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## Genetic Differentiation of *Reticulitermes speratus* in East Asia

By

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### Abstract

In this study, samples of *Reticulitermes speratus* (Kolbe) were collected in China and a portion of the mitochondrial gene encoding for the cytochrome oxidase subunit II (COII) was sequenced. A phylogenetic tree was constructed using neighbor-joining (NJ) and Maximum likelihood (ML). To compare the phylogenetic relationship and migration route of *R. speratus* in East Asia, we used GenBank data of populations from Korea and Japan. Results indicated:

1 The 655bp length sequence consisted of 581 conserved sites, 74 variable sites, 62 information sites. G+C content averaged 37.0%, A+T content averaged 63.0%.

2 Results showed that *R. speratus* was mainly divided into two groups, with a correlation between phylogenetic distance and locality of collection. *R. speratus* of southern Japan and Korea flock together in one branch, *R. speratus* of northern Japan to other branch, *R. speratus* of China divided to two branches.

3 According to the comparative analysis of the sequences, we suggested that *R. speratus* is found in China and Japan, China and Korea, and there were at least two subspecies of *R. speratus* in China included nominate subspecies *R. speratus speratus* and *R. speratus kyushuensis*. 4 Our results indicated that *R. speratus* migrated into the Japanese main islands from East China, and that Korean populations diverged from Japanese populations.

**Key words:** *Reticulitermes speratus*; COII; migration route; phylogenetic relationship

### Introduction

*Reticulitermes speratus* (Kolbe) was described by Kolbe 1885 where type locality was Japan, Snyder (1949) moved it into the genus *Reticulitermes* where it remains (Huang, *et al.*, 2000).

This economically important termite pest widely distributed in northern China, Japan and Korea, and its speciation, genetic variation and migration route among those three countries is fascinating.

### Materials and Methods

#### Materials information

Termite samples were collected from 6 cities in Liaoning and Shandong Province, 3~5 colonies per city. All specimens were kept in 75% ethyl alcohol for morphological comparison, and a backup sample kept in 75% ethyl alcohol at -20°C for molecular comparison (Table 1).

*Morphological comparison*

Digital images for illustrations were taken using a NIKON SMZ1500 camera (NIKON Microsystems, Japan). Measurements data were compared and analyzed with original ones in taxonomy references to identify the samples.

*Molecular comparison*

DNA templates was extracted with “TianGen”-kits and kept at -20°C.

*PCR amplification*

DNA sequence COII universe primer pairs (Folmer *et al.*,1994):TL-J-3037 (5-ATGGCAGATTAGTGCAATGG-3), TK-N-3785 (5-GTTTTAAGAGACCAGTACTTG-3) (Xing *et al.*, 2001).PCR program: 94°C 1 min, 46°C 1 min, 72°C 2 min, total 35 cycles and keep -4°C.

