IDENTIFICATION OF 12 YEARS OLD MUSEUM SAMPLES OF ISOMYIA TIBIALIS, COSMINA PRASINA AND COSMINA LIMBIPENNIS (CALLIPHORIDAE: RHINIINAE)

Devinder Singh*and Naina Khullar

Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India 147002

Email: devinder.ss.61@gmail.com

(Received on Date: 15th March 2015 Date of Acceptance: 17th May 2015)

ABSTRACT

Mitochondrial deoxyribonucleic acid (mtDNA) is a prudent and effective tool in identification and phylogenetic analysis of various dipterans insects. The most intermittently used gene is Cytochrome oxidase subunit I (COI) which is immensely informative and considered valuable for predicting divergence. In the present study 180 bp region of COI gene has been successfully amplified for 12-13 years old, dried museum samples, namely Isomyia tibialis (Villeneuve, 1927), Cosmina prasina (Brauer and Bergenstamm, 1889) and Cosmina limbipennis (Macquart, 1848) belonging to subfamily Rhiniinae. DNA sequencing was performed and finally phylogenetic tree was constructed for deciphering phylogenetic interrelationship. DNA in such old samples usually becomes highly fragmented and shows inter strand crosslinks, which further hamper the amplification process, thus this short fragment of COI gene may prove to be a valuable identification and phylogenetic marker.

Keywords: Cytochrome oxidase subunit I (COI); Mitochondrial DNA Typing; Rhiniinae; museum samples; identification; phylogenetic analysis

No. of Tables: 3 No. of Figures: 1 No. of References: 4
INTRODUCTION

Insects are diverse, vivid, proficient creatures, which are cosmopolitan in their distribution. Among these, the dipterans represent one of the largest insect orders. Rhiniinae is now categorised as a subfamily in forensically important Calliphoridae family (Bharti, 2011). In the present study, an attempt has been made to sequence 3 different species belonging to two genera. The challenge with the old and dried museum samples is that, the DNA gets highly fragmented and the samples despite of proper preservation do get infected with certain bacteria and fungi. Thus it is a tedious task to extract DNA and successfully sequence such samples. Worldwide, valuable alpha and beta level of taxonomic work, based on morphological and genitalial attributes have been undertaken on these flies. Many foreign authors have also been known to contribute significantly in the molecular field pertaining to the family Calliphoridae but this subfamily has remained completely ignored. This is a first attempt to sequence and molecularly analyse members of this subfamily. Rhiniinae is an important subfamily of family Calliphoridae. Members of this group are metallic to dull grey-brown and have glossy occiput. Epistome is strongly projecting and thoracic squama is bare. The most striking diagnostic feature of genus Cosmina is the absence of presuturalacrostrichals and that of genus Isomyia is the presence of black setae in the upper part of mesopleuron.

MATERIALS AND METHODS

Specimens The three species Isomyiatibialis, Cosminalimbipennis and Cosminaparsina were chosen for the present work. These were approximately 12 -13 years old. These flies were killed using ethyl acetate vapours and the samples were dried, preserved in insect boxes using naphthalene powder. The samples were old and degraded, so extraction of DNA was a challenging task. Morphological description of these flies is as under:

Table 1:

<table>
<thead>
<tr>
<th>Name</th>
<th>Frons</th>
<th>Epistome</th>
<th>Genae</th>
<th>Facial carina</th>
<th>Parafrontal</th>
<th>Thorax</th>
<th>Chaetotaxy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isomyiatibialis</td>
<td>reddish brown</td>
<td>Dull golden, bare</td>
<td>Metalllicpurplish</td>
<td></td>
<td></td>
<td></td>
<td>Acrostichals0+2, dorsocentrals 2+4, intra alars 1+2, presutural present, humeral 1, posthumeral</td>
</tr>
<tr>
<td>Cosminalimbipennis</td>
<td>reddish brown</td>
<td>shining black</td>
<td>Shining black</td>
<td>Ash coloured with black spots</td>
<td>Dull greenish with coppery reflections, silver dusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Face shining dark grey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>blakpaipi, vertical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DNA extraction: The old museum samples were first rehydrated and then washed in distilled water to remove any foreign DNA or microbes. These samples were then dissected using sterilized surgical blade. Only legs were dissected out and used for DNA isolation. The tissue was crushed using liquid nitrogen. Then cell lysing (0.01M EDTA, 0.03 M Tris- HCl , 0.01 M sucrose) solution and proteinase K was added and incubated overnight at 55°C in 1.5ul eppendorf tubes. These were then subjected to centrifugation at 13,000 rpm for 7 minutes. After this, the pellet containing the debris was discarded and the supernatant was carefully procured and used for further extraction(Kambhapat and Rai, 1991). Equal proportions of isopropanol and chilled ethanol approximately double in volume to the supernatant were added. To this supernatant 1ul of glycogen solution (Qiagen) was added which is a vital ingredient for the precipitation of traces of DNA. These were then left in the deep freeze for DNA precipitation for overnight. Then these were centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded and pellet was washed with ethanol and air dried. PCI treatment was done for further purification. This purified pellet was then dissolved in TE and stored at -20°C.

