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Plant Protection

Identification of a Calliphorid Fly, *Pollenia Rudis* (Diptera: Calliphoridae) Based on its Fragments through DNA Barcoding

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Abstract

A bottle was received from a pharmaceutical company containing fragments of insect species like antennae, two leg pieces, a portion of the abdomen and two intact wing pieces at ICAR-National Bureau of Agricultural Insect Resources, Bangalore, India, for the possible identification of the insect specimen. The wing pieces given to the taxonomists suggested that the wing fragments belonged to a calyptrate dipteran - Sarcophagidae/Calliphoridae relative. Furthermore, DNA barcoding based identification was employed to determine the identity by amplifying COX1 mitochondrial gene, which was 658 bp size and GenBank accession number and barcode were generated, *viz.*, KT368817 and VETIP006-15, respectively. Our sequence matched 100% with GenBank accession nos. GQ409351 and JF439551 and identity were determined as *Pollenia rudis* (Fabricius) (Diptera: Calliphoridae). The present work highlights that DNA barcoding based identification tool a powerful and imperative in determining the identity of insect, even if a part or fragment of the specimen is available. This method can be used for insect identification wherever fragments are available, which can lead to preventive measures.

Highlights

- DNA barcoding based identification determined the identity of the insect as *Pollenia rudis*.
- This method can be employed for identification based on fragments for other insects.

Keywords: DNA barcoding, *pollenia rudis*, fragments, identification

Pollenia species are commonly known as the attic fly, cluster fly, loft fly and buckwheat fly belonging to family Calliphoridae and are widely distributed all over world. Often, the flies are found "bunching" near the interior windows of a warm structure. They are generally not harmful to human beings as they neither lay eggs nor contaminate human food, however, do cause nuisance, when the adult swarm enter houses to hibernate during late summer or autumn. Among the caliphorids, *Pollenia rudis* (Fabricius) is one of the most important species. It has been recorded from Europe, USA and Canada (Rognes 1987,Whitworth 2006). In India, Bharti (2011) updated checklist of blowflies and listed *P. rudis* as one species occurring in India. Identification of insect species is generally done based on the morphological characters. However, molecular techniques using mitochondrial gene COX1 have been recently used for identification, which are reliable and rapid in view of the problems faced in morphology based identification.

A bottle from pharmaceutical company was received that contained fragments of insect including wings for identification. The fragments of wings were subjected for possible morphology based identification and further the fragments were subjected to DNA barcoding based identification based on mitochondrial gene COX1. DNA based identification of species can be a very useful tool particularly in cryptic species or where fragments are available, which cannot be resolved through conventional identification. Andriæ *et al.* (2014) emphasized the larval molecular based identification to be effective in identifying unknown larvae of many insect

species. The DNA can be acquired virtually from any part of the insect body. A project termed "the barcode of life" was initiated, which described identification method based on DNA. This difference in sequences helps entomologists to easily identify two similar species, invasive species or determine the identity even if fragments are available (Nagoshi *et al.* 2011). In the present study, the objective was to study if DNA barcode-based identification can be useful for identification of insect species based on fragments.

Materials and Methods

Morphological identification

Fragments of insect including a single pair of wings were obtained from a medicine bottle sent for identification. The wing fragments were relaxed in a container with cotton swabs soaked in 5% acetic acid and covered with butter paper (to avoid entangling of the specimen remains with cotton fibers) for 30 minutes. . They were subsequently exposed to normal dehydration process keeping 15 minutes each in 50%, 70%, 90% and 100% alcohol, respectively. Later specimens were transferred to 100 % alcohol plus clove oil (M/s Vasa Scientific, Bangalore, India) in the ratio 50:50 for 15 minutes and finally to 100% clove oil before mounting in natural Canada balsam. Photographs of the wings were taken using Leica M 205 A stereo zoom microscope with Leica DC 420 inbuilt camera using auto montage software (version 3.8). The specimen remains are deposited in the ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India.

DNA extraction and sequencing

DNA was extracted from the dry tissues (leg, antennae, a portion of abdomen and wings) of insect specimens rich in mitochondria using Qiagen DNeasy® kit, following the manufacturer's protocols. The extracts were subjected to polymerase chain reaction (PCR) amplification of a 658bp region near the 5' terminus of the *COX1* gene following standard protocol (Hebert *et al.* 2003). Primers used were: forward primer (LCO 1490: 5'- GGTCAACAAATCATAAAGATATTGG-3'), and reverse primer (HCO 2198: 5'- TAAACTTCAGGGT GACCAAAAAATCA-3'). PCR reactions were carried out in 96-well plates, 50 µL reaction volume containing: 5 µL GeNeiTM Taq buffer, 1 µL GeNeiTM 10mM dNTP mix, 2.5 µL (20 pmol/µL) forward primer, 2.5 µL (20 pmol/µL) reverse primer, 1 µL GeNeiTM Taq DNA polymerase (1 U/µL), 2 µL DNA (50 ng/µL), and 36 µL sterile water. Thermocycling consisted of an initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. PCR was performed using a C1000™ Thermal Cycler. The amplified products were analyzed on a 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001). The amplified products were sent to M/s Chromous Biotech, Bangalore, India, for sequencing. The species was bi-directionally sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI BLAST and ORF finder. A maximum parsimony tree was constructed in the program MEGA 5.05 by applying 1000 bootstrap support and tree was inferred by Subtree-Regrafting (SPR) method in the dataset and the most parsimonious tree was selected. The sequence was uploaded to GenBank and the Barcode of Life Database (BOLD, http://www.boldsystems.org).

