



## New Host Record of Blossom Midge, *Contarinia maculipennis* (Felt) (Cecidomyiidae: Diptera) in Tamil Nadu, India

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### ABSTRACT

The blossom midge *C. maculipennis*, a serious pest in orchids which infests blooms and mainly hampered the cut flower production have been newly found in Tamil Nadu in recent years. Those infesting the flower buds of *Dendrobium* spp. (Orchidaceae) was identified, on the basis of morphological key characters and further, the identity of midge specimen was confirmed through molecular techniques with mitochondrial Cytochrome Oxidase subunit I (COI) gene specific primers, as *C. maculipennis*. The COI sequence of *Contarinia* species was closely (100% similarities) related with already sequenced *C. maculipennis* with 678 bp. *Dendrobium* spp. is newly regarded as one of the host plants of *C. maculipennis*.

**Keywords:** *Contarinia maculipennis*, *Dendrobium* spp., morphological, molecular

### 1. INTRODUCTION

Orchids are the most elegant and colourful flowers widely used as cut flowers and decorative flowers. Orchids are the most important flowering plants valued for cut flower production due to their long lasting vase life and high price in the international market (Gupta, 2017). Among the orchids, *Dendrobium* spp. are the most popular tropical orchids widely used as cut flowers in the world (Sugapriya *et al.*, 2012) and occupies nearly 90 per cent of the area under orchid cultivation due to the advancement in management practices and availability of plant materials (Sujatha, 2009). In South India, Kanyakumari district of Tamil Nadu and Southern and Coastal district of Kerala are having the congenial climate for commercial cultivation of orchids.

*C. maculipennis* has been recorded as an important insect pest infesting the flower buds of *Jasminum sambac* Linn. In Andhra Pradesh (Thirumala Rao *et al.*, 1954) and *J. auriculatum* Vahl. In Tamil Nadu by David (1958) for the first time. Further, it is primarily a pest of hibiscus and dendrobium orchids, tomato, jasmine, plumeria, eggplant, pepper, bitter melon and many vegetables and ornamentals (Kawate and Sewake, 2014).

### 2. Materials and methods

#### 2.1. Morphological identification of blossom midge on orchids

Flower buds showing the infestation or damage symptoms were brought to the laboratory and different life stages were mounted on slides using Canada balsam as medium and observed for morphological characters under Magnus MLX<sup>®</sup> microscope (ZENITH, India).

The larvae and adults of orchid blossom midge were collected from the study area and preserved in 70% ethanol and sent to University of Agricultural Sciences, Dharwad for taxonomical confirmation.

#### 2.2. Molecular characterization of blossom midge, *C. maculipennis* on orchids

Total nucleic acid was extracted from the adults of *C. Maculipennis* following Gem-CTAB method (Rouhibakhsh *et al.*, 2008) and agarose gel electrophoresis was carried out and examined under an UV light and documented utilizing Gel Doc<sup>™</sup>. The unpurified fragment of Cytochrome oxidase subunit I (COI) region was sent to the sequencing facility,

Eurofins Genomics India Private Limited, Bangalore, for the amplification of PCR product and single pass DNA sequencing with COI forward and reverse primers.

Fragment of mitochondrial COI region amplified by PCR was carried out with HCO-2198 and LCO-1490 primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. PCR product was resolved on 1% agarose gel and it was purified before sequencing. Consensus sequence of the PCR amplicon was generated from forward and reverse sequence data using aligner software. Local alignment was performed using NCBI-BLAST and ten sequences based on maximum identity score were selected. Multiple alignments was performed using Clustal W.

### 3. Results

#### 3.1. Morphological identification of blossom midge on orchids

The midge infested orchid buds were brought to the laboratory and dissected out for the presence of various life stages *viz.*, egg and maggot. The field collected pupa and adults were also brought to the laboratory. Specimens of egg, different larval stages, pupae and adults were mounted on glass slides and observed under microscope. The morphological key characters of adult blossom midge were observed for identification of the species. In female midge, moniliform antennae with whorl hairs nearly twice the length of the body was observed. In male, antennal length is three fourth of the body. Both sexes had reddish brown mesonotum with black head. Wings distinctly sub hyaline and abdomen yellowish brown in colour. Based on the morphological key characters observed as described by Felt (1933) the midge species was identified as *C. maculipennis*. Further the specimens were sent to Dr. Kumar Ghorpade, Scientist Emeritus and Honorary Research Associate in Systematic Entomology, University of Agricultural Sciences, Dharwad, who confirmed the midge species as *C. maculipennis*.

#### 3.2. Identification and confirmation of blossom midge by molecular technique

The midge species collected from orchids was identified as *C. maculipennis* based on the morphological characters. But, confirmation on the identity using molecular techniques would be more accurate and appropriate. For this, genomic DNA from the midge specimen was isolated using CTAB

method and a single band of intact genomic DNA was visualized on the agarose gel (Fig. 1). From this, mitochondrial COI gene of the midge specimen was amplified with primers HCO-2198 (forward) and LCO-1490 (reverse) using a thermocycler and the product was visualized as a single band in agarose gels stained with ethidium bromide. The amplicon size of a mitochondrial COI gene fragment was 678 bp (Fig. 2).

