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GENETIC DIVERSITY OF THE CIGARETTE BEETLE, *LASIODERMA SERRICORNE* (FABRICIUS), DERIVED FROM MITOCHONDRIAL DNA SEQUENCES

RUKHSANA, K. AND SEBASTIAN, C. D.*

Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India

ABSTRACT

The cigarette beetle, *Lasioderma serricorne* (Fabricius) is one of the major pests for stored tobacco which is widely distributed in the world. It is a pest of many stored products like spices, pet food, dried flowers etc. This study highlights the DNA barcoding and genetic diversity of the cigarette beetle and in discriminating global phylogeographical variants among the *Lasioderma* species. From this study, *L. serricorne* in different phylogeographical areas are in a same clade reveals its common ancestry. Here we report the partial sequence of cytochrome oxidase subunit I gene (COI) of *L. serricorne* (GenBank Accession No. KM 216269) and its phylogenetic relationship with other related insect species. The COI DNA barcode developed in this study can be used for its accurate identification and phylogeographical analysis.

Keywords: ***Lasioderma serricorne*, cytochrome oxidase subunit I gene, DNA barcode, molecular phylogeny**

***Corresponding author**

SEBASTIAN, C. D
Molecular Biology Laboratory, Department of Zoology,
University of Calicut, Kerala, India

INTRODUCTION

The cigarette beetle, *Lasioderma serricorne (*Fabricius) is coming under the Family Anobiidae of Order Coleoptera, Class Insecta. It is a small, measuring about 2-3 mm and reddish brown in colour. Larval feeding causes direct damage to stored food products through contamination by the presence of adult beetles, larvae, pupae, frays and insect parts. The female lays egg on the food material and emerged larvae eat and grow. They spin a cocoon when it is time to change in to adults. The lifespan of cigarette beetles in captivity is 26 days to 1 year, with an expected lifespan of 44 days[1]. The optimal conditions for growth and development are between 30° to 37°C and 70 to 75% relative humidity. A constant temperature of greater than 40°C or less than -18°C is fatal to all stages of life and low humidity significantly shortens their lifespan. Beetles raised on wheat flour have the highest body size and fecundity, laying an average of 10 times more eggs than beetles living on cigar tobacco[2, 3,4]. Cigarette beetles are found worldwide, everywhere that stored tobacco. The beetle spread widely as it was transported in packaged tobacco or other packaged products. It is believed that the cigarette beetle originated in Egypt because their carcasses have been found in Egyptian tombs [2,5,6]. The cigeratte beetle is a serious pest of stored product throughout the world causing severe damage to spices, tobacco, pet food and dried flowers. DNA barcoding using specific marker nucleotide sequences are used for unambiguous species identification. Short mitochondrial nucleotide gene sequences like mitochondrial cytochrome oxidase subunit I and II (COI and COII) genes are used for insect identification and phylogenetic studies[7]. The phylogenetic analysis of different insect orders using mitochondrial COI gene sequences were reported in odonates[8], butterflies[9], bees[10], bugs[11], flies[12,13] and calliphorid flies [14]. The partial coding sequence of mitochondrial COI gene of *L. serricorne* isolate from different areas were reported in NCBI database (GenBank accession No. KJ 964758, KJ 680546 and KC 569549). The phylogeographical analysis of *L. serricorne* are very important because of its world-wide distribution and currently there is no report on the COI gene structure analysis of *L. serricorne* species from Kerala.

MATERIALS AND METHODS

The *L. serricorne* were collected from the Kozhikkode, Kerala. . The insects were stored at $^{-}20^{0}$C until the DNA was extracted. The mitochondrial genomic DNA from the adults were isolated using GeNei Ultrapure Mammalian Genomic DNA Prep Kit (GeNei, Banglore) as per the Manufacturer's instruction. About 2ng of DNA was amplified for mitochondrial cytochrome oxidase subunit I (CO I) gene using the forward primer with sequence 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer with sequence 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. The PCR reaction mixture consisted of 2 nanogram of genomic DNA,1µl each forward and reverse primer with at a concentration of 10 µM,2.5 µl 10dNTP$_S$ (2mM), 2.5 µl 10X reaction buffer, 0.20 µl Taq polymerase(5U/ µl) and 12.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 5 min at 95°C, followed by 30 cycles of 10s at 95°C, 10s at 55°C and 1 min at 72°C and ending with a final phase of 72°C for 3 min. The PCR products were resolved on a 1% TAE- agarose gel, stained with EtBr and photographed using a gel documentation system. After ascertaining the PCR amplification of the corresponding coI fragment, the remaining portion of the PCR product was column purified using PCR Clean-up Kit (Mo Bio Laboratories Inc., California) as per the manufacturer's instructions. The purified PCR product was sequenced from both ends using the forward and reverse primers used for the PCR using the Sanger's sequencing method[13]. The forward and reverse sequences were assembled by using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2) after removing the primer sequences and the consensus was taken for the analysis. The final

