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Phylogenetic Position of the Western Bangladesh Populations of Weaver Ant, *Oecophylla smaragdina* (Fabricius) (Hymenoptera: Formicidae)

MM Rahman, S Hosoishi, K Ogata

Institute of Tropical Agriculture, Kyushu University, Fukuoka, Japan

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Corresponding author
Md Mamunur Rahman
Institute of Tropical Agriculture
Kyushu University
6-10-1 Hakozaki, Higashi-ku, Fukuoka-shi, Fukuoka, Japan 812-8581
E-Mail: mamunur111@gmail.com

Abstract

The weaver ant species, *Oecophylla smaragdina* is distributed from India through Southeast Asia to Northern Australia including many tropical Western Pacific Islands. A recent phylogenetic study of *O. smaragdina* revealed the central Bangladesh population as belonging to the Southeast Asian mainland clade despite of its geographical proximity to India. However, the Bangladeshi analyzed sample was limited to a single site and the geographical border between Indian and Southeast Asian groups has not been presented. In this study, 19 samples collected from western parts of Bangladesh have been used to infer the phylogenetic position. A total of 20 *O. smaragdina* colonies were sampled from Bangladesh during 2013 to 2014. Their haplotype and phylogenetic relationships were determined by analyzing 2 mitochondrial loci: *Cytochrome b* (*Cytb*) consisting of 606 bp and *Cytochrome c oxidase subunit I* (*COI*) consisting of 775 bp. Bayesian analysis inferred that the western parts of Bangladesh were occupied by mitochondrial haplotype usually found in India, which is recorded first time in the country. The present study revealed that, both the Indian and Southeast Asian mitochondrial haplotypes were occurred on either side of Ganges river.

Introduction

The weaver ant genus *Oecophylla* (Hymenoptera, Formicidae) has two broadly distributed species: *O. smaragdina* and *O. longinoda*. Both species are distributed in tropical and subtropical Asia and Africa, respectively. Their colonies are arboreal, large and polydomous in nature. Workers show polymorphic characters with diversified organizing behavior in the colony. They are aggressive and well known for their predatory behavior (Hölldobler & Wilson, 1977). Weaver ants form their nest in the tree canopy by their unique nest building behavior. Workers construct pendulous bag-like nests from cluster of green leaves which are bound together with silk produced by their mature larvae (Chapuisat & Keller, 2002). *Oecophylla* ants are hosts to a variety of inquilines, such as spiders, which mimic the colony odor to escape detection (Schlüns et al., 2009). *Oecophylla smaragdina* and *O. longinoda* are very similar in morphology and behavior (Bolton, 1995). According to the fossil records, *Oecophylla* might have originated in the early Paleogene (ca. 60 Ma) in the Palaearctic region, and dispersed during the climatic changes of the Eocene–Oligocene transition at ca. 43 Ma (Dlussky et al., 2008). Recently, Blamier et al (2015) estimated the divergence time of the genus *Oecophylla* based on the fossil records and ultra conserved elements (UCEs). They estimated that *Oecophylla* crown group evolved during Oligocene at ca. < 30 Ma and stem-group evolved during early Eocene at ca.50 Ma.

Azuma et al. (2002) first analyzed populations of *O. smaragdina* using molecular data and samples of *O. smaragdina* from Bangladesh. Including additional populations of *O. smaragdina* from India, Southeast Asia and Australia, Azuma et al. (2006) proposed an outline of the phylogeography of *O. smaragdina* and categorized the sampled populations into 7 major clades: group 1 from India; group 2 from Southeast Asian mainland including the Indochinese and Malayan Peninsulas, as well as the Greater

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Sunda Islands; group 3 from the Philippines; group 4 from Flores; group 5 from Sulawesi; group 6 from Halmahera; group 7 from Australia and New Guinea. Hereafter we call their group 1 the Indian clade and group 2 as Southeast Asian clade. Asaka (2010) extended the survey of *O. smaragdina* to South Asia, and collected several samples from India and Sri Lanka. Her phylogenetic analysis showed that all analyzed samples belong to Indian clade with low levels of sequence divergence.

