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Genetic structure and phylogeny analysis of coconut black headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae)

Rukhsana Kokkadan, Chempakassery Devasia Sebastian*

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

E-mail : drcdsebastian@gmail.com Contact No : +91-9447648961

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Abstract

The coconut black headed caterpillar, *Opisina arenosella* is an endemic, frequently outbreaking pest of coconut palm in India, Myanmar and Sri Lanka which feeds on the leaves by scraping the chlorophyll matter, resulting in drying of the leaf and all palm become burnt in appearance. The *O. arenosella* has been reported to be a pest of coconut palm attaining major pest status in coastal areas of many districts of Kerala. Here we report the partial DNA sequence of cytochrome oxidase subunit I (COI) of *O. arenosella* isolated from Parappanangadi of Kerala and its phylogenetic status. The COI partial coding sequence of *O. arenosella* (GenBank Accession No. KM 216268) showed 2.51% difference over 489 bp nucleotides and 3.44% difference over 163 amino acids to that of *O. arenosella* (GenBank Accession No.JQ 807264) isolated from Karnataka.

Key words : Cytochrome oxidase subunit I gene, *Opisina arenosella*, Phylogeny

INTRODUCTION

The black headed coconut caterpillar, *Opisina arenosella* is an endemic, frequently out breaking pest of coconut palm in India, Myanmar and Sri Lanka which feeds on the leaves by scraping the chlorophyll matter, resulting in drying of the leaf and all palms become burnt in appearance. *O. arenosella* is a small and grayish white moth, commonly in coastal areas of Kerala [1]. Taxonomically *O. arenosella* comes under the family Oecophoridae and the genus *Opisina*. The population of *O. arenosella* is found to be present throughout the year and attain peak density during March to May. The major environmental factors regulating the pest population includes maximum temperature, sunshine and rainfall [2]. The different life stages of *O. arenosella* spans 4-6 days for egg, 30-60 days for larval instars, 10-14 days for pupation and a short adult stage altogether completed within 50-75 days. Here we report partial mitochondrial cytochrome oxidase subunit I (COI) DNA sequence of *O. arenosella* and also its phylogenetic status. The first record of this pest in India was from Coimbatore in 1907 on Palmyra plants and on coconut plants from Baptala, Andhra Pradesh, in 1909 [3]. Since the record of major infestation on coconut palms from Travancore district of Kerala in 1917 [4], *O. arenosella* is still one of the most serious pests of coconut in Andhra Pradesh, Bihar, Goa, Karnataka, Kerala, Maharashtra, Orissa, Tamilnadu and West Bengal. Its infestation is also reported from Gujarath [5].

In Kerala, a severe infestation of the pest was noticed in Kollam district during 1917-1918. The pest appeared in Mangalore area in 1922 [6] and was reported from Cochin in 1924 [7]. The pest made its appearance at Kasaragod and Calicut in 1925 and Ponnani area in 1926 [3]. Control of the pest by biological agents were reported and studied by [8], [9] and [10]. With increasing cultivation of the crop in unconventional areas, the pest is also spreading to new areas including interior places. Severe outbreaks occurred in several parts of Karnataka and Andhra Pradesh in 1983-1984 [11]. A review of all works carried out on the bionomics and control measures of the pest were made [12].

The grayish adult moth has 18-30 mm size with head and Forewing elongated, costa greatly arched with whitish edge, apex rounded, tegmen obliquely rounded and pale grayish with some fine scattered blackish scales. The egg is oval in shape, creamy coloured and 0.5-0.8 mm long. The larva emerges within 4-6 days. The *O. arenosella* exhibits five larval instars. The newly hatched larvae are pale white in colour measuring about 1.5mm in length. The fully grown larvae are about 14-18mm in length. The larvae spin silken threads around themselves and live inside. The pupa is brown in colour and moth emerges in about 10-13 days.

The larva of *O. arenosella* is a much harmful coconut pest. It lives in large members in the galleries formed of silk and other materials in the under surface of leaflets. It feed on the leaves by scraping out the green matter. In the cases of severe attack, leaflets dry out and the whole palm looks burnt. This seriously affects the health of the palm, considerably reduces its photosynthetic activity and adversely affects the yield [13], [14] and [15].

MATERIALS AND METHODS

The black headed coconut caterpillar, *O. arenosella*, used in the present study was collected from Parappangadi in Malappuram district of Kerala. For DNA extraction the tissue from one of the thoracic legs was homogenized using a glass pestle and mortar. The mitochondrial genomic DNA in the homogenate was extracted using GeNei Ultrapure Mammalian Genomic DNA Prep Kit as per the manufacturer's instructions. About 2 ng of genomic DNA was PCR amplified for mitochondrial cytochrome oxidase subunit (COI) gene using the forward primer with DNA sequence 5'-TATATTTTATTTTT GGAATTTGAGC-3' and reverse primer with DNA sequence 5'-TTAAATTTCGGTCTGTTAAAAGTAT-3'. The PCR products were resolved on a 1% TAE-agarose gel, stained with ethidium bromide and photographed in gel documentation system. After ascertaining the PCR amplification of the corresponding COI fragment, the remaining portion of the PCR product was column

purified using Mo Bio Ultraclean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California) and was sequenced from both ends using the forward and reverse primers used for the PCR using Sanger's sequencing method. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using ClustalW and the consensus was taken for the analysis. The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov/) and inter and intra specific genetic diversity were calculated using Kimura 2-parameter model with the pair-wise deletion option and the difference in the nucleotide in codon usage partial COI sequence of *O. arenosella* was analyzed using MEGA5 software [16].

