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Research Article

MOLECULAR BARCODING AND PHYLOGENY ANALYSIS OF *GONIOZUS NEPHANTIDIS* (HYMENOPTERA: BETHYLIDAE), A LARVAL PARASITOID OF COCONUT BLACK HEADED CATERPILLAR, *OPISINA ARENOSELLA* (LEPIDOPTERA: OECOPHORIDAE)

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ABSTRACT

Goniozus nephantidis (Hymenoptera: Bethylidae) a larval parasitoid of *O. arenosella* and this study reveals molecular barcoding and phylogeny analysis of *G. nephantidis*. The adult *G. nephantidis* gave excellent control of larvae of *O. arenosella*. We have PCR amplified and sequenced partial fragment of cytochrome oxidase1 gene of (COI) of *G. nephantidis* (Gen Bank Accession: KM 216267) isolated from Kerala, India and its phylogenetic status. In Partial COI DNA sequence of *G. nephantidis* has is 11.44 % difference to that of *G. jacintae* (KF 027190).

Keywords: *Opisina arenosella,* biological control, parasitoid, *Goniozus nephantidis,* DNA barcoding, phylogeny, cytochrome oxidase subunit I gene

INTRODUCTION

The family Bethylidae consists of 2200 species belonging to subfamilies Pristocerinae, Bethylinae, Mesitiinae and Epyrinae[1]. They are parasites of larvae of Coleoptera and Lepidoptera. *G. nephantidis* attack the larvae of coconut black headed caterpillar, *Opisina arenosella* and it is used for the control of this pest. With increase in larval parasitation, reduce the pest population. Insect pest such as *O. arenosella* can cause extensive damage to coconut across India. This pest is very difficult to manage with conventional control methods. This pest population has been shown to progressively diminish through natural parasitoid interactions, thus providing an effective and sustainable approach for controlling this pest. Several investigators have discussed the phylogeny, biology and mode of parasitic interaction of *G. nephantidis*[1-3]. Santhosh and Narendran studied about parasitizing character of *Goniozus* Forster[4] and in Korea, descriptions of two new species of *Goniozus* Forster were done by Lim and Lee[5]. The genus *Goniozus* Forster, 1856 from subfamily Bethylinae consists of approximately 170 species worldwide, of which are recorded from Oriental (53 spp.), Neotropical (35), Nearctic (32), Palaearctic (28), Afrotropical (12), and Australian (9) regions[1,4,6,7]. Molecular phylogeny analysis based on Mitochondrial DNA revealed the proper identification and evolutionary history of various animal species[8]. Phylogenetic analysis using mitochondrial cytochrome oxidase subunit I (COI) gene sequences were extensively carried out by several workers in different group of organisms like southern house mosquito *Culex quinquefasciatus*[9], *Armigeres subalbatus*[10], green bottle fly *Lucilia sericata*[11], *Herpetogramma stultalis*, white backed plant hopper *Sogatella furcifera*[13], Asian honeybee *Apis cerana*[14] and lepidopteran species[15]. A study of the genetic diversity of parasitic hymenoptera may facilitate their precise identification, determination of interspecies relationship and delineating their phylogeny. Here we report the partial DNA sequence of cytochrome oxidase I of *G. nephantidis* that can be used as molecular barcode of the species and its phylogeny analysis.

MATERIALS AND METHODS

G. nephantidis used in the present study was collected from Tirurangadi in Kerala, India. Mitochondrial genomic DNA was extracted from one of the thoracic legs of the experimental insect, *G. nephantidis*. The tissue was homogenized using a glass pestle and mortar. The genomic DNA in the homogenate was extracted using Ge Nei Ultrapure Genomic DNA Prep Kit in accordance to the manufacturer's instructions. About 2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene in both direction using the forward primer with DNA sequence 5'-GGTCAACAAATCATAAAGATATTGG -3' and reverse primer with DNA sequence 5'- TAAACTTCAGGGTGACCAAAAAATCA -3'. The PCR products were resolved on a 1 % TAE-agarose gel, stained with Ethidium bromide 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 Mo Bio Ultraclean 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 Sanger's sequencing method. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using Clustal W 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 *G. nephantidis* was analyzed using MEGA 6 software[16]. Substitution pattern and rates were estimated under the Kimura 2-parameter model[17]. The evolutionary history was inferred using the Neighbor-Joining method[18]. The evolutionary distances were computed using the Maximum Composite Likelihood method[19] and are in the units of the number of base substitutions per site. The analysis involved 18 nucleotide sequences. Codon positions included were

1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated.

RESULTS

The BLAST search using the sequence revealed that the sequence obtained in this study was novel. The average divergence in intra genic comparisons is 11.44 %. Partial COI DNA sequence of *G. nephantidis* (KM 216267) is 11.44 % difference to that of *Goniozus jacintae* (KF 027190) and 13.47 % to *Goniozus jacintae* (KF 027188) sequenced.The evolutionary divergence of *G. nephantidis* (KM 216267) with various Bethylid species is given in Table 1. The average nucleotide composition across the species was T = 47.4 %; A = 33.3 %; C = 12.3 %; G = 7.0 %. This results show that analysis based on mitochondrial gene can be useful for unraveling phylogenetic relationships in the species G. *nephantidis*. The percentage of A + T was higher than that of G + C which reflected further in the codon usage. The estimated Transition/Transversion bias (*R*) is 0.64. The nucleotide frequencies are A = 25.00 %, T/U = 25.00 %, C = 25.00 %, and G = 25.00 %. The maximum Log likelihood for this 18 nucleotide sequences is 556.694. The optimal tree with the sum of branch length = 1.98684877 is shown. The percentage of replicate trees in which the associate taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches[20]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary history was inferred using the Neighbor-joining method using COI partial sequence. The evolutionary history of *G. nephantidis* was inferred using the Neighbor- joining method (Figure 1).

