

Phylogenetic Study and Divergence of Weaver Ant, *Oecophylla smaragdina* Fabricius in Bangladesh (Hymenoptera: Formicidae)

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Abstract: The weaver ant species, *Oecophylla smaragdina*, is widely distributed from India through Southeast Asia to northern Australia including many tropical Western Pacific islands. Recent phylogenetic study of *O. smaragdina* showed that the species is divided into 7 groups based on mtDNA and the Bangladesh populations belong to SE Asian mainland clade despite its geographical proximity to India. The present study aims to reveal the phylogenetic aspect of *O. smaragdina* in Bangladesh with its divergence time estimation based on extensive materials. Sampling was executed according to zonation of 5 areas which are demarcated by 3 main rivers and a total of 95 colonies from 87 localities of 47 Districts were collected during 2013 to 2018 from Bangladesh. The molecular analyses, using 2 mitochondrial loci: Cytochrome b oxidase subunit 2 (Cytb) consisting of 507 bp and Cytochrome c oxidase subunit I (COI) consisting of 639 bp done in Ku, Japan. The analysis of 84 colonies revealed that 47 are the Indian clade (Indian type) and 37 are the SE Asian mainland clade (SE Asian type). The distribution of mtDNA types showed the occurrence of both the two types with some overlapping distribution in the central part of Bangladesh. The divergence time analysis in Bangladesh populations, resulted that Indian type diverged ca. 2.2 Ma and SE Asian type diverged ca. 0.20 Ma corresponding to early to late Pleistocene. This study suggested a comprehensive phylogenetic scenario of *O. smaragdina* in Bangladesh.

Keywords: Distribution Pattern, Divergence Time, Phylogeny, Mitochondrial DNA, Weaver Ant

1. Introduction

The weaver ant, *O. smaragdina* is a broadly distributed genus and has the ability to disperse over ocean islands without human interventions. Azuma et al. first analyzed populations of *O. smaragdina* using molecular data and samples, including the *O. smaragdina* from Bangladesh [3]. In addition, she added comprehensive samples of *O. smaragdina* from India, Southeast Asia and Australia, Azuma et al. 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 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 [4]. Hereafter we will refer the Azuma et al. group 1 as Indian type and Azuma et al. group 2 as SE Asian type [4]. Asaka extended the survey of *O. smaragdina* to South Asia and collected several samples from India and Sri Lanka [1]. Her phylogenetic analysis showed that all analyzed samples belong to Indian clade with low levels of sequence divergence. Azuma et al. characterized the mitochondrial sequence identity of the Bangladesh populations as belonging to the Southeast Asian clade in spite of the geographical proximity of Bangladesh to India [4]. They hypothesized the existence of an Indian refuge for the independence of group 1 from group 2 and concluded that if

the Indian population originated from the Indian refuge and Bangladesh. 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.* are geographically bordered by the sea [4]. In recent phylogeographic study, Rahman *et al.* identified the western Bangladeshi populations as Indian type and some parts of the central area has the overlapping population of both Indian and SE Asian type [22, 23]. This is the first report of occurring Indian type in Bangladesh and this evidence proved that in Bangladesh, there is no such border to restrict the occurrence of Indian type in Bangladesh. A recent study about the site records for *O. smaragdina* in Bangladesh has showed the same results [31]. Similar trends were also observed in the case of Asian elephants, where two highly divergent mtDNA of Asian elephants overlapped geographically due to secondary contact after glaciation during Pleistocene [30].

Oecophylla smaragdina, based on the fossil record 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 [10]. The abrupt cooling during this period had great impact on biodiversity [14]. During this period, Earth's climate shifted from a relatively ice-free world to one with glacial conditions in polar regions characterized by substantial ice sheets [6]. Recently, Blamier *et al.* estimated the divergence time of the genus *Oecophylla* based on the fossil records and ultra-conserved elements (UCEs) [5]. They estimated that *Oecophylla* crown group evolved during Oligocene at ca. < 30 Ma and stem-group evolved during early Eocene at ca. 50 Ma. Wetterer gave a glimpse of distribution of *O. smaragdina* in this continent with some interesting evidence of distribution [31]. *Oecophylla smaragdina* and *O. longinoda* have diverged in 13.3 to 11.3 Ma ago, in the late Miocene. Diversification of seven groups occurred between the middle of Miocene to early Pliocene. While the diversification within groups was recorded between middle Pliocene to early Pleistocene [4]. There was no evidence regarding the divergence time of group 1 or Indian types. Due to widespread distribution, *O. smaragdina* was considered valuable material for biogeographic study in tropical Asia.

The purpose of the present experiment was to examine the phylogenetic relationship of weaver ant populations from northern, eastern and central parts of Bangladesh for covering its distribution range in the country and is to get insights into the estimation of the time of divergence of Indian and SE Asian types of *O. smaragdina* in Bangladesh. The previous sampling by Azuma *et al.* was limited to only one site of Nurbag, Gazipur, located at the central part of Bangladesh, distinguished as Southeast Asian clades [4]. However, *O. smaragdina* from the western parts of Bangladesh were identified as Indian clade. This result

suggested the importance of phylogenetic study of central and eastern Bangladeshi populations to identify the nature of distribution with its divergence history.

2. Materials and Methods

2.1. The Setting of the Study Areas and Its Characterizations

Based on three main rivers, Ganges, Jamuna, and Meghna, the study area in Bangladesh was broadly categorized into five areas under 8 divisions. The detailed information of that 5 broad areas is presented in the following figure (Figure 1).