**Table 1 List and sampling localities of the termites used in this study**

Species	Sites	Reference	GeneBank No.
<i>R. speratus</i>	Korea	Kim <i>et al.</i> ,2012	GU732288
<i>R. speratus</i>	Korea	Kim <i>et al.</i> ,2012	HM560007
<i>R. speratus</i>	Korea	Kim <i>et al.</i> ,2012	HM560010
<i>R. speratus</i>	Korea	Ohkuma <i>et al.</i> ,2004	AB109530
<i>R. speratus</i>	North Japan	Yashiro <i>et al.</i> ,2007	DQ493737
<i>R. speratus</i>	North Japan	Yashiro <i>et al.</i> ,2007	DQ493740
<i>R. speratus</i>	North Japan	Austin <i>et al.</i> ,2002	AF525344
<i>R. speratus</i>	North Japan	Xing <i>et al.</i> ,2001	AB050706
<i>R. speratus</i>	South Japan	Yashiro <i>et al.</i> ,2007	DQ493736
<i>R. speratus</i>	South Japan	Yashiro <i>et al.</i> ,2007	DQ493739
<i>R. speratus</i>	South Japan	Yashiro <i>et al.</i> ,2007	EF016101
<i>R. speratus</i>	Yantai, CN	—	KU061240
<i>R. speratus</i>	Longkou, , CN	—	KU061241
<i>R. speratus</i>	Gongzhuling	—	KU061242
<i>R. speratus</i>	Dalian, CN	—	KU061243
<i>R. speratus</i>	Qixia, CN	—	KU061244
<i>R. speratus</i>	Dalian, CN	—	KU061245
<i>R. speratus</i>	Qingdao, CN	—	KU061246
<i>R. speratus</i>	Changsha, CN	Long <i>et al.</i> ,2009	FJ423457
<i>R. speratus</i>	Changsha, CN	Long <i>et al.</i> ,2009	FJ423463
<i>R. speratus</i>	Changsha, CN	Long <i>et al.</i> ,2009	FJ423464
<i>R. speratus</i>	Beijing, CN	Chen <i>et al.</i> ,2012	JX142152
<i>R. speratus</i>	Beijing, CN	Chen <i>et al.</i> ,2012	JX142153
<i>R. speratus</i>	Beijing, CN	Chen <i>et al.</i> ,2012	JX142154
<i>R. aculabialis</i>	Hangzhou, CN	Chen <i>et al.</i> ,2012	JX142172○
<i>R. chinensis</i>	Beijing, CN	Xing <i>et al.</i> ,2001	AB050705○

○With potential for wrong identify.

*Data analysis*

The sequences were edited with DNASTar software (Burland,1999), homologous sequence searched via Blast on GenBank, an evolutionary tree was built by MEGA 5.0 software (Tamura *et al.*, 2011), the genetic distant, polymorphic sites and haplotypes were analyzed.

## Results and discussion

### Morphological comparison

The samples in this study were *R. speratus* revealed similar morphologies with description in the key.

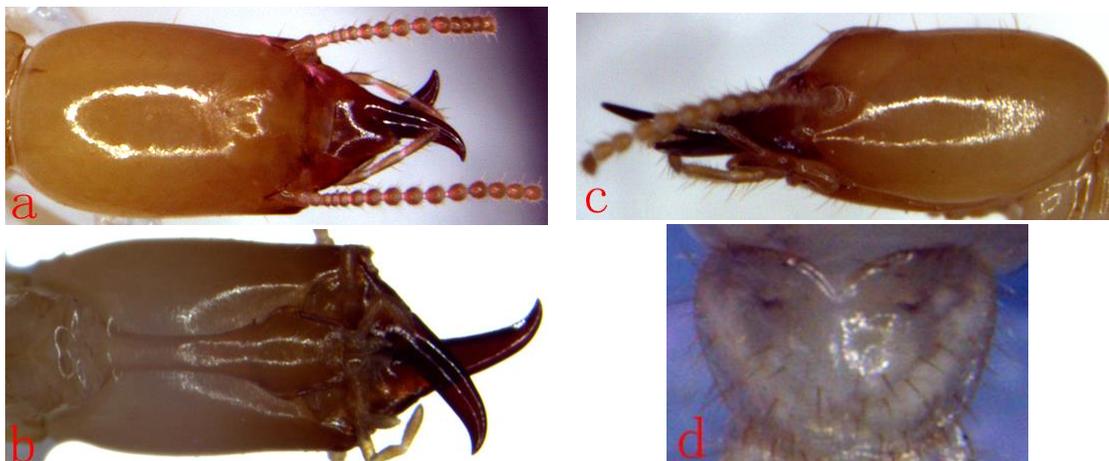


Fig.1 Morphological characteristics of the soldiers' head and the pronotum of *R. speratus*  
 Head of *R. speratus*: a. dorsal view; b. ventral view; c. lateral view. d. Pronotum.

### Molecular analysis

The 655bp length sequences were selected a phylogenetic tree constructed and analyzed, which consisted of 581 conserved sites, 74 variable sites, 62 information sites with the G+C average content of 37.0%, while A+T averaged 63.0%.

The tree was constructed by neighbor-joining method (NJ) and Maximum likelihood (ML) method (Fig. 2~3).