Azuma et al. (2006) characterized the mitochondrial sequence identity of the Bangladesh populations as belonging to the Southeast Asian clade in spite of geographical proximity of Bangladesh to India. Based on those data, Bangladesh is considered a major transition zone between Indian and Southeast Asian populations. This is the unique case of population boundaries without any distinguished geographical borders (e.g., deep sea or high mountains) although the seven groups of *O. smaragdina* based on haplotype grouping by Azuma et al. (2006) are geographically bordered by the sea. As Bangladesh is a riverine country with three main rivers Ganges, Jamuna and Meghna crisscrossed throughout the mostly flat territories of the country, the river might have some influence of separating the Indian clade and the Southeast Asian clade in Bangladesh.

The goal of the present study is to test whether the western Bangladesh populations of *O. smaragdina* all belong to the SE Asian clade. The previous sampling by Azuma et al. (2002) was limited to a single site, Nurbag, Gazipur, which is located in the central part of Bangladesh. Surveying the western part of Bangladesh provides additional information on the phylogeography of *O. smaragdina*. The analysis of mitochondrial haplotype identity of these populations will shed light on the geographic distribution of the Southeast Asian clade in Bangladesh.

Materials and Methods

Sampling and preparation of specimens

In 2013 to 2014 we collected adult *Oecophylla smaragdina* workers from 20 colonies at 19 localities in 12 districts belonging to 4 divisions of Bangladesh (Fig 1 and Table 1). The specimens were preserved in 99% ethanol prior to DNA extraction.

Molecular studies

Genomic DNA was extracted from the fore, middle and hind legs of specimens that were preserved in alcohol by using *QIAGEN DNeasy Blood and Tissue kit* (Qiagen, Meryland, USA). Amplification of mitochondrial DNA was done by polymerase chain reaction (PCR). The thermal cycling parameters for *Cytb* and *COI* basically followed the protocols established by Crozier and Crozier (1993) and Sameshima et al. (1999), including 95 °C for 5 min for initial denaturation, 35 cycles of dissociation (92 °C, 1 min),

Table 1. Specimen data and GenBank accession numbers.The list of figures.

Locality code	Locality Name	No. of colonies	Upazila	District	Division	Collection Date	Accession number	
							COI	Cytb
L01	Ishwardi	1	Ishwardi	Pabna	Rajshahi	18 Mar. 2014	KX385842	KX430217
L02	Bonpara	1	Baraigram	Natore	Rajshahi	19 Mar. 2014	KX385843	KX430218
L03	Tarash	1	Tarash	Sirajganj	Rajshahi	18 Mar. 2014	KX385841	KX430216
L04	Chauhali	1	Belkuchi	Sirajganj	Rajshahi	19 Mar. 2014	KX389168	KX398946
L05	w side of Jamuna Bridge	1	Sirajganj sadar	Sirajganj	Rajshahi	18 Mar. 2014	KX385840	KX430215
L06	Panjia	1	Keshabpur	Jessore	Khulna	04 Mar. 2014	KX371575	KX398943
L07	Manirampur	1	Manirampur	Jessore	Khulna	14 Sep. 2013	KX355139	KX430212
L08	Khulna Univ. Campus	1	Batiaghata	Khulna	Khulna	03 Mar. 2014	KX379493	KX398942
L08	Khulna Univ. Campus	1	Batiaghata	Khulna	Khulna	03 Mar. 2014	KX379494	KX430213
L09	Chuknagar	1	Dumuria	Khulna	Khulna	04 Mar. 2014	KX385837	KX398944
L10	Batiaghata	1	Batiaghata	Khulna	Khulna	15 Sep. 2013	KX389167	
L11	Atulia	1	Shyamnagar	Satkhira	Khulna	24 Mar. 2014	KX385844	KX398947
L12	Modonpur	1	Tala	Satkhira	Khulna	25 Mar. 2014	KX385845	KX430219
L13	Mollarhat Bazar	1	Mollarhat	Bagerhat	Khulna	29 Oct. 2014		KX430220
L14	Bhanga	1	Bhanga	Faridpur	Dhaka	09 Nov. 2014	KX389172	
L15	Elenga	1	Kalihati	Tangail	Dhaka	18 Mar. 2014	KX385839	KX398945
L16	Kumrail	1	Dharmrai	Dhaka	Dhaka	19 Oct. 2014	KX389169	
L17	Thanamore	1	Dohar	Dhaka	Dhaka	21 Oct. 2014	KX389170	
L18	Bhawal National park	1	Joydebpur	Gazipur	Dhaka	17 Mar. 2014	KX385838	KX430214
L19	Nurbag	1	Kaliakoir	Gazipur	Dhaka	22 Oct. 2014	KX389171	KX430221