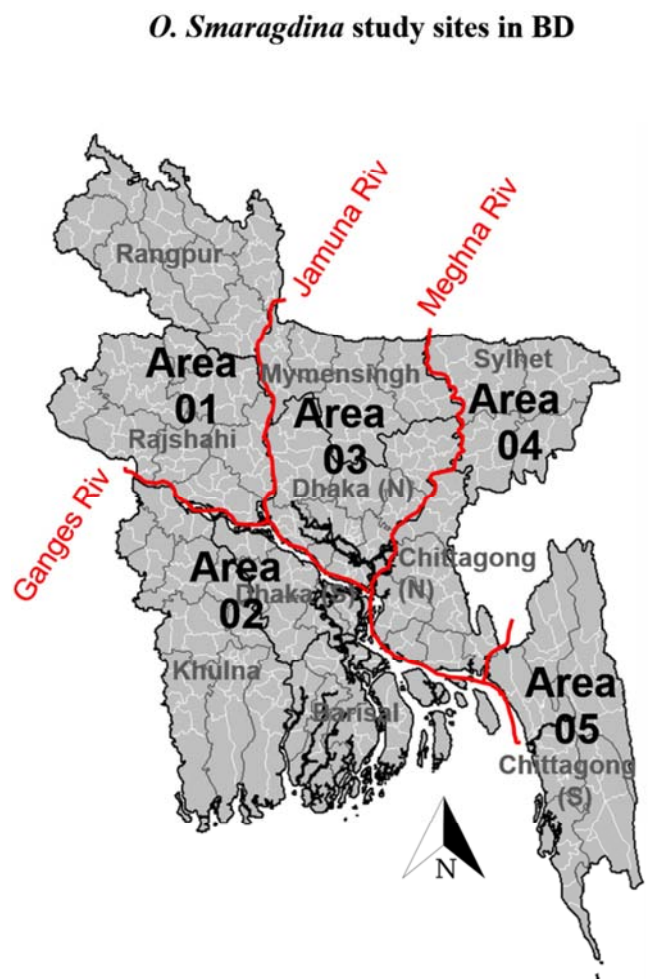


Figure 1. Sampling sites of 5 broad areas based on three main rivers in Bangladesh. The red line across the map showing the river flow that separated 5 broad areas in Bangladesh.

Area 01 included the northwestern part of Bangladesh. This area include 16 districts under 2 divisions. The climate of this area is comparatively dry with very hot summer and cold winter to other areas. This area is surrounded by the river Ganges and Jamuna. The study area lies between 24°07' N to 25°13' N latitude and 88°00' E to 89°10' E longitude [32]. This northwestern part of Bangladesh has been evolved during Pleistocene Terraces due to tectonic upliftment and /or exists as an erosional geomorphic feature [24]. Area 02 was

located at the western districts of the country consisting Khulna, Barisal and western part of Dhaka divisions. The important feature of this area is the existence of Mangrove forest, "The Sundarbans". So, this area possesses some unique vegetation. The lower boundary of that area 02 is flooded by the Bay of Bengal. The major river channels are derived from this Bay of Bengal along with the flow of Ganges. Area 03 was located in the central and northern part of the country with the northern part of Dhaka division and Mymensingh division. The major characteristics of this area is the availability of Shal forest and it is mainly covered by the plain land. This area is characterized by Pleistocene upliftment resulted Bhawal and Madhupur tract [25]. Some hilly areas including tectonically uplifted blocks created during Pleistocene are also the major characteristics in this area. This area is developed on the Ganges-Brahmaputra delta on late quaternary stratification [13]. Area 04 was located in the northern part of Chittagong division and Sylhet. Northern Chittagong is a plane landed area with plenty of fresh water sources and many rivers crisscross across while Sylhet division is mountainous with enormous number of tea garden with natural water fall. The major geological feature of that area is the Bengal basin. The Surma Basin is a sub-basin of the Bengal Basin situated in the northeastern part of Bangladesh. The basin is bounded on the north by the Shillong plateau, east and southeast by the Chittagong-Tripura fold belt of the Indo-Burman ranges, and west by The Indian Shield platform. This area is mainly created during Holocene [18]. Area 05 includes the southern Chittagong division. This area is divided from other regions of Bangladesh by the border of Meghna rivers. It is a mountainous area and the longest sea beach, Coxsbazar, is situated in this area. The area is mainly composed of Hill Tracts (CHT) in the south-eastern part of Bangladesh encompassing three hill districts: Rangamati, Khagrachari and Bandarban. It shares borders with Myanmar on the south and southeast, India on the north and northeast, and the Chittagong district of Bangladesh on the west. CHT is located between 21°40' degrees and 23°47' degrees north latitude and 91°40' degrees and 92°42' degrees east longitude.

2.2. Localities of *O. smaragdina* Sampling in Bangladesh

A total of 95 *O. smaragdina* colonies had been collected from 87 localities of 47 districts belonging to 8 divisions of Bangladesh during September 2013 to December 2018. The locality position and detailed information are presented in Figure 2 and Table 1.

