DNA barcoding of commonly prevalent *Culicoides* midges in South India

ARCHANA M1, PLACID E D’ SOUZA2, S K JALALI3, C RENUKAPRASAD4 and RAKSHITH OJHA5

Karnataka Veterinary, Animal and Fisheries Science University, Hebbal, Bengaluru, Karnataka 560 024 India

Received: 19 June 2014; Accepted: 11 September 2014

**ABSTRACT**

DNA barcoding has gained increased recognition as a molecular tool for species identification of insects. Interspecific variation in DNA sequences of some genes is much higher than intraspecific and provides an opportunity to use DNA sequences for species identification. A study was therefore undertaken to barcode 5 commonly prevalent *Culico* ides species in farming regions of Bengaluru districts in Karnataka state. The barcoding of Cytochrome oxidase I (COI) gene of *C. anophelis*, *C. palpifer*, *C. huffi*, *C. innoxius* and *C. circumpictus* yielded an amplified fragment of 648 bp sequence. Barcode for all 5 species was generated using BoldSystems v3 and submitted to genbank for accession numbers. DNA barcoding enabled exact identification of 5 prevalent species.

**Key word**: Bold Systems v3, *Culico* ides, Cytochrome oxidase I, DNA barcoding

*Culico* ides, biting midges, are nematocerous flies which are one of the smallest haematophagous flies measuring from 1 to 3 mm in size. More than 1,400 species of genus *Culico* ides were identified worldwide of which about 96% are obligate blood feeders attacking mammals and birds (Mellor et al. 2000). *Culico* ides is a prime vector for various viruses causing bluetongue disease, African horse sickness, epizootic hemorrhagic disease, Akabane, Aino, Chu-zan, and bovine ephemeral fever, vesicular stomatitis, equine encephalitis and schmallenberg viruses, protozoans such as haemoproteus, leucocytozoon, hepatocystis, avian trypanosomes, lizard and avians plasmodia and helminths such as *Onchocerca cervicalis*, dipetalonema and other filarid worms of birds and mammals (Prasad and Bhatanagar 2000).

*Culico* ides anophelis is a predator of engorged mosquitoes, parasitizing on another dipteran *Anopheles stephensi*. At least 19 mosquito species in the genera *Anopheles*, *Culex*, *Aedes* and *Armigeres* were documented as hosts of *C. anophelis*. The mosquito females infested with this ectoparasite failed to lay eggs and did not survive for long (Ma et al. 2013). *C. circumpictus* is regarded as ornithophilic in Israel, man biting and also feeding on cattle in Thailand. It is also a vector for *Leucocytozoon* in birds (Dasgupta 1995).

The morphological identification of *Culico* ides based on morphological features is tedious, confusing and time-consuming because its size ranges from 1 to 3 mm. Therefore the molecular method of identification is an aid for identification of species. Among the molecular methods DNA barcoding is widely used in species identification and biodiversity research (Kim et al. 2012). Cytochrome oxidase I (COI) barcoding sequences can be used to discover cryptic species: closely related and morphologically similar ones (Rivera et al. 2009) and is reliable, cost-effective and accessible solution to the current problem of species identification (Hebert et al. 2003). Mitochondrial (Mt) DNA is used for DNA barcoding because Mt DNA is much smaller than nuclear DNA and sequencing is easy. COI is used for DNA barcoding and very efficient for species identification, easy to isolate from wide range of organisms, therefore, in the present study COI was used for DNA barcoding. A study was therefore undertaken to barcode commonly prevalent *Culico* ides species in farming regions of Bengaluru districts in Karnataka state in view of their vector potential and importance as pests of animals and man.

**MATERIALS AND METHODS**

**Traps and collection**: Flies were collected by using UV-light trap from 11 different farms of cattle, buffalo, sheep and goat in rural and urban districts of Bengaluru. Collections were made during dawn and dusk and the traps were set from 6 pm to 6 am. The light trap was located within 25 m of livestock premises and were suspended from the walls of building at 1.5–2.0 m above the ground level at night.

To 300 ml of clean water 2 drops of detergent was added into the collecting beaker to break the surface tension of the water allowing collected insects to sink into the solution.
RESULTS AND DISCUSSION


PCR amplification of COI for barcoding of Culicoides spp.: Hebert et al. (2003) established that the mitochondrial gene cytochrome c oxidase I (COI) could serve as the core of a global bioidentification system for animals. COI gene of insects was amplified by PCR, and they yielded specific amplicon of 658 bp. In the present study C. anophelis, C. palpifer, C. huffi, C. innoxius and C. circumscriptus yielded a specific amplicon at 648 bp (Fig. 1) which is in agreement with the above findings. The above results were also in accordance with others (Ander et al. 2012, Puente et al. 2012, Kim et al. 2012). Therefore molecular method of identification was found to be faster with increased sensitivity and specificity. DNA barcoding has been widely used in species identification and biodiversity research (Kim et al. 2012) and provide a reliable, cost-effective and accessible solution to the current problem of species identification (Hebert et al. 2003).

Sequencing of PCR products: PCR products were sequenced in both forward as well as reverse directions and sequencing results were obtained in .ab1 file format and .txt format. Sequences were then checked for homology online with the bioinformatics tool BLAST (Basic local alignment search tool) from NCBI (National Centre for Biotechnology Information) server which confirmed the specificity of primers. The sequences showing maximum similarity confirmed the species.

The sequences were edited and the allotted accession numbers by genbank, (> Culicoides anophelis ACCESSION NO: KF145178, > Culicoides circumscriptus ACCESSION NO: EF506557).

Fig. 1. PCR amplification of COI for barcoding of Culicoides. (L: 100 bp Ladder, Lane 1: C. innoxius, Lane 2: C. huffi, Lane 3: C. anophelis, Lane 4: C. palpifer, Lane 5: C. circumscriptus, Lane 6: NTC).

Table 1. Nucleotide sequence of COIF/COIR primers

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Nucleotide sequence</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>COIF</td>
<td>5'-GGTCAACAATCATAAGATATTG-3'</td>
<td>648-658</td>
<td>Hamada et al. (2010), Rivera and Currie (2009), Hebert et al. (2003), Pramual et al. (2011), Puente. et al. (2012), Kim et al. (2012)</td>
</tr>
<tr>
<td>COIR</td>
<td>5'-TAACTTCAGGGTGACCCAAAAATCA-3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NO: KF145180, > Culicoides huffi  ACCESSION NO: KF145177, > Culicoides innoxius ACCESSION NO: KF145176, Culicoides palpifer  ACCESSION NO: KF145179). The BoldSystem V3 was used to generate barcode for the five species of *Culicoides*. A project was created at BoldSystems v3; Project Name is VETIP (Veterinary important insects), where all the specimen data and sequences were uploaded. The present aim is creation of DNA barcode library and automated identification of all eukaryotes based on DNA barcode library.

Identification of 5 different species of *Culicoides* based on morphology were confirmed by DNA barcoding to prove correct identity method. Interspecific variation in DNA sequences of COI genes is much higher than intraspecific correct identity method. Interspecific variation in DNA was confirmed by DNA barcoding to prove the principal vector of bluetongue (BT) and African horse sickness (AHS) in Europe. *Veterinary Research* 32: 325–37.

ACKNOWLEDGEMENT

The provision of insect light trap for the research work by Dr S. M. Byregowda Joint Director, IAH & VB is gratefully acknowledged. The necessary lab facilities provided by the Biotechnology unit of NBAIL Bangalore and facilities provided by ICAR through the Centre of Advanced Faculty Training in Veterinary Parasitology is gratefully acknowledged.

REFERENCES