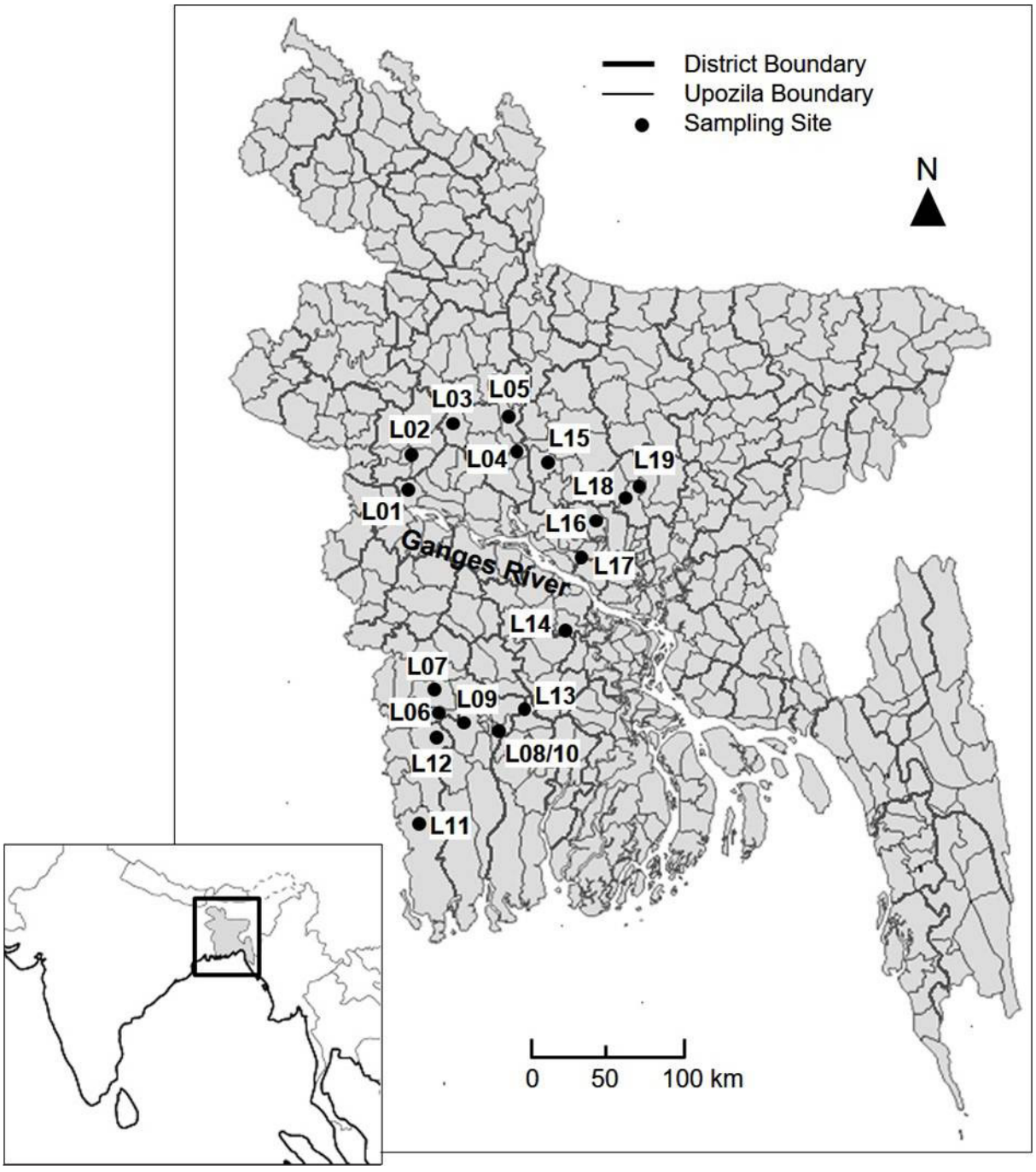

Fig 1. The sampling sites of *Oecophylla smaragdina* in Bangladesh. Locality codes correspond to those in Table 1.

annealing (50 °C for *Cytb* and 54 °C for *COI*, 1 min), and extension (70 °C, 2 min). The primers used for amplification are identical to primers reported by Crozier et al. (1994), Lunt et al. (1996), Azuma et al. (2002), and Azuma et al. (2006). Primers for the *Cytb* gene fragment were Cb1 (5'TATGTACTACCATGAGGACAAATATC'3) and tRs (5'TATTTCTTTATTATGTTTTCAAAAC'3). For the *COI* gene fragment, COI 1-3 (5'ATAATTTTTTTTATAGTTATACC'3) and COI 2-4 (5'TCCTAAAAAATGTTGAGGAAA'3) were used as forward and reverse primers, respectively (Crozier & Crozier, 1993). Illustra and ExoProStar were followed according to the instruction of the manufacturer GE Healtcare. For cycle sequencing, ABI PRISM Big Dye Terminator v3.1 cycle sequencing kits from Applied Biosystems were used in an automated sequencer. Primers for the sequencing reaction were identical to those used in the amplification step. Sequencing reaction was performed by using ABI 3100 Avant DNA Sequencer (Applied Biosystems).

For the phylogenetic analysis of western Bangladeshi *O. smaragdina* populations, 16 samples for *Cytb* and 19 samples for *COI* genes have been used with 606 bp and 775 bp, respectively. In addition, in this analysis, sequence data of both *COI* and *Cytb* were used from Azuma et al. (2002), Azuma et al. (2006) and Asaka (2010) retrieved from DDBJ GenBank. Sequence data of both *COI* and *Cytb* of *Oeocophylla longinoda* from Cameroon were used as outgroup