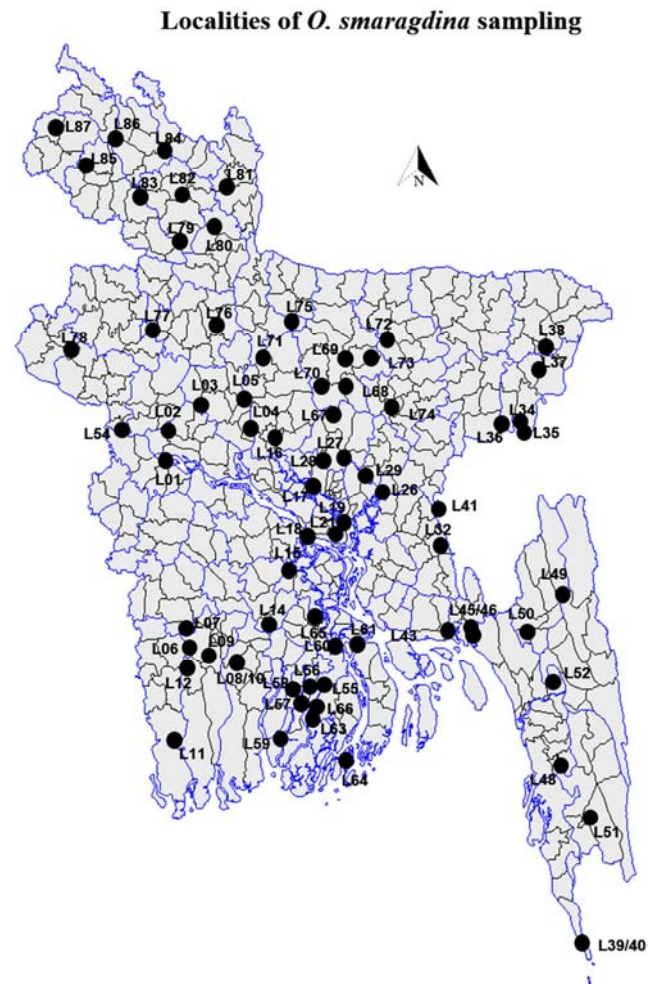


Figure 2. Locality based sampling sites of *Oecophylla smaragdina* in Bangladesh.

Table 1. Detailed locality information of *O. smaragdina* sampling sites in Bangladesh.

Locality No.	Locality Name	District	Division	Area Code	GenBank accession no.		GPS Coordinatoin	
					Col	Cytl	N Latitude	E Longitude
L01	Ishwardi	Pabna	Rajshahi	A01	KX385842	KX430217	24°06'52"	89°04'52"
L02	Bonpara	Natore	Rajshahi	A01	KX385843	KX430218	24°16'29"	89°05'19"
L03	Tarash	Sirajganj	Rajshahi	A01	KX385841	KX430216	24°21'59"	89°21'40"
L04	Chauhali	Sirajganj	Rajshahi	A01	KX389168	KX398946	24°30'61"	89°72'73"
L05	w side of Jamuna Bridge	Sirajganj	Rajshahi	A01	KX385840	KX430215	24°23'43"	89°44'44"
L06	Panjia	Jessore	Khulna	A02	KX371575	KX398943	24°15'	89°30'
L07	Monirampur	Jessore	Khulna	A02	KX355139	KX430212	24°15'	89°30'
L08	Khulna Univ. Campus	Khulna	Khulna	A02	KX379493/ KX379494	KX398942	24°46'	89°31'
L09	Chuknagar	Khulna	Khulna	A02	KX385837	KX398944	22°49'	89°18'
L10	Batiaghata	Khulna	Khulna	A02	KX389167		24°45'	89°31'
L11	Atulia	Satkhira	Khulna	A02	KX385844	KX398947	24°16'	89°03'
L12	Modonpur	Satkhira	Khulna	A02	KX385845	KX430219	24°46'	89°03'
L13	Mollahat Bazar	Bagerhat	Khulna	A02		KX430220	24°01'16.6"	90°17'53.7"
L14	Pachuria	Gapalgonj	Dhaka	A02	KY618816		23°00'3.6"	89°49'13.8"