sequence was searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov/). The genetic diversity, nucleotide substitution and the phylogenetic tree were plotted using neighbor joining method using by MEGA6 software [15,16,17].

RESULTS AND DISCUSSION

The PCR of the COI gene fragment of *L. serricorne* yielded a single product of 574 bp. The BLAST search using the sequence revealed that the sequence obtained in this study was novel (GenBank Acession No KM 216269). The evolutionary divergence of *L. serricorne* within beetle group is given in the Table 1. The partial COI gene sequence of *L. serricorne* obtained in this study showed 100% identities with *L. serricorne* isolated from South Korea and 99.7% with identities with Europe. The composition of nucleotides of *L. serricorne* in each codon position was analysed and compared with other species of Coleoptera. The result indicates that the composition of each nucleotide in COI sequence of *L. serricorne* showed similarity with other Coleopterans species. But the use of nucleotide in each codon position showed difference in the COI sequence of *L. serricorne* compared to other Coleopteran species. Variation in the nucleotide sequence is a fundamental property of all living organisms which can be used for their identification and phylogenetic status. DNA barcoding provide rapid and automatable species identification by short standardized DNA fragment as species tag and its makes the Linnaean classification system more accessible. The COI sequence obtained in this study showed significant variation with other species. NJ clustering analysis showed single monophyletic clade of the sequences belonging to the same species without any overlap, even though these sequences are from the specimens separated by a large geographic distances. The average nucleotide composition proportions for these twelve sequences were G, 17.8%; A, 26.8%; T, 40.7%; and C, 14.7% (Table 2). The present results indicate that an identification system for insect life based on the COI gene will be highly effective. Although COI divergences appear too low to regularly enable species diagnosis within the insects, generic-level identifications in these organisms remain a prospect. More importantly, the mitochondrial genomes of closely allied species in other phyla, those that comprise the bulk of animal diversity, regularly show sufficient sequence diversity to enable their discrimination. The evolutionary history of *L. serricorne* was inferred using the Neighbour- joining method (Figure 1). Phylogeneticaly *L. serricorne* from Bangalore and South Korea is the nearest relative of *L. serricorne* (Acession No. KJ 680546, KC 569549) isolated from Kerala. The species *L. serricorne,* which comes under the Family Anobiidae shows higher AT content. The NJ clustering analysis showed inter and intra species divergence. The intra species nucleotide divergence between *L. serricorne* calculated shows 0.3% divergence with *L. serricorne* from Europian region (GenBank Acession No. KJ 964758). Interspecies nucleotide divergence for 16 species shows divergence from 16-25%. This shows a deep interspecies nucleotide divergence. The estimated Transition/Transversion bias (R) is 0.47. The maximum Log likelihood for this computation was -2201.900 and the analysis involved 20 nucleotide sequences. There were a total of 388 positions in the final dataset. Maximum transitional substitutions are C to T and transversional substitutions are A to T.

Table 1
Percentage of genetic divergence of L. serricorne and other related species.

Sl. No.	Accession No. and Species Name	Divergence
1.	KJ 680546 *Lasioderma serricorne*	0.0%
2.	KC 569549 *Lasioderma serricorne*	0.0%
3.	KJ 964758 *Lasioderma serricorne*	0.3%
4.	GU 142831 *Zizyphomyia* sp.	16.6%
5.	KC 407724 *Stegobium paniceum*	19.9%
6.	KC 407723 *Stegobium paniceum*	19.9%
7.	KC 407722 *Stegobium paniceum*	19.9%
8.	KC 407721 *Stegobium paniceum*	19.9%
9.	KJ 965846 *Bradycellus caucasicus*	20.0%
10.	KJ 964447 *Bradycellus caucasicus*	20.0%
11.	HM 891887 *Potamia littoralis*	20.3%
12.	HM 389015 *Potamia littoralis*	20.3%
13.	HQ 248105 *Verticia orientalis*	20.6%
14.	EU 710809 *Harpalus puncticeps*	21.5%
15.	KJ 964636 *Ochthebius minimus*	21.9%
16.	KF 930572 *Platycheirus atlasi*	22.1%
17.	KJ 962153 *Ochthebius minimus*	22.4%
18.	EU 409170 *Toxomerus procrastinatus*	23.2%