#### 3.3. DNA sequence analysis of COI region

COI products of the midge specimen obtained by PCR were cleaned with PCR cleanup kit to remove the residual primers, polymerases and salts in the PCR product. The purified PCR product of midge specimen was sequenced at Eurofins genomics Private Limited Bengaluru, India (Fig. 3). The mitochondrial DNA homology was performed using the BLAST program searched in the database of National Center for Biotechnology Information (NCBI), USA (Fig. 4). In this homology analysis, the sequence of COI region of mitochondrial DNA of the specimen showed 100 per cent identity with COI region of mitochondrial DNA of *C. maculipennis*

### 4. Discussion

The morphological key characters of blossom midge, *C. maculipennis* used for its identification and description by the earlier workers by Felt (1933), and David *et al.* (1990) are used for identification in the present study. The distinguishable characters of the midge such as tiny insect, moniliform antennae with whorl of hairs; black head; sub hyaline wings; yellowish brown abdomen are in concurrence with earlier findings and the midge species has been identified as *C. maculipennis*.

However, the species confirmation was done with the help of molecular techniques involving the genomic DNA of the organism. For identification of species genera and species, genomic DNA is interrupted by the Cytochrome oxidase subunit I (COI) which was employed due to their specific sequences as a target region. Though a number of DNA based identification methods are available, the specific advantage of COI sequencing using HCO-2198 and LCO-1490 primers used for identification of many gall midges using the database containing the corresponding sequence of previously identified midge species or closely related species was in line with Uechi *et al.* (2007). In the present investigation, COI sequence of genomic DNA was PCR amplified and mitochondrial DNA fragment

from COI PCR amplicon was 678 bp. Sequencing of COI region indicated that the sequence is similar to *C. maculipennis*. Thus, the blossom midge specimen was confirmed as *C. maculipennis*. The earlier studies done by Yukuwa *et al.* (2003) and Hebert *et al.* (2003) utilized the COI primers for molecular characterization of midges.

## 5. Conclusion

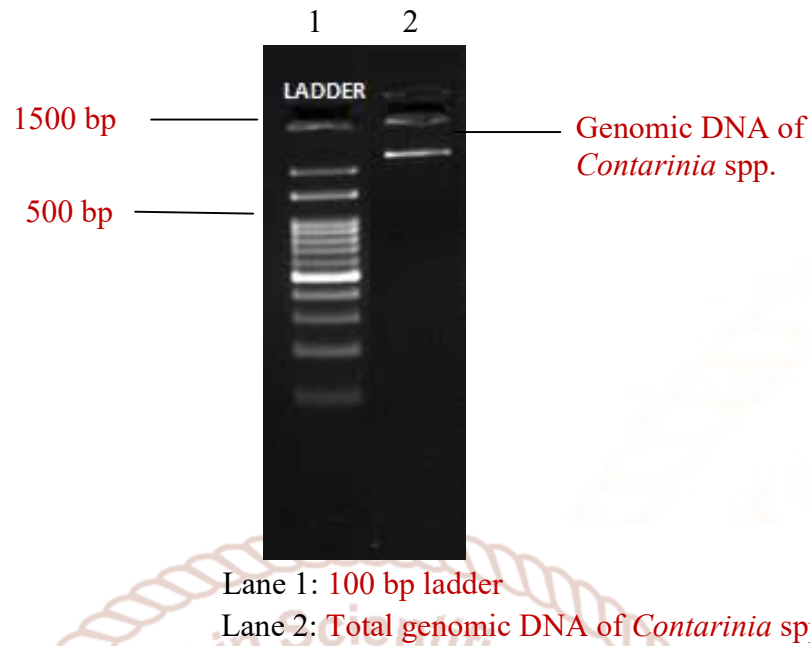
Increased incidence due to the introduction of this pest from Thailand during importation of orchids into Tamil Nadu and the absence of efficient natural enemies, with prevailing agro climate conditions coupled with increase in reproductive capacity.