in this analysis. The sequencing analysis was done by using Vector NTI Advance ver. 11.5 software. Haplotypes of *Cytb* and *COI* were aligned by using MEGA 6.0 software (Tamura et al., 2013). Phylogenetic trees inferred from concatenated matrix conducted by Bayesian methods based on MrBayes 3.1.2. For the selection of best- fit model MrModeltest 2.3 was performed with PAUP*4.0 Beta version10. For both mitochondrial *COI* and *Cytb* genes, substitution model GTR + I + G with 1,000,000 generations were used. The nucleotide sequences for both *Cytb* and *COI* have been deposited in the GenBank with accession number mentioned in Table 1.

Results and Discussion

We recognized a total of 211 variable characters including 140 in *COI* and 71 in *Cytb* from *O. smaragdina* samples, of which 80 and 40 characters were parsimony informative in *COI* and *Cytb*, respectively. The phylogenetic tree obtained from the Bayesian analysis of the mitochondrial concatenated matrix dataset showed that the samples collected from the western part of Bangladesh were nested within the Indian clade (posterior probability 100%) (Fig 2). This is the first record of Indian mitochondrial haplotypes in Bangladesh.

We recognized that Indian mitochondrial haplotypes occurred on both sides of Ganges river. *Oecophylla* species may disperse via nuptial flight of queens and/or rafting method which is very effective between island to island dispersal (Peng et al., 1998). Since *O. smaragdina* is an arboreal species, the inseminated queen dispersed more likely by wind than ground-dwelling ants (Azuma et al., 2006). Thornton (1996) also reported that the relatively frequent colonization by rafting between two neighboring islands is very common.