Locality No.	Locality Name	District	Division	Area Code	GenBank accession no.		GPS Coordinatoin	
					Col	Cytb	N Latitude	E Longitude
L15	Bhanga	Faridpur	Dhaka	A02	KX389172		23° 23' 0"	89° 59' 0"
L16	Elenga	Tangail	Dhaka	A03	KX385839	KX398945	24°23'01"	89°50'05"
L17	Kumrail	Dhaka	Dhaka	A03	KX389169		23°13'26.1"	90°13'26.1"
L18	Thanamore	Dhaka	Dhaka	A03	KX389170		23°36'49.4"	90°07'13.3"
L19	Ruhitpur	Dhaka	Dhaka	A03		KY562567	23°40'07"	90°17'54"
L20	Baluakandi	Munshiganj	Dhaka	A03	MG967475		23°38'57.2"	90°35'8.2"
L21	Nimitali	Munshiganj	Dhaka	A03	KY628426		23°36'55.3"	90°19'55.6"
L22	Bejgaon	Munshiganj	Dhaka	A03		KY583087	23°32'21.3"	90°17'48.2"
L23	Nababganj Bazar	Narayanganj	Dhaka	A03		MG967471	23°39'39.2"	90°09'41.9"
L24	Shiddirganj	Narayanganj	Dhaka	A03		KY562571	23°41'41.8"	90°29'26.5"
L25	Vulta	Narayanganji	Dhaka	A03		KY562573	23°46'50"	90°34'16.5"
L26	Panchdona	Norsingdi	Dhaka	A03	KY657490	KY562569	23°53'36.2"	90°34'16.5"
L27	Bhawal National Park	Gazipur	Dhaka	A03	KX385838	KX430214	24°04'43"	90°24'06"
L28	Nurbag	Gazipur	Dhaka	A03	KX389171	KX430221	24°01'16.6"	90°17'53.7"
L29	Charpara	Gazipur	Dhaka	A03	KY628425		23°55'31.9"	90°36'0.8"
L30	Zamirarchala	Gazipur	Dhaka	A03			24°06'6.2"	90°28'25.8"
L31	Rajbari	Gazipur	Dhaka	A03		KY562568	24°03'5.7"	90°23'17.8"
L32	Nimshar	Comilla	Chittagong	A05	MG967473	MG967472	23°06'6.2"	90°28'25.8"
L33	Madhoyia	Comilla	Chittagong	A05			23°30'08.5"	91° 0' 30"
L34	Tea Resort Center	Moulvibazar	Sylhet	A04	KY618809	KY583084	24°18'7.2"	91°45'34.92
L35	Lauachra National Park	Moulvibazar	Sylhet	A04	KY618815	KY583085	24°18'05.7"	91°45'41.0"
L36	Bahubal	Habiganj	Sylhet	A04	KY618818	KY583083	24°22'49"	91°24'46.0"
L37	Tarau	Sylhet	Sylhet	A04	KY618817		24°19'42.6"	91°47'05.2"
L38	Doradarpur	Sylhet	Sylhet	A04	KY618810	KY657484	24°46'40.0"	91°47'01.8"
L39	Marishbunia	Cox's Bazar	Chittagong	A05	MG967474		21°05'25"	92°20'
L40	Noakhali para	Cox's Bazar	Chittagong	A05			24°46'40.0"	91°47'01.8"
L41	Mondabag	Bramhanbaria	Chittagong	A04	KY608802	KY657492	23° 44' 0"	91° 10' 0"
L42	Jogotpur	Comilla	Chittagong	A04			23° 33' 0"	91° 7' 36"
L43	Sebarhat	Noakhali	Chittagong	A04	KY608803	KY550396	22° 58' 59.88"	91° 13' 59.88"
L44	Senbag Upozilla Hospital	Noakhali	Chittagong	A04		KY657493	22° 58' 59.18"	91° 13' 59.08"
L45	Mohipal	Feni	Chittagong	A04	KY618811	KY550397	23° 1' 0.89"	91° 23' 30"
L46	Mohipal Primary School	Feni	Chittagong	A04	KY628427	KY550398	23° 1' 1.10"	91° 23' 50"
L47	Raujan Bazar	Chittagong	Chittagong	A05		KY550399	22° 32' 0"	91° 56' 0"
L48	Satkania	Chittagong	Chittagong	A05	KY657489	KY550400	22° 6' 12"	92° 4' 50"
L49	Dighinala HRC	Khagrachari	Chittagong	A05	KY608804	KY550401	23° 15' 30"	92° 3' 30"
L50	Matiranga Dhibi	Khagrachari	Chittagong	A05	KY608805	KY550402	23° 2' 30.12"	91° 52' 30"
L51	Ruma Karai	Bandarban	Chittagong	A05	KY657488	KY550403	22° 3' 0"	92° 25' 0.12"
L52	Kawkhali Bazar	Rangamati	Chittagong	A05	KY583089		22° 32' 0"	92° 1' 0"
L53	Patia	Chittagong	Chittagong	A05		KY562572	22° 18' 0"	91° 59' 0"
L54	Thanapara Sadah	Rajshahi	Rajshahi	A01	KY628429		24° 17' 0"	88° 46' 30"
L55	Nalchiti prim. sch. field	Jhalokati	Barisal	A02	KY618814	KY657585	22° 37' 19.92"	90° 16' 14.88"
L56	BRAC more	Jhalokati	Barisal	A02	KY657491	KY550389	22° 38' 36"	90° 12' 0"
L57	Baghribazar	Jhalokati	Barisal	A02	KY618813	KY550388	22° 40' 0.12"	90° 8' 30.12"
L58	Kawkhali Upz P Chottor	Pirojpur	Barisal	A02	KY583090	KY550390	22° 37' 13.08"	90° 4' 9.84"
L59	Shakharikathi	Pirojpur	Barisal	A02	KY583091	KY550391	22° 17' 12.84"	89° 58' 0.12"
L60	Rupatoli	Barisal	Barisal	A02		KY550395	22° 48' 0"	90° 30' 0"
L61	Patarhat	Barisal	Barisal	A02	KY583092	KY550392	22° 49' 54.84"	90° 31' 59.88"
L62	Barguna Sadar bus stand	Barguna	Barisal	A02		KY550393	22° 9' 2.88"	90° 7' 35.04"
L63	PSTU	Patuakhali	Barisal	A02		KY550394	22° 26' 0"	90° 22' 0"
L64	Panpatti	Patuakhali	Barisal	A02	KY583093	KY583088	22° 9' 48"	90° 25' 48"
L65	Agailjhara Uni P office	Barisal	Barisal	A02	KY628430		22° 58' 0"	90° 9' 0"
L66	Mohespur	Barisal	Barisal	A02	KY618812	KY657486	22° 33' 0"	90° 20' 18"
L67	Bhaluka Bazar	Mymensingh	Mymensingh	A03	KY657499		24° 22' 30"	90° 22' 42"
L68	Trishal Primary School	Mymensingh	Mymensingh	A03	KY657500		24° 34' 30"	90° 23' 30"
L69	BAU Campus	Mymensingh	Mymensingh	A03	KY657501		24°45'8.39"	90°24'6.59"
L69	BAU, Sesh Matha More	Mymensingh	Mymensingh	A03	KY657502		24°45'8.39"	90°24'6.59"
L70	Nandail	Mymensingh	Mymensingh	A03	KY657503	KY657505	24° 34' 0"	90° 41' 0"
L71	Sarisha Bari High School	Jamalpur	Mymensingh	A03	KY657504	KY657506	24° 44' 30"	89° 50' 0"
L71	Bazar Pukur	Jamalpur	Mymensingh	A03		KY65707	24° 44' 32"	89° 50' 01"
L72	Sadar Hospital	Netrokona	Mymensingh	A03	KY657494		24° 52' 30"	90° 44' 0"
L72	Sadar Primary School	Netrokona	Mymensingh	A03	KY657495		24° 52'28"	90° 44' 03"
L73	Gauripur Upz Complex	Mymensingh	Mymensingh	A03	KY657496		24° 45' 30"	90° 34' 30"
L74	Higher par	Kishorgonj	Mymensingh	A03	KY657497		24° 25' 59.88	90° 46' 59.88"
L75	Sadar Thana more	Sherpur	Mymensingh	A03	KY657498		25° 0' 0"	90° 1' 0"
L76	Dhunat Upz. Chattor	Bogra	Rajshahi	A01	MG873538	MG886852	24°41'10.00"	89°32'0.03"
L77	Municipality orchard	Naogaon	Rajshahi	A01	MG873539	MG886851	24°48'18.00"	88°57'0.18"