Amplification
A specific region of COI gene of mitochondrial DNA was chosen as the target for amplification. Amplification was the most challenging part as optimum annealing temperature was required to be set for every sample. All polymerase chain reactions were performed using Bio-rad T100 TM thermal cycler. The thermal cycler conditions were the following: initial denaturation at 98°C for 2 minutes followed by 40 cycles at 98°C for 30 seconds, annealing at 55°C for 40 seconds, elongation at 72°C for 1 minute and final elongation at 72°C for 7 minutes. 50μl PCR cocktail constituted of Phusiontaq DNA polymerase enzyme 1U/50μl reaction, 5X Buffer 10μl, 10pm dNTP, 50Mm MgCl₂ 1μl, 10pm primers 1ul each and MQ water (Thermo Fisher Scientific, India). The primers used were modified universal primers (Junqueira et al., 2002).

Electrophoresis
PCR products were detected by gel electrophoresis in 1.2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.
Fig 1: Gel image of successfully amplified PCR products of 14 years old blow fly species namely; IT- Isomyiatibialis; CL- Cosminalimbipennis; CP- Cosminaparsina and L- Ladder

Table 2: Primers used

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI –F</td>
<td>ITGATTTTTTTGGTCATCCAGAAGT</td>
</tr>
<tr>
<td>COI - R</td>
<td>ACTGTAAATATATGATGAGGCTCA</td>
</tr>
</tbody>
</table>

Sequencing

>Seqit1 [organism= Isomyiatibialis]
ATAAAGTTTTCTTTAAAATTCGGTATTTTCTCCAGATTTTCTCAAAAGAAGCGCCCTTTTCGGATACCTCTCAA
TGGTATCCGAAACGTTTCTTATTTGCTTTTCTAGGTATGAGCTCATCACAATGTTACATTTAAA
>Seqit2 [organism= Isomyiatibialis]
GTATTTGCTCAGAAGACGATCAAAGACGTTGGCAGTACCTGGAAGAGATCCGAAAAGGCGCTTTCTTGA
AAAATACCGAATTCGCCGAGAAATACCCGAAAGCGCGCAATACCAAGATGAAACTCTGGATGATCCAAAAGAA
TCAAAATTTAGT
>Seqcl1 [organism= Cosminalimbipennis]
CATTACAGCCCTGGTATATCCCATATTTGTAACCTATACTCCTCCGAGAAAAGAACCATTITGGGATACATGTAAT
GGTCTGACTATGATATCAATGCTAAGGTTTATCTGAGCTCATATATTACAGAAAA
>Seqcl1 [organism= Cosminalimbipennis]
CTAAGGAGGGCTTGGTATATCCCATATGTGAAATCCCTTTTCTTTTCTCCGGAGATAGGATATATG
CTTATGAAAGGATTTATCCGAGAAATCCGAGTGAAGAAATATATAATAGGCTTGAGATTATATGAAAGGAA
>Seqcp1 [organism= Cosminaparsina]
GAAATTTCCCTGCCTTTGGCAGCATTATACTGCTTCAGACCCCATATTTGTAACCTATACTCCTCCGAGAAAAGAACCATTITGGGATACATGTAAT
GGTCTGACTATGATATCAATGCTAAGGTTTATCTGAGCTCATATATTACAGAAAA
>Seqcp1 [organism= Cosminaparsina]
TTCGCCCACCGCCGATCTTGGCACTATCCTGGCAGTATGCTGCCTCCGACTTTTGCCAGAGCCTGTGTTTATGATAGGATATATG
CTTATGAAAGGATTTATCCGAGAAATCCGAGTGAAGAAATATATAATAGGCTTGAGATTATATGAAAGGAA
RESULT AND DISCUSSION
The present study pertains to the use of small COI marker for the identification of old dried pinned museum samples. The old samples generally loose the intact DNA as the tissue and cells decompose and thus only small amount of fragmented DNA is available for amplification. The author has been able to successfully amplify and sequence small region of COI gene for three species of family Calliphoridae, namely Isomyia fulvicornis, Cosminalimbipennis and Cosminaparsina. Sequencing results showed that this primer has worked satisfactorily well for these old museum samples. The attempt was to try if this small fragment of COI gene holds some signature sequences specific for identification. The results shows that this small fragment does provide small percentage of match to the species but the percentage match is not very significant thus the author suggests cloning of these small fragments in a suitable vector.

REFERENCES
Bharti, M. 2011 "An updated checklist of blowflies (Diptera: Calliphoridae) from India." Halteres


Table 3:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reference</th>
<th>Protein translation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isomyia fulvicornis</td>
<td>Seqif1</td>
<td>IKFSLKSVFS PRFCDFLKKA PFRILFNGIR NGSYWFRLFH GSSSHVYII</td>
</tr>
<tr>
<td>Isomyia fulvicornis</td>
<td>Seqif2</td>
<td>YWLEARSRQL RYLEEIRKGA FLKKSQKFRR KYRKPAIPRC KLLDVQRIKI</td>
</tr>
<tr>
<td>Cosminalimbipennis</td>
<td>Seqcl1</td>
<td>TYSFGYLPYC NLLLRKKRTI WIHRYGLSYD INWLPRVYRV SSSYIYRK</td>
</tr>
<tr>
<td>Cosminalimbipennis</td>
<td>Seqcl2</td>
<td>SLIFWSSRSL YSYPTRLRNN LPYCNLLLRK KRTIWIHRYG LSYDIKGLL</td>
</tr>
<tr>
<td>Cosminaparsina</td>
<td>Seqcp1</td>
<td>NFPCLPLASSR RCRLPEAA VWLCINGVNCN VYRAAVIGF VSSSYIYSN</td>
</tr>
<tr>
<td>Cosminaparsina</td>
<td>Seqcp2</td>
<td>YFDLVIQKC TSGFCLPLAS SRLYPNTFAR KPLFAYACNG VCNVKDWRWA</td>
</tr>
</tbody>
</table>