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Research Article

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Genetic diversity and phylogenetic analysis of *Apanteles taragamae* viereck by molecular barcoding using COI gene sequences

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Abstract: *Apantelestaragamae* (Hymenoptera: Braconidae) a larval parasitoid of *Opisinaarenosella*and this study revealsmolecular barcoding and phylogeny analysis of *A. taragamae*. The adult *A. taragamae* gave excellent control of larvae of *O. arenosella.*We have PCR amplified and sequenced partial fragment of cytochrome oxidase1 gene (COI) of *A. taragamae* (KU510321) isolated from Keralaand its phylogenetic status was studied. In Partial COI DNA sequence of *A. taragamae* (KU510321) has is 9.836% difference to that of*Apantelesfumiferanae* (KJ627789) and 9.141% difference to other Microgastrinae species (HM373821).

Key words: *Opisina arenosella,* parasitoid, *Apantelestaragamae,* phylogeny, cytochrome oxidase

Introduction

A. taragamae isa parasitoid of *O. arenosella* which belongs to the family Braconidae of Hymenoptera.Braconidae is a family of parasitoid wasps and one of the richest family of insects and the approximate 12,000 described species. The family Braconidaebelonging to subfamiliesBraconinae, Adeliinae, Dirrhopinae, Exothecinae, Hormiinae, Masoninae, Meteorideinae, Microtypinae, Pambolinae, Rhysipolinae, Rhyssalinaeetc (Jones, 2009). *A. taragamae* attack the larvae of coconut black headed caterpillar, *O. 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 are difficult to manage with conventional methods. This pest population have been shown to progressively diminish through natural parasitoid interactions. Thus providing an effective and sustainable approach for controlling this pest. *A. taragamae* is cosmopolitan parasitoid and mainly reported from Kerala, Karnataka, West Bengal, Tamil Nadu and Orissa. It is a larval parasitoid of *opisinaarenosella.* Ovipositor sheaths of this species is more slender and ovipositor is shorter than hind tibia. Forewings are with stigma pale and

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colorless, but with distinct dark brown border. First tergite is parallel sided and narrow at apex and one and a half times as long as wide at apex.

Phylogeny of genus *Braconbrevicornis* were studied(Rukhsana and Sebastian, 2015). Molecular barcoding of *A. cerana* demonstrates the efficiency of the barcoding gene in discriminating global phylogeographical variants among the *Apis*species complex (Rukhsana*et al*., 2014). The Braconidae comprises a large and economically important family of parasitoid hymenopteran that represented in all zoogeographic regions (excluding Antarctica), consisting of some 1056 genera and about 19652 known species (Yu *et al*., 2012). Thirty six of 46 subfamilies of the Braconidae are recorded from the western Palaearctic region (Jones, 2009). The greatest diversity of Braconidae occurs in old world tropical and subtropical regions.The subfamily Braconinae currently comprises more than 2900 described species worldwide (Yu *et al.*, 2012).

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 *A. taragamae*that can be used as molecular barcode of the species and its phylogeny analysis. In this study, we conduct molecular systematic studies of the*A. taragamae*to clarify deep divergences and evolutionary affinities with related groups.

Materials and Methods

*A. taragamae*used in the present study was collected from Kerala. Mitochondrial genomic DNA was extracted from one of the thoracic legs of the experimental insect,*A. taragamae*. The tissue was homogenized using a glass pestle and mortar. The genomic DNA in the homogenate was extracted using aGeNei Ultrapure Mammalian Genomic DNA Prep Kit in accordance to the manufacturer's instructions (BangloreGeNei, Banglore). About 2 nanogram of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit (COI) gene using the foreward primer with DNA sequence 5'-GGTCAACAAATCATAAAGATATTGG -3' and reverse primer with DNA sequence 5'-TAAACTTCAGGGTGACCAAAAAATCA -3'. The PCR reaction mixture consisted of 2 nanogram of genomic DNA, 1µl each forward and reverse primers at a concentration of 2.5 µM, 2.5 µl of dNTPs (2mM), 2.5 µl of 10X reaction buffer, 0.20µl of Taq polymerase (3U/µl) and 11.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 2

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minutes at 95^0C, followed by 30 cycles of 5s at 95^0C, 45s at 50^0C and 45s at 72^0 C and ending with a final phase of 72^0C for 3 minutes. 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 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 at SciGenom Labs Pvt. Ltd., Cochin. 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 specific genetic diversity were calculated using Kimura 2-parameter model with the pairwise deletion option and the difference in the nucleotide in codon usage partial COI sequence of *A. taragamae*was analyzed using MEGA5 software (Tamura *et al*., 2011). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site.