Locality No.	Locality Name	District	Division	Area Code	GenBank accession no.		GPS Coordinatoin	
					CoI	Cytb	N Latitude	E Longitude
L78	Nijampur	Chapainawabganj	Rajshahi	A01	MG873540	MG886850	24°43'48.03"	88°25'12.13"
L79	Hakimpur Nursery	Dinajpur	Rangpur	A01	MG873541	MG886847	25°16'59.88"	89°1'0.12"
L80	Shibpur	Gaibandha	Rangpur	A01	MG873542	MG886849	25°07'59.88"	89°23'30.12"
L81	Ghariaidanga	Kurigram	Rangpur	A01	MG873543		25°48'00"	89°33'0.02"
L82	Rasulpur School ground	Rangpur	Rangpur	A01	MG873544	MG886845	25°24'54"	89°19'0.10"
L83	Saidpur airport surrounding	Nilfamari	Rangpur	A01	MG873545		25°47'0.04"	88°54'0.02"
L84	Barobala	Rangpur	Rangpur	A01	MG873546	MG886846	25°32'30"	89°17'0.00"
L85	Pirganj fire station orchard	Thakurgaon	Rangpur	A01	MG873547	MG886848	25°51'15.12"	88°22'0.12"
L86	Debiganj bus stand	Panchagar	Rangpur	A01	MG873548	MG886844	26°07'9.89"	88°45'33.47"
L87	Atwari sadar thana more	Panchagar	Rangpur	A01	MG873549		26°18'29.88"	88°27'29.88"

2.3. Sample Preparation

The collected samples from Bangladesh were analyzed for sequencing in the laboratory Institute of Tropical Agriculture of Kyushu University, Japan. The collected colonies of *Oecophylla smaragdina* were preserved in 99% ethanol prior to DNA extraction.

2.4. Molecular Study

Genomic DNA was extracted from the legs of specimens that were preserved in alcohol by using QIAGEN DNeasy Blood and Tissue kit (Qiagen, Maryland, USA). Amplification of both mitochondrial and nuclear DNA was done by polymerase chain reaction (PCR). The primers used for amplification are identical to primers reported by Crozier et al., Lunt et al., Azuma et al., and Azuma et al. [9, 17, 3, 4]. For, mitochondrial DNA analysis, Primers for the Cytb gene fragment were Cb1 (5'TATGTACTACCATGAGGACAAATATC'3) and tRs (5'TATTTCTTTATTATGTTTTCACAAAC'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 by Crozier and Crozier [8]. The thermal cycling parameters for Cytb and COI basically followed the protocols established by Crozier and Crozier and Sameshima et al., including 95°C for 5 min for initial denaturation, 35 cycles of dissociation (92°C, 1 min), annealing (50°C for Cytb and 54°C for COI, 1 min), and extension (70°C, 2 min) [8, 28]. Illustra ExoProStar was followed according to the instruction of the manufacturer GE Healthcare. For cycle sequencing, ABI PRISM Big Dye Terminator v 3.1 cycle sequencing kits from Applied Biosystems were used in an automated sequencer. Sequencing reactions were performed by using ABI 3100 Avant DNA Sequencer (Applied Biosystems).

2.5. Phylogenetic and Divergence Tree Inference

For the phylogenetic analysis of *O. smaragdina* populations, combination of mitochondrial cytochrome b and cytochrome oxidase subunit-1 gene of 1143 bp were used in the analysis. Of which, 63 samples for Cytb and 72 samples for COI genes have been used with 504 bp and 639 bp, respectively. In addition, sequence data of both COI and Cytb were used from Azuma et al., Azuma et al. and Asaka as reference [3, 4, 1]. The sequence

