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Short Communication

DNA barcoding for identification of *Conocephalus dorsalis* (Orthoptera: Tettigoniidae) from Northern Kerala using Cytochrome Oxidase Subunit I Gene

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Abstract

Grasshoppers are widely distributed in all ecosystems. Tettigoniidae is a family of grasshoppers including katydids or bush crickets in the order Orthoptera. Approximately 6,400 species of Tettigoniids are found around the world. Conocephalus dorsalis, Tettigoniidae family, was collected from rice fields of Northern Kerala. It is an omnivorous insect that feeds grasses, seeds and small insects including pests. They are predators on rice field, controlling pest populations. The present study deals with species identification and revealed phylogenetic history of C. dorsalis using cytochrome oxidase subunit I (COI) gene encoded as mitochondria. The COI gene of C. dorsalis, 589bp are sequenced and obtained was deposited in the NCBI GenBank.

Keywords: *Conocephalus dorsalis*, mitochondrial COI gene, DNA barcoding.

Introduction

Orthoptera is one of the largest order having over 20,000 species worldwide. Tettigoniidae is a major family of long horned grasshoppers, placed in the suborder Ensifera[1]. They have long and thread like antenna which may exceed their own body length. Tettigoniids play an important role in the ecosystem. Many species are exclusively predatory which make the balance between pests and their natural enemies. Some Tettigoniids are considered as insect pests by commercial crop growers and are applying pesticide to limit the population[2].

Conocephalus dorsalis is widely distributed over central Europe to Western Siberia. They are small green bush cricket, size from 11-18 mm in lengths. They are characterized by strong hind limbs for leaping, long and thread like antenna, the wings are short, abdomen entirely blackish and sometimes dark brown colour. It is an omnivorous insect but feeds mainly on seed heads, grasses and also captures small insects like pests[3]. There are reports from Indonesia have reported that *C. dorsalis* are predators on rice fields[4]. Therefore, they will benefit in reducing pest population and are naturally quite important. They colonize wetlands, reed edges, fen meadows and ditches. The nymphs are arises from May to June and the adults from July to mid October.

DNA barcoding is one of the most important taxonomic method which helps in the identification of organisms using short genetic markers. The barcode region, cytochrome oxidase subunit I gene (COI) was proposed by Paul Hebert[5] in 2003, based on intra specific and inter specific variation. The sequence data is also used to develop barcode libraries for identification of unknown species by matching sequences with the known species. Phylogenetic tree of related individuals are clustered together and established species relationships[6]. DNA barcoding is proposed as a powerful tool for taxonomic studies and the bioidentification of organisms.

Phylogenetic analysis using mitochondrial COI gene sequence were extensively carried out in various insect groups like grasshopper, *Microcentrum rhombifolium*[7], damsel flies *Ceriagrion coromandelianum*[8], cigarette beetle *Lasioderma serricorne*[9], leaf hopper *Thaia subrufa*[10] and moth *Herpetogramma stulasis*[11].

Materials and methods

Sample collection and preservation: *C. dorsalis* used in present study was collected from rice fields of Parappanangadi, Northern Kerala. Sampling was done manually, using sweep net method and the sample was transferred to 70% of alcohol contained tube. The collected sample was identified morphologically and which preserved as cooling storage at -20° C.

DNA extraction, amplification, sequencing: The Mitochondrial genomic DNA of cytochrome oxidase subunit I gene (COI) was extracted from one of the thoracic leg. The

tissue was homogenized and the genomic DNA was extracted to using genomic DNA kit (Origin genomic DNA kit). Approximately 2 ng of genomic DNA was amplified for COI gene using forward primer, 5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3' and the reverse primer 5'- TAA ACT TCA GGG TGA CCA AAA AAT CA- 3'. The PCR reaction mixture contained 2 ng of 1µl genomic DNA, 1µl each forward and reverse primer at a concentration of 10µM, 2µl dNTPs (2Mm), 10µl of 10X reaction buffer, 1µl Taq polymerase (5U/µl) and 84 µl water. The PCR profile involved initial denaturation step of 5 minutes at 95ºC, followed by 30 cycles of 10 second at 95ºC, 1 minute 50ºC and 1 minute at 72ºC and ending with final phase of 72ºC for 3 minutes. The amplified product of COI gene was analyzed on 2% TAE - agarose gel electrophoresis for the confirmation. The remaining portion of amplified product was column purified using GeneJET PCR purification kit (Fermentas Life Science). The purified PCR product of COI was sequenced from both ends using forward and reverse primers by Sanger's dideoxy chain termination sequencing method, with ABI 3730XL Automated Sequencer[12]. Sequences were aligned to using the MEGA6 software package and the estimation of residue and pair wise distances were using the Clustal W tool of MEGA6 software. The final sequences were used for its similarity using BLAST programme of NCBI GenkBank. The Sequence divergences were estimated and Neighbour Joining tree was developed to exhibit evolutionary history of species.