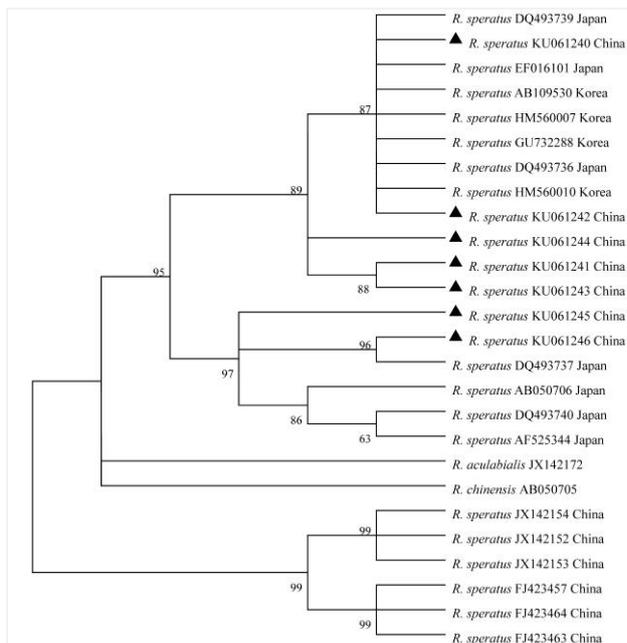


Fig.2 Maximum Likelihood tree based on the COII gene  
 ▲: The specimens were collected in China, the same below.

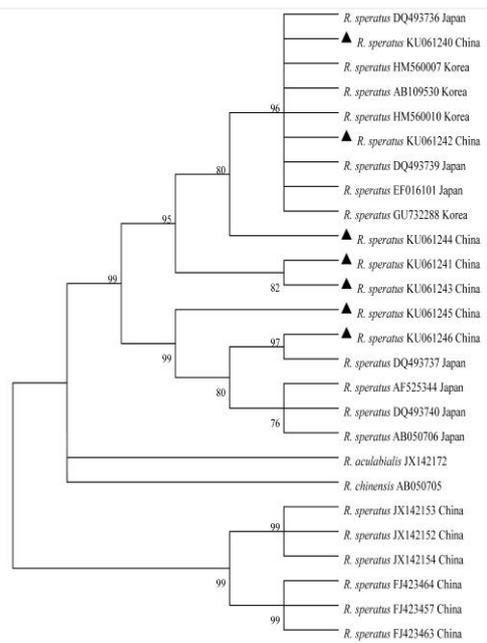


Fig. 3 Neighbor-joining tree based on the COII gene

Analysis results showed that *R. speratus* was mainly divided into two groups, with correlation the phylogenetic distance and the locality of collection. *R. speratus* of southern Japan and Korea flock together into one branch, *R. speratus* of northern Japan to other branch, *R. speratus* of China divided to six branches.

## Discussion

### *Subspecies discuss*

According to the comparative analysis of the sequences, we suggested that the same *R. speratus* subspecies are existed in China and Japan, China and Korea, respectively, there were at least two subspecies of *R. speratus* in China including *R. speratus speratus* and *R. speratus kyushuensis*.

### *Migration route of R. speratus in East Asia*

Our results supported the hypothesis that ancestral *R. speratus* separated into northern and southern Japanese populations after its migration into the Japanese main islands from East China during the early Pleistocene via the East China Sea basin, which may have been exposed during that period. The Korean populations seem to have diverged recently from southern Japanese populations; this may explain the current distribution of *R. speratus* in the Japanese Archipelago (Park *et al.*,2006).

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**Table 2 Genetic distances among the populations of *Reticulitermes speratus***