data of both COI and Cytb of *Oecophylla longinoda* from Cameroon were used as outgroup in this analysis. The sequencing alignment was done by using Vector NTI Advance ver. 11.5 software. The sequences of Cytb and COI were aligned by using MEGA 6.0 software [29]. Phylogenetic trees were inferred from 106 concatenated matrix sequences of both COI and Cytb genes, conducted by MrBayes 3.1.2 with 1,000,000 generations [19, 27, 12]. For the selection of best-fit model, MrModeltest 2.3 was performed with PAUP*4.0b10. [21, 20]. The substitution model, GTR + I + G was used in mitochondrial COI and Cytb genes were used the alignment was partitioned into 1st, 2nd and 3rd nucleotide positions. The nucleotide sequences for both Cytb and COI were deposited in the GenBank with accession number are corresponding to Table 1. For estimating the divergence time of *O. smaragdina* in Bangladesh, a total of seven localities nucleotide sequence data of Cytochrome oxidase subunit 1 (COI) and Cytb data were used. Those seven localities include L07, L08, L26, L32, L41, L49, L66, respectively. The detailed locality information are presented in Table 1. Like the phylogeny analysis, in this case, sequence data of both COI and Cytb were used from Azuma et al., Azuma et al. and Asaka retrieved from DDBJ GenBank with the samples from Malaysian and Cameroon as references [3, 4, 1]. The sequences of *Oecophylla 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 [29]. A total of 1149 bp of COI and Cytb nucleotide sequences of the samples collected from those 7 localities were used along. A Bayesian Markov chain Monte Carlo (MCMC) packages [11], which relies on a relaxed molecular clock approach, were used to estimate the divergence time. Optimal nucleotide substitutional model were chosen by Mr. ModelTest.

3. Results

3.1. Bayesian Phylogenetic Tree Inferences

Among identified 197 variable characters, 133 were parsimony informative. The Bayesian analysis of the mitochondrial concatenated matrix dataset of 1143 bp showed that the Bangladeshi *O. smaragdina* samples were nested into two distinct clades (posterior probability > 90%) (Figure 3). Bangladeshi 48 weaver ant samples were nested with Indian clade of *O. smaragdina*, whereas 39 samples were nested with the Southeast Asian clades. Therefore, the occurrence of both

the Indian and SE Asian types was found within Bangladesh. Based on the phylogenetic tree obtained, the Bangladeshi populations showed the overlapping distribution of the Indian and SE Asian types of *O. smaragdina* (Figure 3).

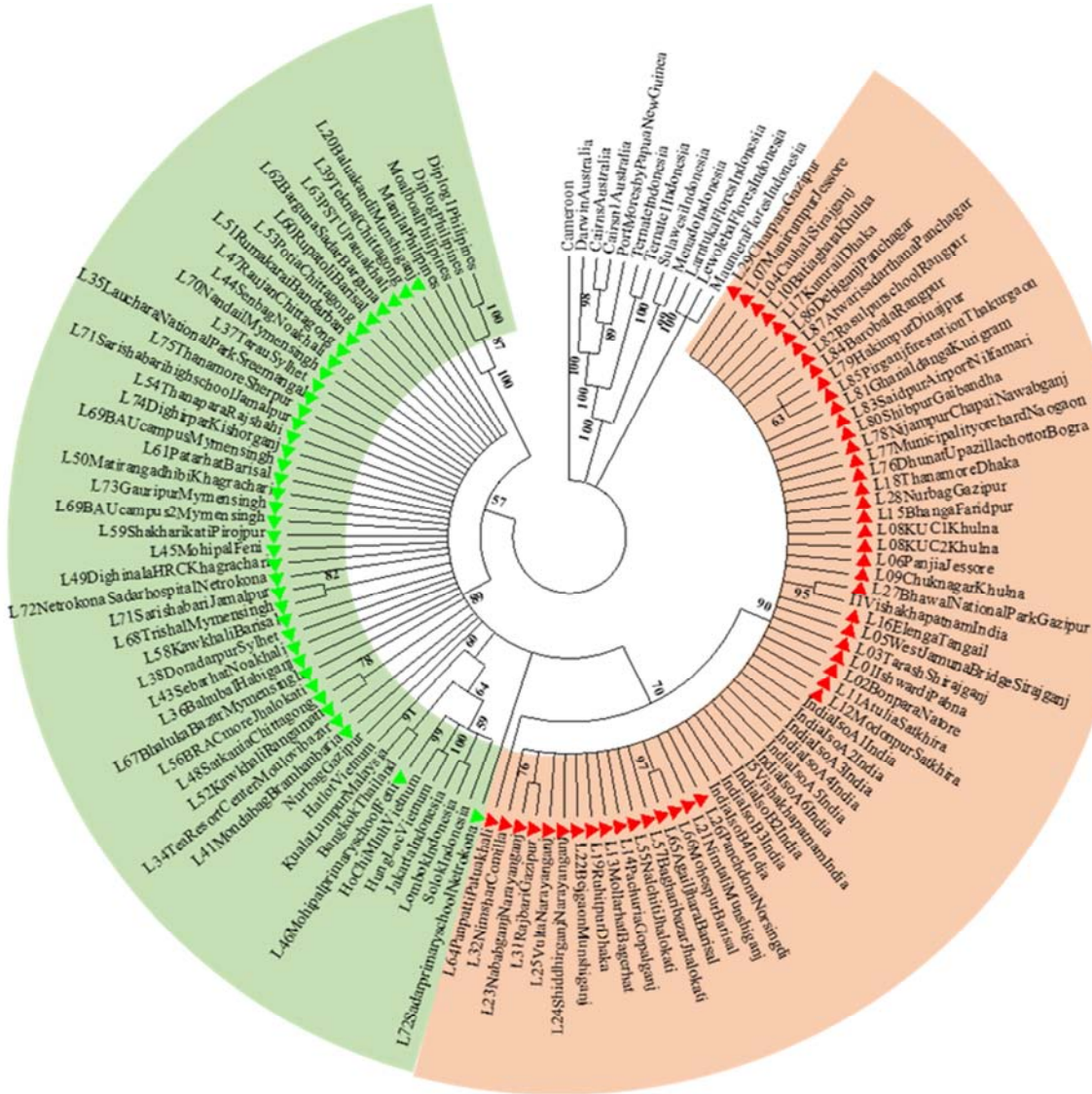


Figure 3. Bayesian phylogenetic tree of Bangladeshi *O. smaragdina* populations as inferred from the mitochondrial gene fragments (1143bp) of the COI (639 bp) and the Cytb (504 bp) genes. The number ahead of each locality indicates the locality number. Red and green triangle denotes the samples from Bangladesh clustering with Indian and SE Asian types, respectively.

