# Molecular Taxonomy and Phylogenetic Status of Metaleptea Brevicornis Orthoptera: Acrididae Using Cytochrome Oxidase Subunit I Gene

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## Abstract

Grasshoppers are the most prevalent insects in pastures and grasslands. The present study was regarded as find a solution to the identification of grasshopper species of the family Acrididae order Orthoptera by DNA barcoding. Approximately 11,000 known species of Acridids are found around the world often inhabiting grass fields, meadows and forest areas. Metaleptea brevicornis is a species belonging to Acrididae family and were collected from a paddy field of Northern Kerala. The specimen was identified to use mitochondrial cytochrome oxidase subunit I gene (COI) and makes a phylogenetic tree for realizing of evolutionary relationships. The partial sequence of mitochondrial COI gene obtained was deposited in the NCBI GenBank for worldwide accession which can be used as DNA barcode of this species.

**KEYWORDS:** Metaleptea brevicornis, COI gene, DNA barcoding

## Introduction

Grasshoppers are herbivores insects and it is found all over the world. Acrididae is one of the enormous families of grasshoppers in the order Orthoptera. They are generally known as short horned grasshoppers or short horned locusts placed in suborder Caelifera. Acrididae represented as economically important insects that sometimes becoming pest due to serious damages to all crops (Hirdesh et al., 2014).

Metaleptea brevicornis is a grasshopper species, commonly called as clipped-wing grasshopper. They are widely distributed in the wetlands of East and North America. This species was founded by Johansson and Carl Linnaeus in his 1763 work Centuria Insectorum (Judith, 1983). It is an agricultural pest. They have chewing mouth parts that remove large section of leaves and flowers (Rusconi et al., 2017). The body may be long and slender or short and stout with green colour. They have a slant face, angled forewing tips, sword like antennas, short ovipositor and rear pointing spines on hind knees are distinctive. They prefer wetlands with sedges, paddy fields, grasses and sometimes occur in salt marshes (Kathryn and Carles, 2009). This organism has been emerging on July to October.

Taxonomy is the science of defining groups of organisms on the basis of shared features. The morphological taxonomy is extremely time consuming process and required an experienced taxonomist. DNA barcoding is a cost effective solution which identify the species of insects at any developmental stages. DNA barcoding intended to an able technique for molecular level species identification that uses a short fragment of cytochrome oxidase subunit I (COI) gene from mitochondria. Obviously

the molecular level species identification system contribute a powerful tool for taxonomic and biodiversity research (Mehrad, 2009). The main advantages of DNA barcoding is the molecular identification of already recognized species and the discovery of unrecognized species (Sukhamrit, 2015). The mitochondrial genetic marker was used to discriminating the phylogenetic evaluation of related organisms, since the cytochrome oxidase subunit I gene is maternally inherited which involves low recombination (Matthew et al., 2010).

The phylogenetic assessment using cytochrome oxidase subunit I gene sequences were performed in various groups of insects like grasshopper Microcentrum rhombifoli, (Mashhoor et al., 2012) and Sigaus australis (Steven, 2008), mormon butterfly Papilio polytes (Akhilesh and Sebastian, 2016), dragonfly Neurothemis tullia (Jisha and Sebastian, 2016), larval parasitoid Bracon brevicornis (Rukhsana and Sebastian, 2015), green bottle fly Lucilia sericata (Priya and Sebastian, 2014), Sand fly Lutzomyia sp. (Luis, 2016) and Bugs Pentatomid species (Sanket, 2014). Previous studies proved that mitochondrial COI gene is an efficient tool for identification of insects. The present study focuses in sequencing COI gene of Metaleptea brevicornis collected from Northern Kerala and its phylogenetic analysis was done. This is important pest of paddy fields the result can be used for its correct identification. The molecular based identification system can be used as helpful tool for the pest management.

## Materials and methods

### Sample collection and preservation

The M. brevicornis were collected from paddy field of Parappanangadi, Northern Kerala. At this site, healthy and uninfected samples were collected using sweep net method and transferred to 70% alcohol containing tubes. The organism was identified by morphologically then it stored at -20° C for the future purpose.