Results

The PCR amplification of partial COI sequence of *A. taragamae* isolated from Kerala yielded a product with 482 bp.The partial COI gene sequence of *A.taragamae*obtained in this study showed 90% similarity withMicrogastrinaespecies isolated from Sweden. The composition of nucleotides of all *Apanteles*species showed clear bias to nucleotide 'AT'. The nucleotide composition analysis revealed the high AT content in the COI gene of *A.taragamae*(74.0%). The nucleotides T, C, A and G present in the COI sequence in the following concentrations 40.2%, 13.5%, 33.8% and 12.4% respectively. *A.taragamae*and *Apantelesfumiferanae*are 9.83% difference in their nucleotides percentage.

The nucleotide divergence analysis revealed that, the *A.taragamae*COI sequence showed 10.03% divergence from the COI sequence of *Apantelesmilleri*. The phylogeny analysis using NJ tree revealed that*A.taragamae*, *A.fumiferanae andA. milleri*are originated from one ancestor, the main clade is divided in to branches (Fig. 1). Among the COI

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sequences of hymenopteran parasitoids used in this study, *Apantelesrosibelelizondoae*was the distant relative of *A.taragamae*.

Table 1. The evolutionary divergence of*A.taragamae*with related Hymenopteran species

Name of species name and GenBank Accession No.	*Percentage of nucleotide divergence*
HM373821 Microgastrinae	9.141%
HM374100 Apantelesfumiferanae	9.836%
HQ106552 Apantelesmilleri	10.037%
KF462128 Apantelescinthiabarrantesae	12.291%
KF461997 Apantelesjaviercontrerasi	11.840%
KF462050 Apantelesedithlopezae	11.332%
KF462169 Apantelesciriloumanai	14.862%
KF461885 Apantelesedithlopezae	11.332%
KF462083 Apantelesadrianaguilarae	14.060%
KF462235 Apantelesedgarjimenezi	13.331%
KF461937 Apantelesluisgaritai	13.373%
KF461962 Apanteleserickduartei	12.558%
KF462100 Apantelesgarygibsoni	12.277%
KF461978 Apanteleschristianzunigai	13.000%
KF461927 Apantelesraulacevedoi	16.027%
KF462073 Apantelesluisgaritai	13.373%
KF461973 Apantelesrosibelelizondoae	16.964%
KF461905 Apanteleschristianzunigai	13.000%
KF462118Apanteles bienvenidachavarriae	13.519%
KF462248 Apantelesraulacevedoi	16.027%
KF462257 Apantelesgarygibsoni	12.277%
KF462055 Apantelesmarisolarroyoae	12.801%
KF461951 Apantelesfelixcarmonai	12.568%
KF462079 Apantelesfernandochavarriai	11.891%

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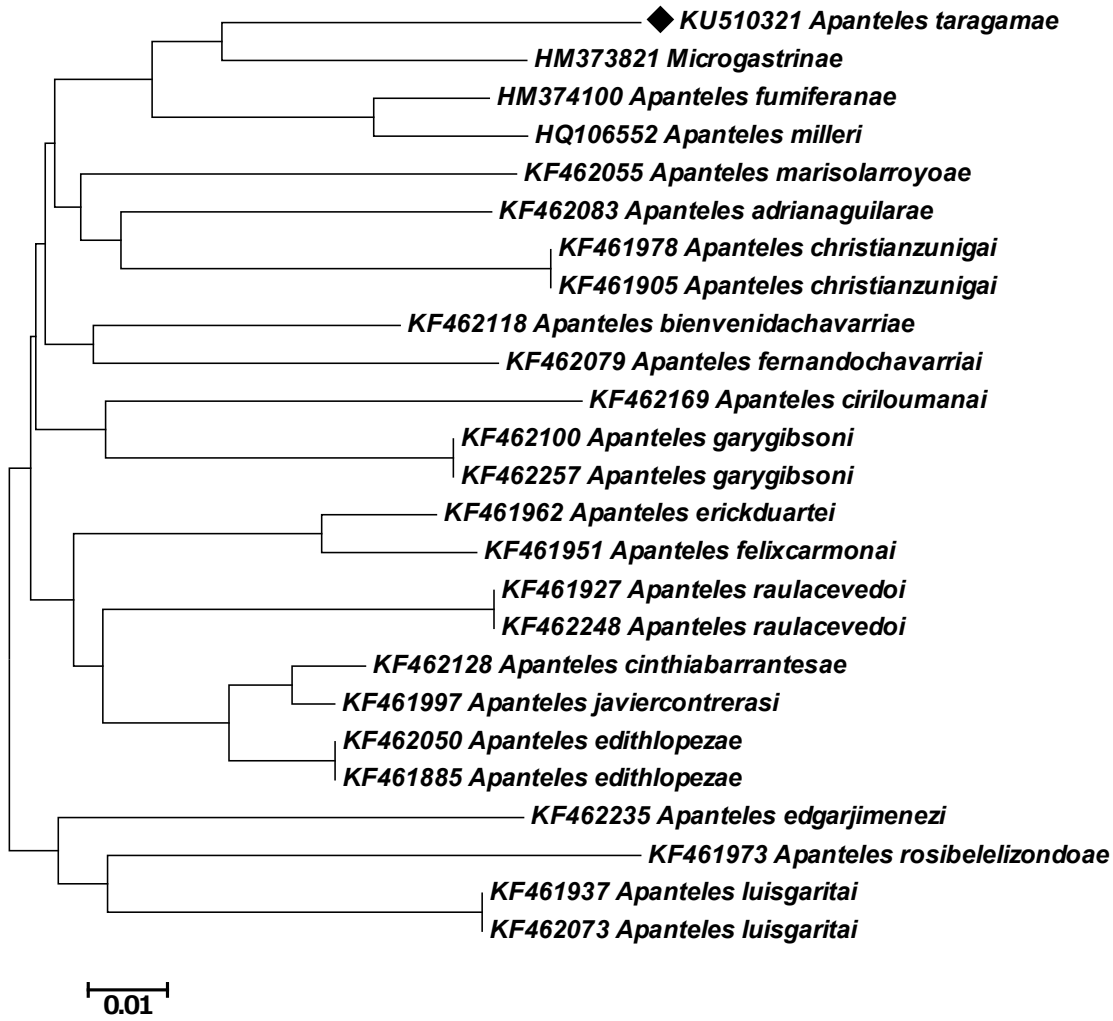