3.2. Summary of the Distribution Pattern

The western part of Bangladesh was mainly occupied by Indian types, the Eastern part was dominated by SE Asian types, while, the mixture of two clades was found in the central parts of Bangladesh (Figure 4). The detailed samples

results based on type are showed in Table 2. Out of 87 localities, we failed to detect 3 locality types of *Oecophylla*. The samples from rest 47 localities were identified as Indian type and 37 locality samples as SE Asian types (Table 2).

Table 2. Detailed summary of the distribution pattern of weaver ant in Bangladesh.

Sampled area	Surveyed district	Sampled colonies	Sampled localities	Type (in locality)		Failed
				Indian	SE Asian	
Area 01	14	18	18	17	1	
Area 02	11	23	22	15	7	
Area 03	10	30	25	13	11	1
Area 04	7	15	13	2	10	1
Area 05	5	9	9	0	8	1
Total	47	95	87	47	37	3

Malaysian and Indian haplotypes were used as reference. Although these divergence tree is not showing quite a good resolution due to lack of so many resolved sister groups but we can get some interesting evidence. According to this divergence tree, Indian type diverged ca. 2.2 Ma and SE

Asian type diverged ca. 0.20 Ma corresponding to early to late Pleistocene.

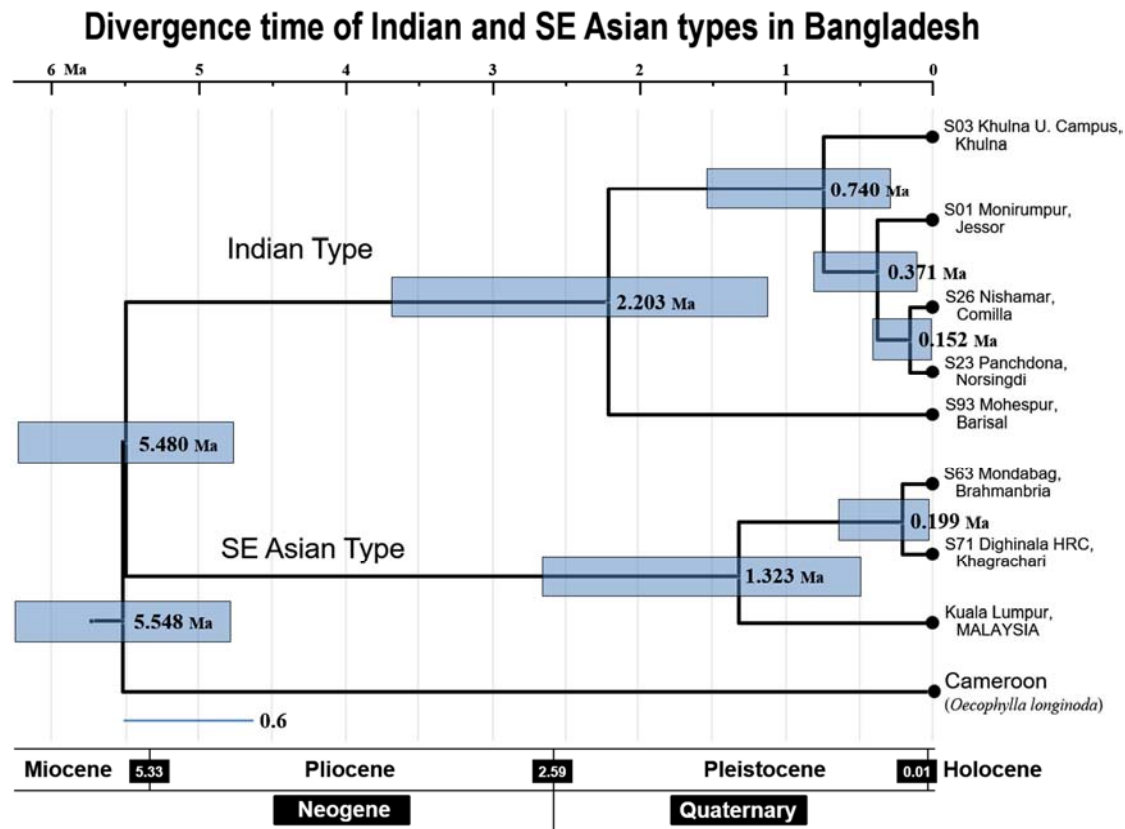


Figure 5. Divergence time of Indian and SE Asian types of *O. smaragdina* in Bangladesh.