Results and discussion

In the present study, COI gene of *Conocephalus dorsalis* yielded as 589bp size of fragment. The sequence obtained was deposited in the NCBI GenBank (GenBank Accession: KX 503055). BLAST analysis revealed sequence similarity between the species and *C. dorsalis* has 97% similarity with *Acrida exaltata* (GenBank Accession: GU226877) from Tamilnadu, India, which doesn't belongs to same family. The two species of *Atractomorpha lata* (GenBank Accession: KF966602) and *Atractomorpha sinensis* (GenBank Accession: KJ889692) from USA showed 91% similarity with the present result. The percentage of COI evolutionary divergence of *C. dorsalis* with other related species is presented in Table-1. Phylogenetic analysis also depicted that *C. dorsalis* is closer to Othopteran grasshopper species and a phylogenetic tree (Figure-1) constructed using Neighbour Joining method revealed monophyletic lineage. The majority of insects under order Orthoptera showed 85 to 97% of sequence similarity to that *C. dorsalis* COI gene sequences.

The percentage of COI evolutionary divergence of *C. dorsalis* showed 9% evolutionary divergence between *Atractomorpha sinensis* (KJ889692.1) and *Atractomorpha lata* (KF 966602.1). *C. dorsalis* shows 15% evolutionary divergence between *Cibolacris parvileps* (JQ 513033.1), *Melanoplus sanguinipes* (KR 148046.1), *Sinopodisma housanda* (KC 139912.1) and *Acrida wellemsei* (KJ 8889501.1).

The study established that DNA barcoding can provide the complement taxonomical studies of organisms. The combination of DNA sequenced data with the classical taxonomy will serve as a model which can revealed on many disciplines. It would enhance the rate of species identification, which in turn helps in conservation of insect biodiversity. The results reveal that mitochondrial COI gene permits the unambiguous identification of grasshopper species. The partial sequenced gene of *C. dorsalis* was more diverge from the sister taxon *viz. Acrida exaltata*. This is also supported by the geographical aspects. The barcode generated for *C. dorsalis* in the present study can be used for accurate identification of the organism.

Table-1: Evolutionary divergence between *Conocephalus dorsalis* and other related species.

Species	% of divergence
GU226877.1 *Acrida exaltata*	3%
KJ889692.1 *Atractomorpha sinensis*	9%
KF 966602.1 *Atractomorpha lata*	9%
KP 641752.1 *Ichtyotettix mexicanus*	15%
JQ 513033.1 *Cibolacris parvileps*	15%
KR 148046.1 *Melanoplus sanguinipes*	15%
KR 145454.1 *Melanoplus femurrubrum*	15%
KC 139912.1 *Sinopodisma housanda*	15%
KC 139912.1 *Sinopodisma iushiensis*	15%
KC 139921.1 *Sinopodisma tsinlingensis*	15%
KJ 8889501.1 *Acrida wellemsei*	15%
KM 532301.1 *Opeia obscura*	15%
KM 816659.1 *Notostaurus albicornis*	15%
KR 005939.1 *Dociostaurus kraussi*	15%

Conclusion

The present study indicates that the COI sequence of *C. dorsalis* can be used as taxonomical studies and bioidentification system of the species, which is one of the dominating species in rice fields due to its high predation on pest species. The COI sequence of the *C. dorsalis* revealed that 3% to 15% of sequence divergence with many other grasshopper species. Phylogenetically *C. dorsalis* is closer to *Acridida exaltata* showing 97% of similarity.

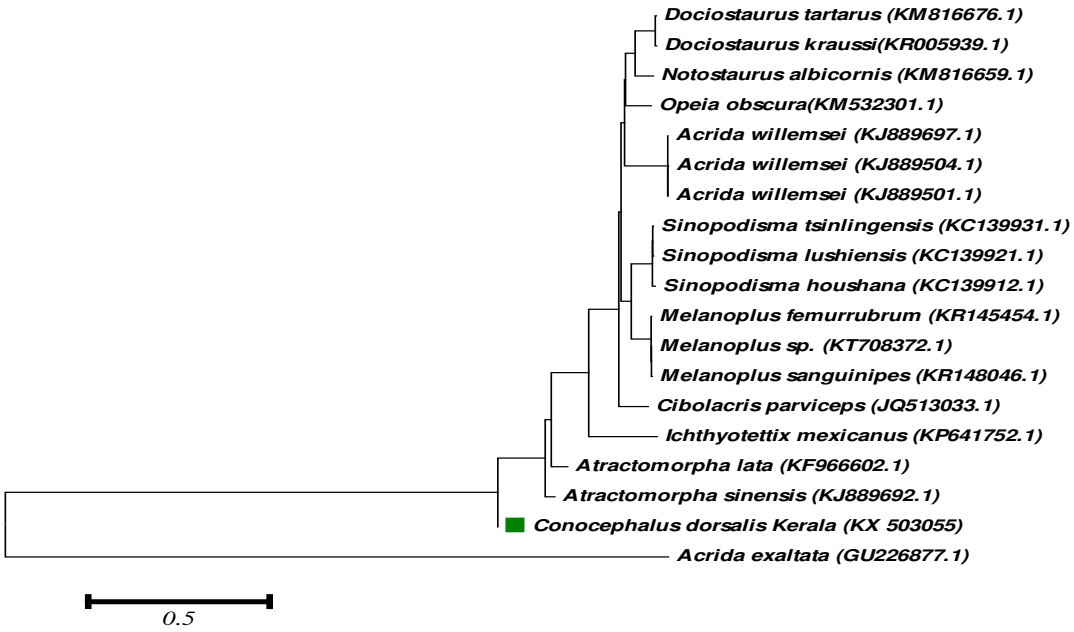

Figure-1: Phylogenetic status of *Conocephalus dorsalis* using Neighbor joining method.

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