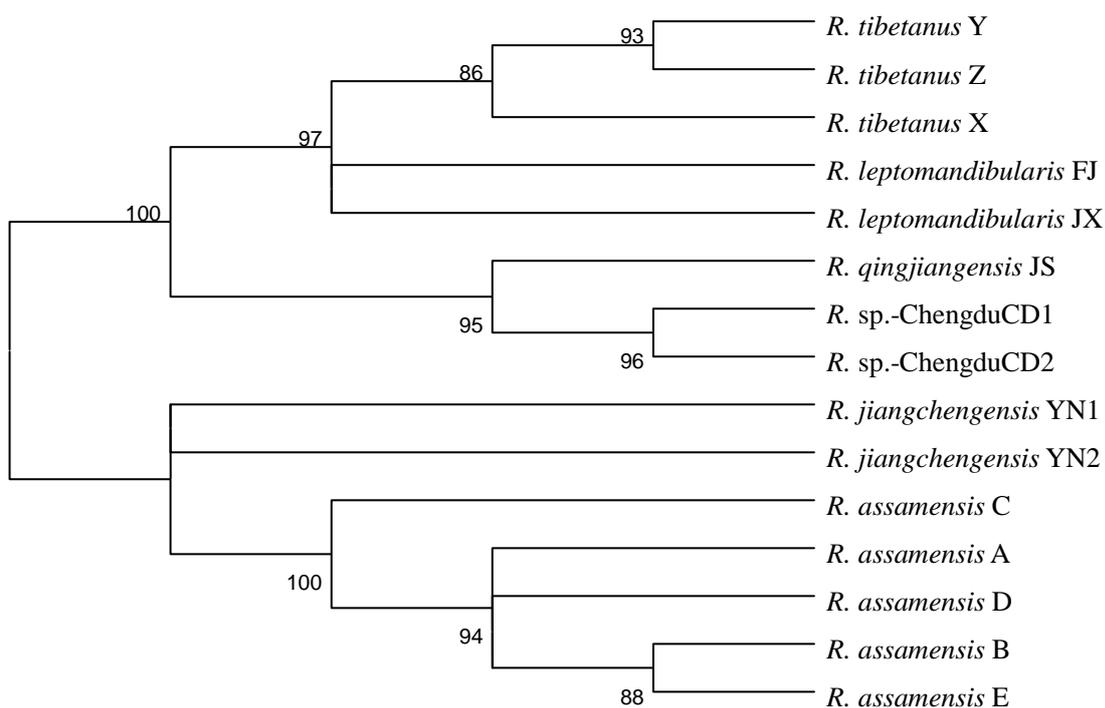
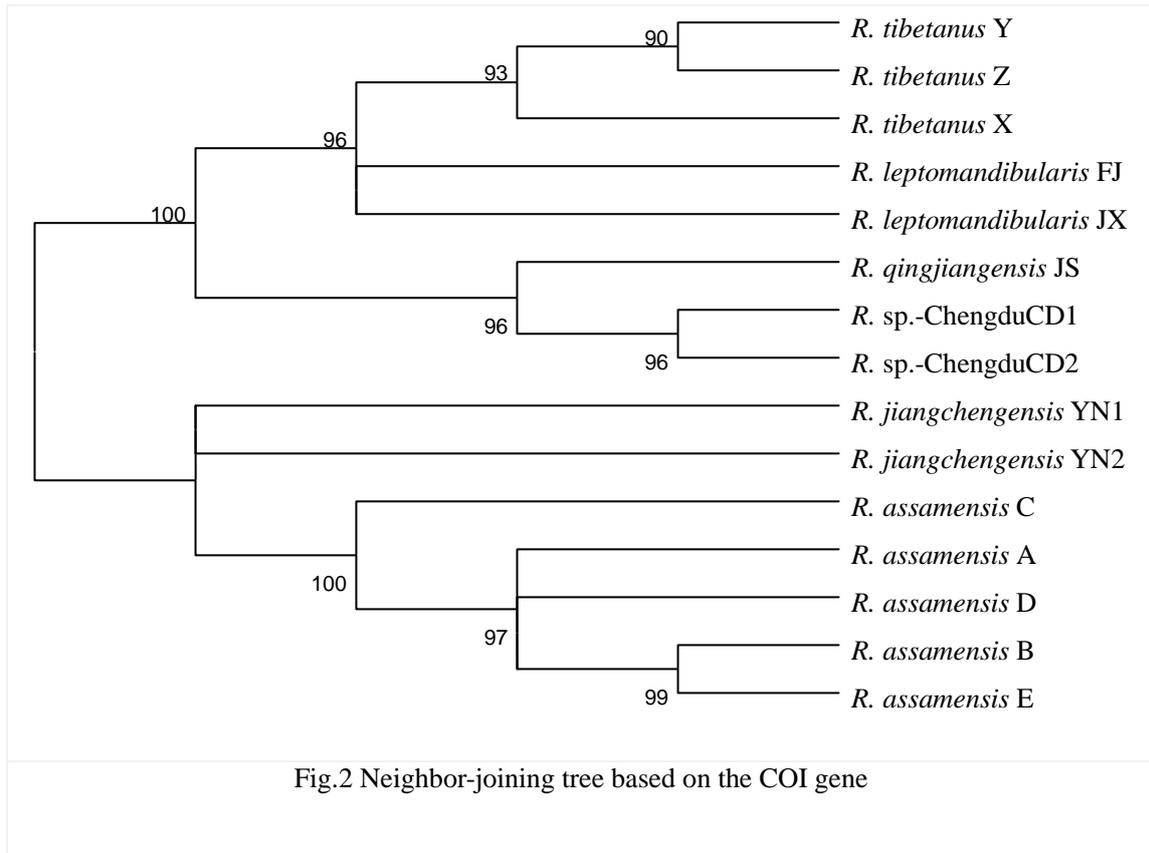


Fig.1 Maximum Likelihood tree based on the COI gene



## Conclusions

The phylogenetic relationship of *Reticulitermes* was analyzed among the termites collected in Tibet and neighboring areas, such as Yunnan, Sichuan, Jiangxi, Jiangsu and Fujian. Molecular evidence supported the morphological identification results, there were 2 species in Tibet. Inter- and inner-species genetic distances are 0.090~0.097 and 0.002~0.005, respectively, both *Reticulitermes* species were distributed in both Chayu City and Motuo City in Tibet. *R. jiangchengensis* is a synonym *R. assamensis*. Two samples from Chendu *Reticulitermes* sp. chengdu CD1 and *Reticulitermes* sp. Chengdu were closely related to *R. assamensis*, and were considered a sister group. The *Reticulitermes* species samples were collected at altitude 2356m in Chayu, which broke the original collecting record 2020m, that's the highest record in East Asia, but is lower than *Archotermopsis wronqhtoni* (Desneux) located in the Kashmir region and northeast of Kabul, Afghanistan that have been found at 2743m. In the samples from Chayu, we strongly suspect the species *R. chayuensis* should be synonymized because we could not detect either a morphological and molecular character that would separate this species., The two *Reticulitermes* species in Tibet are not relict species confined to Tibet.

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