### DNA Extraction, Amplification, Sequencing

Genomic DNA was obtained from one of the thoracic leg of sample using Origin genomic DNA kit. The tissue was homogenized. About 2ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I gene using the 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'[Folmer et al., 1994). The PCR reaction mixture consisted of 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 84µl water. The PCR profile involves initial denaturation step of 5 minutes at 95º C, followed by 30 cycles of 10 second at 95º C. 1 minute 50ºC and 1minute at 72º C and ending with final phase of 72º C for 3 minutes. The PCR product was exhibited on a 2% TAE - agarose gel for the confirmation of the target gene amplification. The remaining portion of PCR product was column purified using GeneJET PCR purification kit (Fermentas Life Science). The purified PCR product was sequenced from both ends using forward and reverse primers by Sanger's method (Sanger and Coulson, 1975). Determination of pairwise distances was using the ClustalW tool of MEGA6 software (Tamura et al., 2013). The final sequence was searched for the similarity using Basic Alignment Search Tool of NCBI. The sequence was submitted in NCBI GenBank for the worldwide accession. Neighbor-Joining method is used for the phylogenetic tree construction. The sequence

divergence of COI gene of M. brevicornis from other species representing various genera of Acididae family was calculated.

**Results and discussion**

The mitochondrial COI gene of M. brevicornis amplified as 623bp yielded product. Afterwards, the obtained sequence was deposited into the NCBI (GenBank Accession: KX524518). The BLAST result also indicated that the nucleotide sequence of M. brevicornis showed 97% similarity with that of the sequence obtained for different genus Acrida exaltata (GenBank Accession: GU226877) from the same family. Further the sequence showed 3% of divergence reported from Tamilnadu, India. The COI gene of M. brevicornis with other associated species of evolutionary divergence presented in Table 1. Phylogenetic evaluation showed that M. brevicornis is adjacent to Orthopteran grasshopper species. The evolutionary history revealed to using Neighbor Joining method for the creation of phylogenetic trees is presented in Figure 1. M. brevicornis shows 91% similarity to Atractomorpha sinensis (KJ889692) isolated from USA. As well as the most of insects under order Orthoptera showed 85 to 97% of sequence similarity to the COI gene of M. brevicornis. The percentage of COI gene sequence divergence of M. brevicornis showed 9% divergence between Atractomorpha sinensis (KJ889692) and 15% sequence divergence between Acrida willemsei (KJ889503), Ceracris kiangu (KJ667354), Opeia obscura (KM 532301), Dociostaurus crassiusculus (KM816679), Sinopodisma housanda (KC139919) and Melanoplus femurrubrum (KM535940).

**Table1:** Sequence divergence between Metaleptea brevicornis and other associated species

| Species | % of divergence |
|---|---|
| GU226877.1 Acrida exaltata | 3% |
| KJ889692.1 Atractomorpha sinensis | 9% |
| KJ 889503.1 Acrida willemsei | 15% |
| KJ 667354.1 Ceracris kiangu | 15% |
| KM 532301.1 Opeia obscura | 15% |
| KM 816679.1 Dociostaurus crassiusculus | 15% |
| KM 816676.1 Diciostaurus tartarus | 15% |
| KM 816659.1 Diciostaurus startarus | 15% |
| KM 16659.1 Notostaurus albicornis | 15% |
| KC 139931.1 Sinopodisma tsinlingensis | 15% |
| KC 139921.1 Sinopodisma iushiensis | 15% |
| KC 139919.1 Sinopodisma housanda | 15% |
| KM 535940.1 Melanoplus femurrubrum | 15% |
| KR 148046.1 Melanoplus sanguinipes | 15% |

Pest is an important anxiety of farmers in all over the world. More than 10,000 insect pests are reported during the damages of crops. In India, the Northern Kerala where farming is well established and products are produced for international and domestic consumption. However, insect pests constitute an important variable in the farming economy of this region. The M. brevicornis is an agricultural pest that damages

various crops (Bhumi et al., 2015). DNA barcoding can help in identification of pests in any stage of life cycle, making easier to control them, saving farmers from pest damage. Fast and accurate identification of pest will help in pest management programme (Ghazali et al., 2014).