Table 2
Composition of nucleotides in each position of codon of the COI sequence of L. serricorne species

Species name	Nucleotide Frequencies in Percentage															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C2	A-2	G-2	T-3	C-3	A-3	G-3
KM216269 Lasioderma serricorne	40.7	14.7	26.8	17.8	42	28.5	11.5	17.7	50	1.6	41.9	7.0	30	14.0	27.1	28.7
KJ680546 Lasioderma serricorne	40.7	14.7	26.8	17.8	42	28.5	11.5	17.7	50	1.6	41.9	7.0	30	14.0	27.1	28.7
KJ964758 Lasioderma serricorne	40.7	14.7	26.5	18.0	42	28.5	11.5	17.7	50	1.6	41.1	7.8	30	14.0	27.1	28.7
KC569549 Lasioderma serricorne	40.7	14.7	26.8	17.8	42	28.5	11.5	17.7	50	1.6	41.9	7.0	30	14.0	27.1	28.7
HQ248105 Verticia orientalis	39.9	14.9	30.2	14.9	42	30.8	11.5	16.2	47	1.6	51.2	.0	31	12.4	27.9	28.7
KC407724 Stegobium paniceum	38.7	16.2	30.2	14.9	42	30.0	10.8	16.9	45	2.3	51.2	1.6	29	16.3	28.7	26.4
KC407723 Stegobium paniceum	38.7	16.2	30.2	14.9	42	30.0	10.8	16.9	45	2.3	51.2	1.6	29	16.3	28.7	26.4
KC407722 Stegobium paniceum	38.7	16.2	30.2	14.9	42	30.0	10.8	16.9	45	2.3	51.2	1.6	29	16.3	28.7	26.4
KC407721 Stegobium paniceum	38.7	16.2	30.2	14.9	42	30.0	10.8	16.9	45	2.3	51.2	1.6	29	16.3	28.7	26.4
KF930572 Platycheirus atlasi	40.5	14.7	29.1	15.7	41	30.0	12.3	16.9	49	1.6	48.1	1.6	32	12.4	27.1	28.7
HM891887 Potamia littoralis	41.8	13.9	28.9	15.5	42	30.0	11.5	16.9	52	0	48.1	.0	32	11.6	27.1	29.5

EU409170 Toxomerus procrastinatus	38.4	15.5	30.7	15.5	41	30.0	13.1	16.2	44	2.3	53.5	.0	30	14.0	25.6	30.2
GU142831 Zizyphomyia sp.	42.0	14.4	28.1	15.5	43	30.0	10.0	16.9	50	0.8	48.1	.8	33	12.4	26.4	28.7
KJ965846 Bradycellus caucasicus	38.7	14.9	27.8	18.6	42	29.2	10.8	18.5	43	3.1	46.5	7.8	32	12.4	26.4	29.5
HM389015 Potamia littoralis	41.8	13.9	28.9	15.5	42	30.0	11.5	16.9	52	0	48.1	.0	32	11.6	27.1	29.5
EU627707 Atherigona orientalis	39.4	16.2	28.6	15.7	42	30.8	11.5	16.2	48	3.1	48.1	.8	29	14.7	26.4	30.2
EU710809 Harpalus puncticeps	38.7	14.7	30.2	16.5	42	29.2	10.0	19.2	46	0	53.5	.8	29	14.7	27.1	29.5
KJ964447 Bradycellus caucasicus	38.7	14.9	28.4	18.0	42	29.2	10.8	18.5	43	3.1	48.1	6.2	32	12.4	26.4	29.5
KJ964636 Ochthebius minimus	38.7	17.3	27.8	16.2	41	30.8	11.5	16.9	46	5.4	47.3	1.6	29	15.5	24.8	30.2
KJ962153 Ochthebius minimus	38.4	17.5	27.8	16.2	41	30.8	11.5	16.9	45	6.2	47.3	1.6	29	15.5	24.8	30.2
Average	**39.7**	**15.3**	**28.7**	**16.3**	**42**	**29.7**	**11.3**	**17.2**	**47**	**2.1**	**47.9**	**2.8**	**30**	**14.0**	**27.0**	**28.7**