RESULTS

The PCR of the COI gene fragment of *O. arenosella* yielded a single product of 489 base pairs. The BLAST search using the sequence revealed that the sequence obtained in this study was novel. The average divergence in intraspecific comparisons is 2.51%. Partial COI DNA sequence of *O. arenosella* (GenBank Accession No.KM 216268) is 2.51% difference to that of *O. arenosella* (GenBank Accession No.JQ 807264) sequenced.

The average nucleotide composition across the species was T=39.9%; A=30.0%; C=15.8%; G=14.3%. This results show that analysis based on mitochondrial gene can be useful for unraveling phylogenetic relationships in the species *O. arenosella.* The percentage of A+T was higher than that of G+C which reflected further in the codon usage. The second codon position contains 92.8% of AT nucleotides and it is decreased 59% in third codon position. AT nucleotide composition of *O. arenosella* from Kerala is less than to that of Karnataka. The evolutionary nucleotide and peptide divergence of *O. arenosella* with various Lepidopteran species is given in table. 1.

Partial COI peptide sequence of *O. arenosella* (from Kerala) is 3.44% difference with *O. arenosella* (GenBank Accession No.JQ807264). The evolutionary history was inferred using the Neighbor-joining method using COI partial sequence. The evolutionary history of *O. arenosella* was inferred using the Neighbor- joining method (Figure 1).

DISCUSSION

DNA sequence based identification technique has revealed the morphological and ecological traits of many species. The phylogeny analysis using NJ tree revealed the sharing of common ancestor of this species, *O. arenosella*. DNA barcoding techniques have been used to demarcate the phylogenitical variants. Using the mitochondrial gene, the phylogeny of *O. arenosella* have been resolved here. Intraspecific divergence of partial coding fragment of COI gene is very efficient for species identification. This region has good discrimination power for *O. arenosella*.

CONCLUSION

Variation in the nucleotide is fundamental property of all living organisms which can be used for their identification and phylogenetic status. The COI sequence obtained in this study showed nucleotide variation of 2.51% with the same species *O. arenosella* sequenced in Karnataka proving the species obtained

Table 1: The evolutionary divergence of *O. arenosella* with related Lepidopteran species

Name of families with species name and GenBank Accession No.	Percentage of nucleotide divergence	Percentage of peptide divergence
Opisina arenosella (KM216268)	0	0
Opisina arenosella (JQ807264)	2.51	3.44
Pseudoscada florula aureola (EU069101)	7.54	3.44
Epibrontis_hemichlaena (KF394543)	7.54	1.36
Epibrontis hemichlaena (KF393262)	7.78	1.36
Melipotis punctifinis (GU336760)	8.04	2.05
Echiomima fabulosa (KF405042)	8.26	0.68
Herpetogramma luctuosalis (KC682506)	8.54	2.74
Godyris_dircenna_dircenna (JX573763)	8.54	3.44
Aporocosmus lamprodeta (KF393140)	8.56	2.74
Godasa orientis (HM3955150)	8.76	0.68
Pseudaegeria hyalina (KF402179)	9.04	2.05
Ardozyga loemias (KF392566)	9.54	1.36