The barcode results in understanding both phylogenetic signal and population level variation. In phylogenetic study exhibit the DNA barcoding technique is initial point for most favorable solution of taxonomy and the sequences are added to GenBank for phylogenetic assessment (David et al., 2013). The genetic exploration provides an essential signal of population divergence and it will make easy the comparative studies of population diversity in many organisms.

Here geographical variations might have brought a genetic divergence in different genus obtained from distant geographical locations in the same family. The present study reveals rapid and accurate identification of cryptic species (Torbjorn et al., 2007).

**Conclusion**The present study reveals that COI barcoding permit the unambiguous identification of pest species Metaleptea brevicornis, agriculturally important pest widely distributed in paddy fields. The mitochondrial COI gene sequences of M. brevicornis disclose assessable variation with all other related species in the Acrididae family. Phylogenetically M. brevicornis is closer to Acrida exaltata, showing 97% of similarity.

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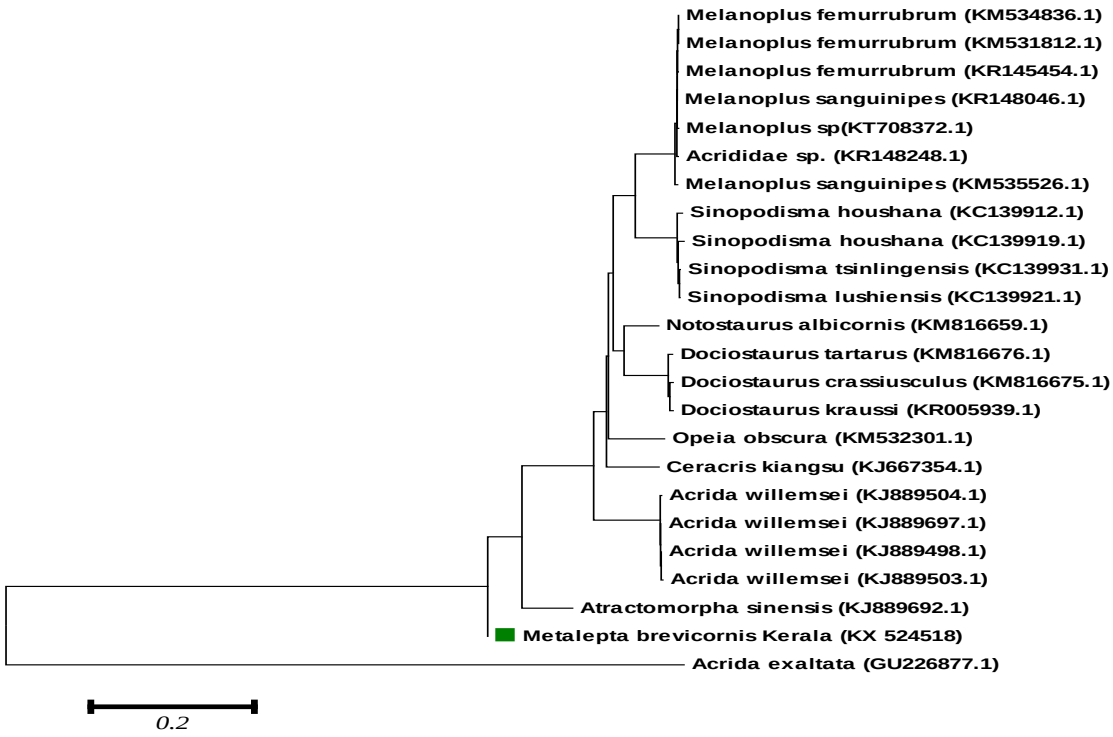


Figure 1: Phylogenetic tree of Metaleptea brevicornis using Neighbor Joining method