Fig. 1. Wings (left and right forewing) of *Pollenia rudis*

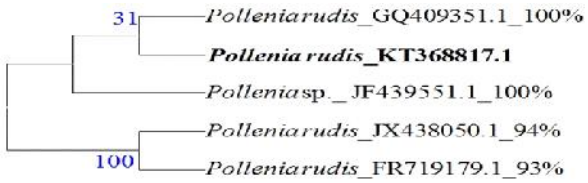

Fig. 2. Maximum parsimony tree for *Pollenia* species

Fig. 3. DNA barcode of *Pollenia rudis* (VETIP006-15)

Results and Discussion

Taxonomic constraints

The fragments of the specimen(s) were identified belonged to Order Diptera and family Sarcophagidae based on morphological characters of wings (Figure 1). For further identity confirmation, the images were sent to two internationally renowned taxonomists for second opinion.

Expert 1 comments: The wings could be either of family Sarcophagidae (Order: Diptera) or of another closely related group such as family Calliphoridae or

family Tachinidae as some species in these groups also show a very similar wing-vein pattern to this, and cannot be distinguished by their wings alone (pers. comm. Nigel Wyatt).

Expert 2 comments: It is difficult to identify the family Sarcophagidae and family Tachinidae based on the wings only as they have similar wing venation. For correct identification, abdomen with intact male genitalia is required (pers. comm. C.A. Viraktamath).

Molecular identification

Good quality of DNA was obtained from the fragments of insect specimen present in the bottle received from a pharmaceutical company eventually resulted into a PCR product. The sequence showed 100% similarity to *Pollenia* sp. JF439551 and *P. rudis* JX438050 and 96% to *P. rudis* FR719179. DNA sequences obtained from BLAST search were combined for phylogenetic analysis.

Maximum parsimony tree (Figure 2) showed 31% bootstrap supports between *P. rudis* (Current study - KT368817) and *P. rudis* GQ409351 (100% BLAST similarity) and *Pollenia* sp. JF439551, which was found sister to the above two sequences also showed 100% similarity in BLAST analysis, however, two *P. rudis* GenBank JX438050 and FR719179 were grouped together and formed a outgroup due to the differences in their COX1 region to the ingroup species. The outgroup species showed 93-94% similarity in BLAST analysis to the *P. rudis* of current study. A DNA barcode (Figure 3) for specimen understudy was developed and submitted to VETIP project at BOLD systems.

Species identification based on fragmented tissues is becoming widely accepted procedure in the molecular laboratories across the globe. Mitochondrial DNA has accomplished the identification of species based on one complete turtle shell (Hsieh *et al.* 2008) to sea food adulteration (Handy *et al.* 2011). Forensically important flesh fly was identified from immature life stages through DNA barcoding (Meiklejohn *et al.* 2013). In another study, species determination was done based fragments of insects received using mitochondrial DNA haplotype. The specimens that produced the same haplotypes for the calliphorid fly species, *Cynomya cadaverina* (Robineau-Desvoidy were identified as such (Wells *et al.* 2001). They also mentioned wherever these haplotypes are not closely related to any previously published data; specimens cannot be identified based solely on haplotype data. Various molecular tools were employed to differentiate zygotic, nucellar seedlings and seed and seedlings proteins in plants (Mahanthi and Usha Rani 2013, Sumathi and Balamurugan, 2013). The present study revealed that DNA barcoding of insects is a powerful technique to identify the insect specimen based on the fragments, which is not possible by morphology based identification.

Based on the DNA sequencing data, our sequence (KT368817) matched 100% with GenBank accession no. GQ409351 and JF439551 and identity was determined as *P. rudis* (Diptera: Calliphoridae), which was not at all possible through morphological characterization as only the fragments were received for identification. The results of the present study can be useful for identification of insects even if fragments are available in different parts of the world.

Conclusion

A bottle was received from a pharmaceutical company containing fragments of insect species for the possible identification of the insect specimen. Based on the fragments, which contained intact wings, tentative identity was determined to family level. DNA barcoding based identification was employed to determine the identity by amplifying COX1 mitochondrial gene, which was 658 bp size and identity determined was *P. rudis* (Diptera: Calliphoridae). GenBank accession number and barcode were generated, *viz.*, KT368817 and VETIP006-15, respectively. This method can be used for insect identification wherever fragments are available, which can lead to preventive measures.

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