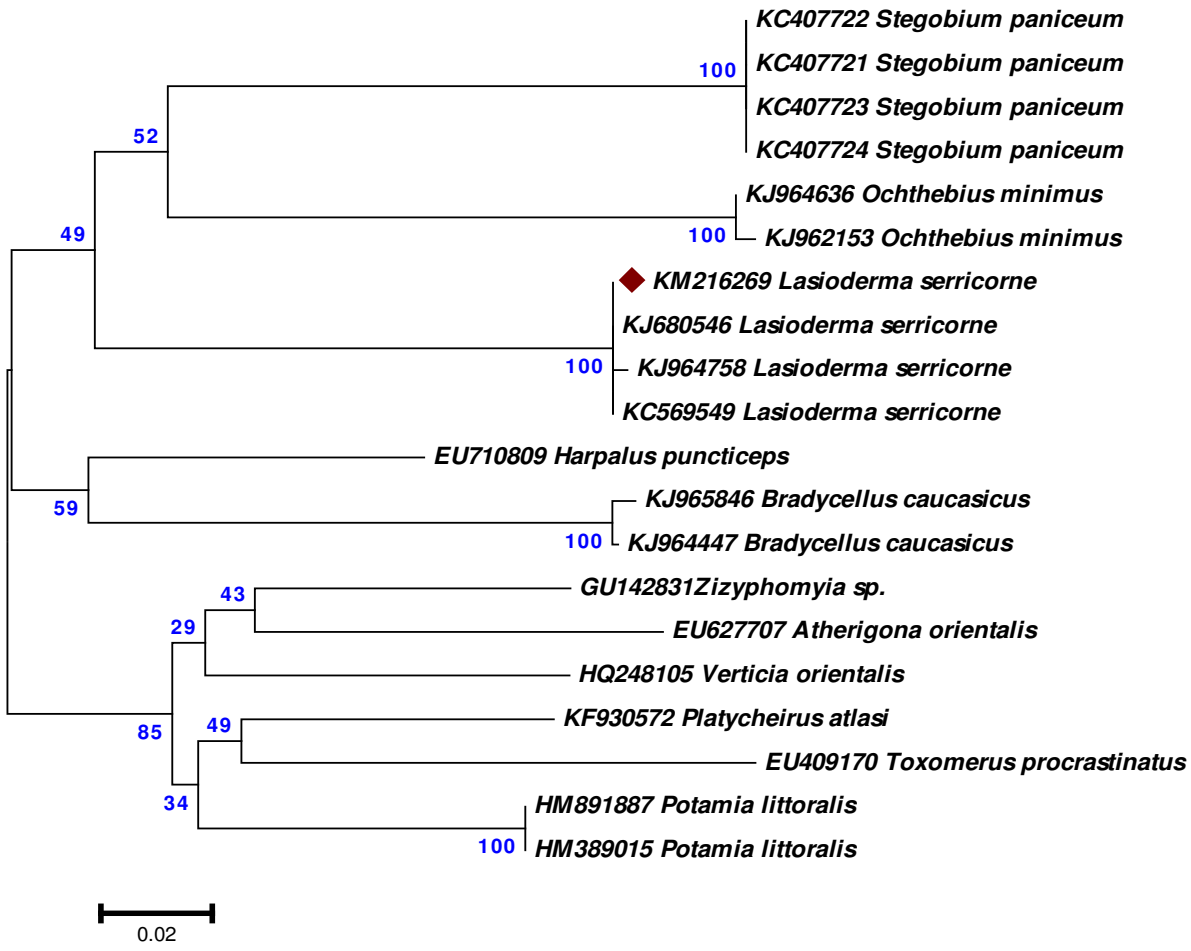

Figure 1

Phylogenetic relationship of L. serricorne isolated from Kerala inferred by Neighbor joining

CONCLUSION

It can be concluded that the COI sequence of *L. serricorne* showed considerable variation with all other related species in the Coleopteran family, therefore the COI sequence identified in this study can be used as barcode for identification of this insect at any stage of its life cycle.

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