No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	<i>R. speratus</i> DQ493736 Japan	—																								
2	<i>R. speratus</i> DQ493739 Japan	0.002	—																							
3	<i>R. speratus</i> EF016101 Japan	0.002	0.000	—																						
4	<i>R. speratus</i> HM560007 Korea	0.002	0.000	0.000	—																					
5	<i>R. speratus</i> HM560010 Korea	0.003	0.002	0.002	0.002	—																				
6	<i>R. speratus</i> AB109530 Korea	0.002	0.000	0.000	0.000	0.002	—																			
7	<i>R. speratus</i> GU732288 Korea	0.002	0.000	0.000	0.000	0.002	0.000	—																		
8	<i>R. speratus</i> KU061242 China	0.002	0.000	0.000	0.000	0.002	0.000	0.000	—																	
9	<i>R. speratus</i> KU061240 China	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	—																
10	<i>R. speratus</i> KU061244 China	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.002	0.002	—															
11	<i>R. speratus</i> KU061241 China	0.005	0.003	0.003	0.003	0.005	0.003	0.003	0.003	0.003	0.002	—														
12	<i>R. speratus</i> KU061243 China	0.005	0.003	0.003	0.003	0.005	0.003	0.003	0.003	0.003	0.002	0.000	—													
13	<i>R. speratus</i> KU061245 China	0.025	0.023	0.023	0.023	0.025	0.023	0.023	0.023	0.023	0.022	0.023	0.023	—												
14	<i>R. speratus</i> KU061246 China	0.027	0.025	0.025	0.025	0.027	0.025	0.025	0.025	0.025	0.023	0.025	0.025	0.014	—											
15	<i>R. speratus</i> DQ493737 Japan	0.025	0.023	0.023	0.023	0.025	0.023	0.023	0.023	0.023	0.022	0.023	0.023	0.012	0.002	—										
16	<i>R. speratus</i> DQ493740 Japan	0.025	0.023	0.023	0.023	0.025	0.023	0.023	0.023	0.023	0.022	0.023	0.023	0.012	0.011	0.009	—									
17	<i>R. speratus</i> AF525344 Japan	0.036	0.035	0.035	0.035	0.036	0.035	0.035	0.035	0.035	0.033	0.035	0.035	0.025	0.023	0.022	0.012	—								
18	<i>R. speratus</i> AB050706 Japan	0.028	0.027	0.027	0.027	0.028	0.027	0.027	0.027	0.027	0.025	0.027	0.027	0.012	0.011	0.009	0.003	0.015	—							
19	<i>R. speratus</i> JX142153 China	0.060	0.058	0.058	0.058	0.060	0.058	0.058	0.058	0.058	0.057	0.058	0.058	0.074	0.076	0.074	0.074	0.084	0.077	—						
20	<i>R. speratus</i> JX142154 China	0.060	0.058	0.058	0.058	0.060	0.058	0.058	0.058	0.058	0.057	0.058	0.058	0.074	0.076	0.074	0.074	0.084	0.077	0.000	—					
21	<i>R. speratus</i> JX142152 China	0.060	0.058	0.058	0.058	0.060	0.058	0.058	0.058	0.058	0.057	0.058	0.058	0.074	0.076	0.074	0.074	0.084	0.077	0.000	0.000	—				
22	<i>R. speratus</i> FJ423457 China	0.065	0.063	0.063	0.063	0.065	0.063	0.063	0.063	0.063	0.062	0.063	0.063	0.075	0.077	0.076	0.075	0.085	0.079	0.032	0.032	0.032	—			
23	<i>R. speratus</i> FJ423464 China	0.065	0.063	0.063	0.063	0.065	0.063	0.063	0.063	0.063	0.062	0.063	0.063	0.075	0.077	0.076	0.075	0.085	0.079	0.032	0.032	0.032	0.000	—		
24	<i>R. speratus</i> FJ423463 China	0.065	0.063	0.063	0.063	0.065	0.063	0.063	0.063	0.063	0.062	0.063	0.063	0.075	0.077	0.076	0.075	0.085	0.079	0.032	0.032	0.032	0.000	0.000	—	
25	<i>R. aculabialis</i> JX142172	0.046	0.045	0.045	0.045	0.046	0.045	0.045	0.045	0.045	0.043	0.041	0.041	0.055	0.056	0.055	0.051	0.063	0.055	0.063	0.063	0.063	0.068	0.068	0.068	—
26	<i>R. chinensis</i> AB050705	0.044	0.043	0.043	0.043	0.044	0.043	0.043	0.043	0.043	0.041	0.040	0.040	0.053	0.051	0.050	0.050	0.061	0.053	0.065	0.065	0.065	0.068	0.068	0.068	0.045