Table 1: The evolutionary divergence of *G. nephantidis* (KM 216267) with various *Bethylid* sp.

S. No.	Species name with Accession No.	% of diversity
1	*Goniozus jacintae* (KF027190)	11.44 %
2	*Goniozus jacintae* (KF027188)	13.47 %
3	*Blattidae* gen. (JQ574571)	13.73 %
4	*Goniozus* sp. (KF 027184)	17.79 %
5	*Goniozus* sp. (KF 027186)	17.79 %
6	*Rhabdepyris* sp. (KF 027194)	20.45 %
7	*Apenesia* sp. (KF 027181)	20.45 %
8	*Sierola* sp. (KF 027199)	23.01 %
9	*Eupsenella* sp. (KF 027183)	23.30 %
10	*Odontomachus meinerti* (KC 418719)	23.92 %
11	*Sclerodermus niveifemur* (KF 027195)	28.52 %
12	*Camptothlipsis* sp. (JF 271239)	28.52 %
13	*Neoapenesia* sp. (AB 795312)	31.06 %
14	*Rhabdepyris* sp. (AJ 514364)	32.09 %
15	*Cephalonomia* sp. (KF 027182)	35.15 %
16	*Sclerodermus harmandi* (AB 795306)	40.22 %
17	*Laelius* sp. (KF 027191)	40.85 %

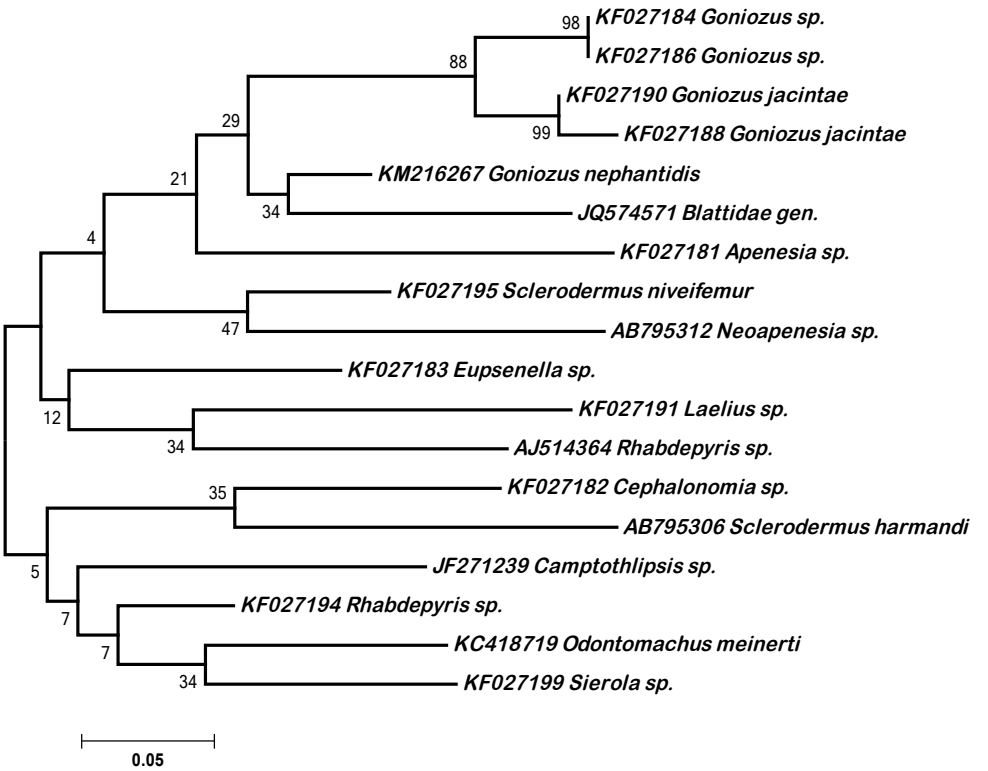

Figure 1: Phylogenetic status of *G. nephantidis* compared with closely related *Bethylid* species

DISCUSSION

Variation in the nucleotide is fundamental property of all living organisms which can be used for their identification and phylogenetic status. The COI gene in the mitochondrial genome has been proved to be an excellent source of information for the set of closely related species belonging to the order Hymenoptera. The COI sequence obtained in this study showed nucleotide variation of 11.44 % to that of *G. jacintae* (KF 027190) COI gene and 13.47 % to that of *G. jacintae* (KF 027188) COI gene sequences. The BLAST analysis of 504 bp of the insect *G. nephantidis* showed significant homology with other *Goniozus* species. *Goniozus* species were separated into related clades in phylogenitic tree. *G. nephantidis* and *G. jacintae* species are in different clades. This work is useful for the unambiguous taxonomic identification of *G. nephantidis* and confirms its actual phylogenetic position.

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