Figure.1. Phylogenetic relationship of *A.taragamae* isolated from Kerala inferred by Neighbor joining

Discussion

Using morphological characters, many phylogenetic studies of Hymenoptera was reported earlier. Phylogenetic analysis of Hymenoptera based on molecular data are revealed Ichneumonoidea very related to Vespoidea and Apoidea.Partial coding sequence of COI was proved as a powerful tool for the identification of organisms (Hebert *et al.*, 2004). The partial COI sequence generated in this study showed considerable variation with other species. The variation in the codons 'A' nucleotide composition in second position of COI sequence of *A.taragamae* and *A.fumiferanae* indicated that it has highest mutation rates. High proportion

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of 'T' in the second position of codon results in a preference of polar and hydrophobic amino acids in the membrane associated proteins. The high interspecific distance observed among *Apanteles* species may be due to the geographical isolation of these species.

The CO1 gene sequence has proved to be suitable for species identification in a huge variety of animal taxa, including wasps (Smith *et al*., 2008). The barcode developed in this study is a diagnostic tool for identification of this parasitoid.

Conclusion

The COI developed in this study can be used for the taxonomy and phylogeny analysis of the *A.taragamae*. Variation in the nucleotide sequence is a fundamental property of all organisms which used for its identification. This study reveals that the former region is capable of differentiating the variation of *A.taragamae*found in world.

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References

1. Rukhsana, K. & Sebastian, C.D. (2015). Genetic Structure and Molecular Phylogeny Analysis of *Braconbrevicornis*Wesmael, a Larval Parasitoid of Coconut Black Headed Caterpillar, *Opisinaarenosella* Walker,*Research in Biotechnology*, 6(3): 17-23.
2. Jones OR. (2009). Using taxonomic revision data to estimate the geographic and taxonomic distribution of undescribed species richness in the Braconidae (Hymenoptera: Ichneumonoidea). *Insect Conservation and Diversity*, 2(3), 204-212.
3. Hebert, P.D.N., Ratnasingham, S. &Dewaard, J. D. (2004). Barcoding animal life, Cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society London Biological Science,* 270: 96-99.
4. Jones, O. R(2009). Using taxonomic revision data to estimate the geographic and taxonomic distribution of undescribed species richness in the Braconidae (Hymenoptera: Ichneumonoidea). *Insect Conservation and Diversity,* 2(3), 204-12.

Corresponding Author: C.D. Sebastian E-mail: drcdsebastian@gmail.com

5. Rukhsana, K.,Akhilesh, V. P. & Sebastian,C.D. (2014). Deciphering the molecular phylogenetics of the asian honey bee, *Apiscerana* (Hymenoptera: Apidae) and inferring the phylogeographical relationships using dna barcoding, *Journal of entomology and zoology studies*, 2(4), 218-220.
6. Saitou, N. &Nei, M. (1987).The neighbor-joining method: A new method for reconstructing phylogenetic trees, Molecular Biology and Evolution, 4:406-425.
7. Tamura, K., Nei, M. & Kumar, S. 2004. Prospects forinferring very large phylogenies by using the neighborjoiningmethod. Proceedings of the National Academyof Sciences (USA) 101:11030-11035.
8. Tamura, K., Peterson,D., Peterson, N., Stecher, G., Nei, M. & Kumar S (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods, *Molecular Biology and Evolution*,28: 2731-2739.2
9. Yu, D.S., C. Van Achterberg, &Horstmann, K. (2012). World Ichneumonoidae, Taxonomy, Biology, Morphology and Distribution. Taxapad (Scientific names for information management) Interactive catalogue on DVD/CDROM. Vancouver. Available from: http://www.taxapad.com (accessed 27 April 2013)

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