It is interesting that our Bangladesh samples of Bhawal National Park (L18) and Nurbag (L19) were geographically close to the former sampling site of Azuma et al. (2002). All of these are located in the Gazipur District, but our samples were inferred to fall into the Indian clade. In contrast, *O. smaragdina* previously sampled from Gazipur was inferred to belong to the Southeast Asian clade (Azuma et al., 2002). Provided that our results are inconsistent with the result previously reported by Azuma et al. (2002) it is possible that: (1) there might be a misidentification of former Bangladesh samples caused by contamination, (2) the former record of Southeast Asian type in Bangladesh might be an exceptional case. In our opinion, both the first and second cases are less plausible because additional samples from Bangladesh were inferred to belong to the Southeast Asian clade (Asaka, personal communication). The third possible case is that the materials of Azuma et al. (2002) would express the western geographic distribution limit of Southeast Asian mitochondrial haplotypes.

We here confirm that *O. smaragdina* populations with Indian mitochondrial haplotypes exist in the western part of Bangladesh. The result of present study suggested the importance of comprehensive surveys, also taking into account the central and eastern populations of Bangladeshi *O. smaragdina*.

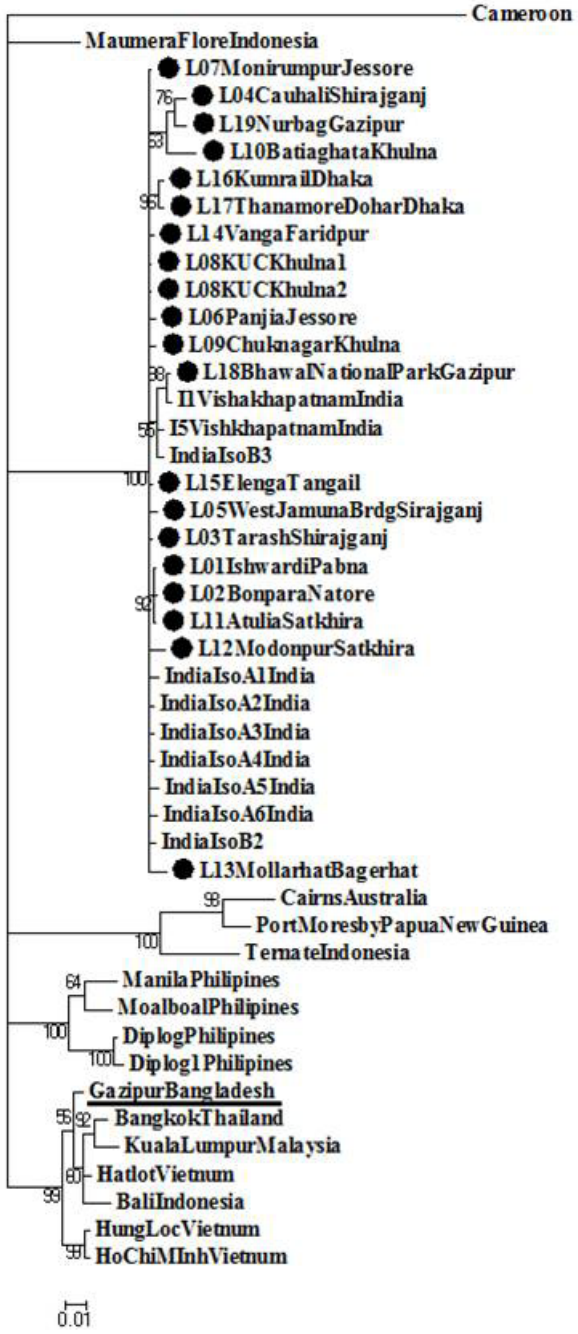

Fig 2. Bayesian phylogenetic tree of Bangladeshi *O. smaragdina* populations as inferred from the mitochondrial gene fragments of the *COI* and the Cytb genes. Numbers adjacent to internal nodes represent bootstrap values (%). Black circles indicate the samples from Bangladesh in the present study. Additional DNA sequence data were downloaded from DDBJ GenBank. The underlined locality shows the Bangladeshi sample in the previous study of Azuma et al. (2006). The number ahead of each locality indicates the locality number.

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