4. Discussions

The result of distribution of *O. smaragdina* based on mtDNA showed that the western parts of Bangladesh are predominantly occupied by the Indian type, whereas the eastern part by the SE Asian type, and that the central parts is the mixture of both types. The study does not show the clear boundary, but suggests the central part as a transition zone. Divergence time of the ant genus *Oecophylla* is thought to be a significant factor of such distribution. Diversification within groups in this continent was recorded from the Middle Pliocene to Early Pleistocene [3]. After this period, the world has encountered a significant climatic change. It might also affect the distribution of *Oecophylla* in different parts of the world. Lokkers suggested two limiting factors, low temperature and humidity for distribution range of *Oecophylla* in Australia [15]. From this tree, we get the information regarding the divergence time of the Indian type that was missing in the previous study by Azuma *et al.* [3]. The quaternary paleo-geographic history of the SE Asia during the ice age showed some significant evidence of the divergence pattern. Vidya *et al.* discussed the distribution of the two clades of Asian elephants and proved the effect of allopatricity in different glacial refugia, the alpha clade in the Myanmar region and the beta clade possibly in southern India–Sri Lanka, 1.6–2.1 Ma ago [30]. Results from nested clade and dispersal–vicariance analyses indicate a subsequent

isolation and independent diversification of the b clade in both Sri Lanka and the Sunda region, followed by northward expansion of the clade. Pleistocene glaciation has significant influential effects on temperature and rainfall that favored the diversification of several ant species including *Oecophylla* distribution [15]. Rainfall moderate the vegetation density while low temperature inhibiting larval development. As Pleistocene glaciation had the effect on both these two limiting factors of *O. smaragdina* distribution so the divergence resulted [15]. According to our study, diversification of both the types occurred in Pleistocene. There is a rapid fluctuation of temperature from late Pliocene to Pleistocene. It might influenced the distribution of *Oecophylla* in different part of the world. During that time, fragmentation and expansion of *Oecophylla* population happened. During LGM, the tropic changed from North to southward and it retained to north after glaciation. Thus, Bangladesh become vacant after glaciation with the tropical climate and hence the occurrence of the both the types have been found subsequently. Effects of temperature and rainfall influence the distribution of *O. smaragdina* as the combination of high temperature and high rainfall is necessary to continue the weaver ant growth rate and distribution, and to a limited extent, higher temperatures can compensate for lower than optimal rainfall levels, and vice versa [15]. During the glaciation period the temperature had fallen down nearly 10°C in Vostok might have significant

influence on the distribution of several species like *Oecophylla* [16], which is markedly affected by low temperature for their colony development. Due to several glaciation during Pleistocene could be a key factor of such effects. Weterrerr showed a comprehensive distribution pattern of *O. smaragdina* in the arid, tropical and subtropical climatic region that also shed light on the effects of temperature on the distribution [31].

The occurrence of two types (Indian type and SE Asian type) in Bangladesh will be made by the following dispersal scenario: The disjunction of populations would occur several times as in shown of divergent time in 7 major groups of mtDNA, and the isolated populations may develop their unique haplotypes in India, in SE Asia, or others during the history of *O. smaragdina* which was estimated to diverged from *longinoda* about 13 - 11 Ma [4, 5]. Before the Last Glacial Maximum (LGM: 25,000 - 15,000 BP) the haplotypes observed in Bangladesh would be already established. During the LGM, the Bengal Delta was supposed to be vacant for *Oecophylla smaragdina* distribution because the northern limit of the species would be located down to the south. After the LGM, the populations expanded to north, and because of the geographical shape the east and west populations met in the Bengal Delta. When the two types of population groups met again in the Bengal Delta, each of the types already contained several haplotypes of mtDNA. This vacant-reunion hypothesis requires a temperature drop in LGM, and diversification of populations before LGM. The present distribution of *Oecophylla smaragdina*, in particular, the northern limit of distribution almost agrees with the isothermal line of 10°C in the average temperature of January. Braconnot et al. estimated the mean temperature of the coldest month (MTCO) in Asia is around 10°C below [7]. Although northern limit of distribution of *O. smaragdina* in LGM is not clear, the Bengal Delta is supposed to be out of the limit. After LGM, as the climate changed to warmer, the populations would extended to northward and come to Bengal Delta simultaneously. The populations extended to Bengal Delta were not uniform, because each of mtDNA types have a history of diversification in somewhere south in India and SE Asia. The diversification time was estimated in the Pliocene to Pleistocene. The recent phylogeographic studies on insects, plants, amphibians or fishes shown the significance of Pleistocene climate changes and biogeographic barriers like mountains, rivers, seas and desserts alter the diversification, radiation and isolation of new genetic lineages within many species [26]. This climatic oscillation played the major role of forming such distribution of species by changing the genetic structure and diversity [2]. The case of the distribution pattern of *O. smaragdina* in Bangladesh can be correlated with the distribution pattern of Asian elephants. Vidya et al. explained that in the case of the elephant haplotypes in Myanmar, rather suggested that these haplotypes did not arise within Myanmar, but instead resulted from a northward range expansion of beta clade haplotypes during warm period from both Sri Lanka and the Sunda region followed by subsequent admixture in this region [30].

The Bengal Basin, which is the major basin of Bengal formation [18], began 127 million years ago that after the age of glaciation, as a new vacant area with newly growing vegetation favored by the tropical climate facilitate the chance of occurrences of both the types.

The results of the study suggest several patterns of dispersals of both Indian and SE type of *O. smaragdina* scenario correlated with the time age of Last Glacial Maximum (LGM).

5. Conclusions

Oecophylla smaragdina has the evolutionary importance in Bangladesh. Based on the phylogenetic study inferred by mitochondrial COI and Cytb genes, it was resulted that western Bangladesh population of *O. smaragdina* is dominated by Indian type, the eastern Bangladesh population are of SE Asian types and the mixture of the two types were found in the central parts of Bangladesh that may lead to hybridized scenario. The divergence time analysis resulted that Indian type diverged ca. 2.2 Mya and SE Asian type diverged ca. 1.32 Mya corresponding to early to late Pleistocene.

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