## Acknowledgments

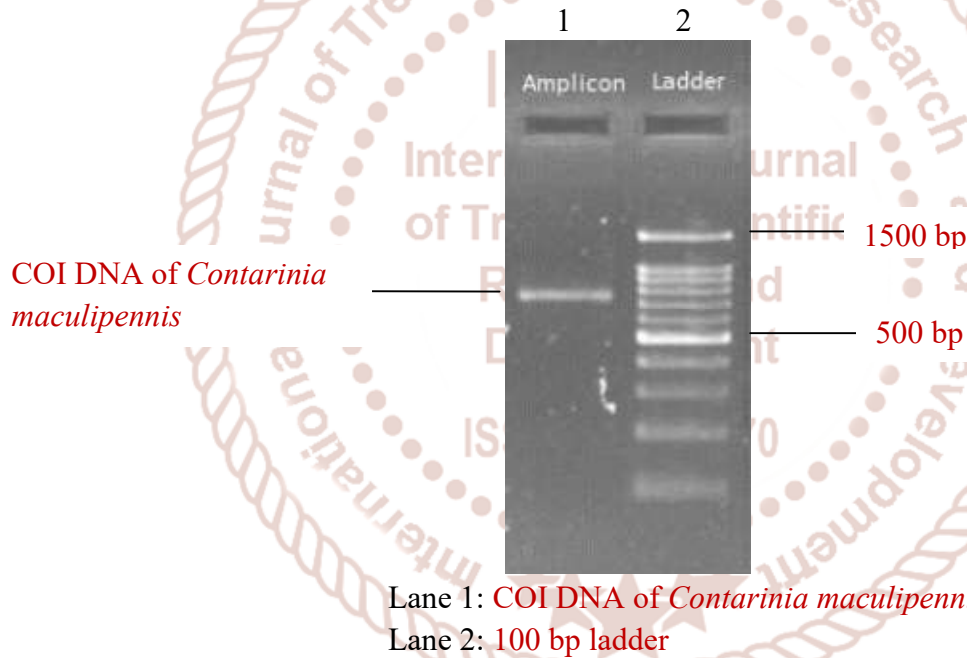
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**Fig. 1. Genomic DNA isolated from *Contarinia* spp.**



**Fig.2. COI - PCR product amplified from DNA of *C. maculipennis***

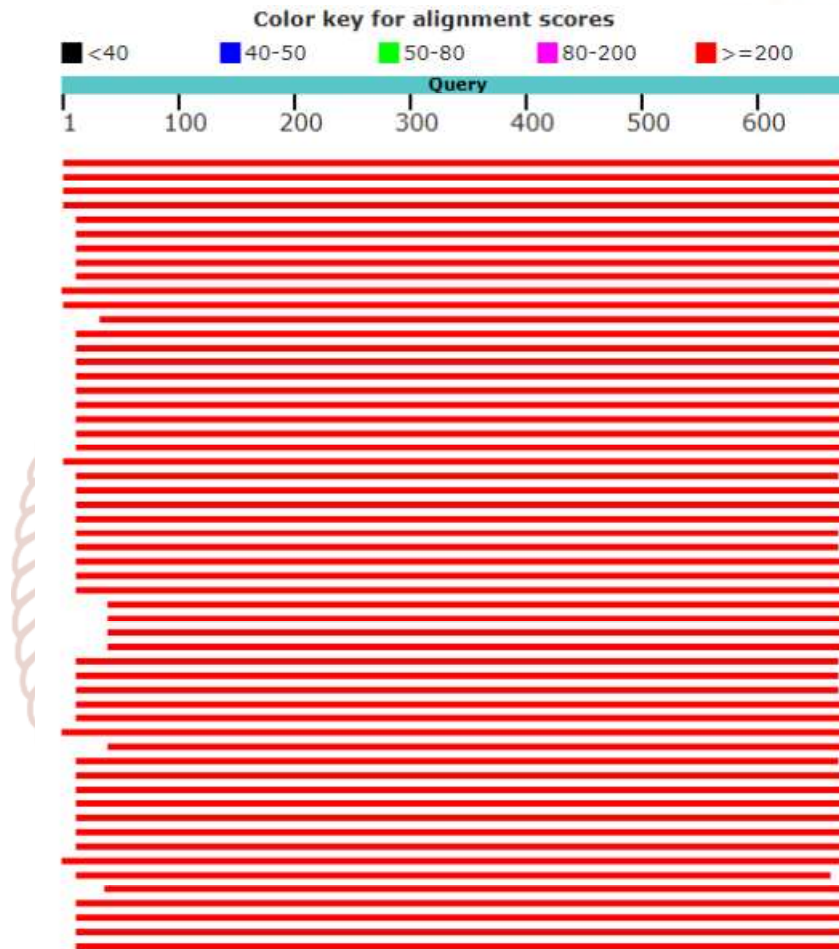
> Midge Consensus

ACCAAAAATCAAATAAATGTTGATAAAGAATAGGATCTCCTCCTCCTATTGGATCAAAGAATG  
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TTAATTTTTATATTAATTATTGTTGAAATAAAATTAATAGCTCCTAAAATTGATGAAATTCCTGCA  
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GAGGATAAACAGTTCATCCTGTTCCCTGTTCCAGTTTCTACTATTCTTCTAATTTAAAAGTAATGAAA  
TTGATGGGGGTAGTAATCAAATCTTATATTATTTATTCGTGGAAAAGCTATATCTGGGGCTCCTA

ATATAATGGGAATTAATCAATTTCCAAATCCTCCAATTAATAAGGTATAACTATAAAAAAATT  
ATAATAAAAGCATGAGCAGTAACAATTACATTATAAATTTGATCATTTCCAATTAATTAGAAATT  
GATCTTAGTTCTAAACGAATTAATAATTCTTAATGATGTACCTAATATTCCTGCTCAAATTCCAAAT  
ATAAAATATAAAGTTCCAATATC

**Fig. 3. Sequence of orchid blossom midge, *C. maculipennis***

**Distribution of 100 Blast Hits on the Query Sequence**



**Fig.4. Alignment view using combination of NCBI GenBank**