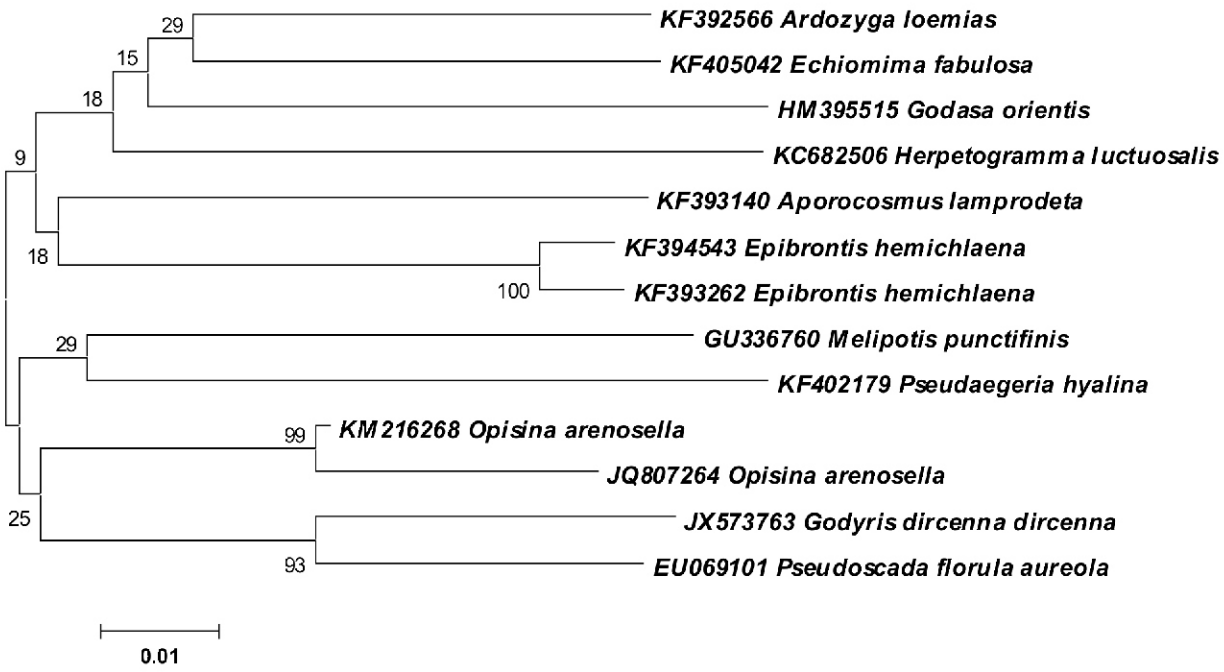

Fig 1: Phylogenetic status of *O. arenosella* compared with closely related Lepidopteran species.

from Kerala is a novel one. Phylogeny analysis using NJ tree revealed the sharing of common ancestor to these two populations and the branch length of *O. arenosella* from Kerala was less compared to that from Karnataka indicating the diversity. Intraspecific divergence in partial coding fragment of COI gene is very efficient for species identification in the case of these pest populations[17]. Molecular COI barcoding is a powerful tool in proper identification and analysis of phenotypic divergence in *O. arenosella* pest population.

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REFERENCES

1. Mohammed UVK, Abdurahman UC, Ramadevi OK. Coconut caterpillar and its natural enemies. A study of Parasites and Predators of Nephantis serinopa Meyrick. Zoological Monograph, No.2, Dept .of Zoology, University of Calicut, Kerala, India.1982.p.162

2. Pushpalatha NA, Veeresh GK. population fluctuation of coconut black headed caterpillar *Opisina arenosella* Walker (Lepidoptera: Xylorictidae). J.of. Plantation crops, 23(1): 1995: 44-47.

3. Rao YR, Cherian MC, Ananthanarayanan, KP. Infestation of Nephantis serinopa Meyrick in South India and their control by the biological methods. Ind. J. Ent. 1948:10(2): 205-247.

4. Madhavan Pillai, R. An attack of *Nephantis serinopa* on coconut palm in Travancore. Agric. J. Ind: 1919: 24, 668-669.

5. Anjaria PI, Kikania KP, Shah AH, Vora VI. Studies on the effects of some commonly used insecticides against coconut black headed caterpillar (Nephantis serinopa Meyr), Coconut Bulletin. 1976: 6: 1-2.

6. Rao YR. An outbreak of Nephantis serinopa at Manglore in1922. Rept. proc. 5th Ent. Meeting. Pusa, 1924: 92-98.

7. Venkatasubban, CS. The coconut caterpillar in Cochin. Madras Agric. J. 1932: 20: 192-198.

8. Hutson, J. C. Report of the Entomologist .Ceylon Dept. Agri. Administr. Repts. 1919: 8-10.

9. Rao YR. Control of coconut caterpillar by its parasites. Agri. J. India, 1926: 21: 452-459.

10. Dharmaraju E. The biological control of the black headed caterpillar, *Nephantis serinopa* in the East Godvari Dist. of Madras State. Indian Cocon. J. 1952: 5: 171-176.

11. Manjunath TM. Coconut black headed caterpillar on banana and coconut. FAO Plant Prot. Bull. 1985 32(2): 71-72.

12. Ramachandran CP, Ponnamma KN, Abdullakoya KM, Kurian, C. The coconut leaf eating caterpillar, *Nephantis serinopa*, A review. Phil. J. Coco.study.1979: 4(2): 9-17.

13. Joy PJ, Joseph KJ. Notes on the biology of *Brachymeria nosatoi* and its importance in the control of *Nephantis serinopa*. Entomophaga. 1972: 18(3): 317-319.

14. Cock MJ, Perera PACR. Biological control of *Opisina arenosella* (Lepidoptera: Oecophoridae). Biocontrol News and Information. 1987: 8: 283-310.

15. Chandrika Mohan, Radhakrishnan Nair, CP, Kesavan Namboothiri, C, Rajan, P. Leaf-eating caterpillar (*Opisina arenosella*) induced yield loss in coconut palm. International J. of

Tropical Insect Science, 2010: 30: 132-137.

16. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA-molecular evolutionary genetics analysis. Mol. Bio.and Evol. 2011: 28: 2731-9.

17. Hebert PDN, Ratnasingham S, Dewaard JD. Barcoding animal life, Cytochrome c oxidase subunit I divergences among closely related species, Proc. R. Soc. Lond. S. B. Biological Science. 2003: 270: 96-99.