



FIRST RECORD OF CITRUS LEAF ROLLER *ARCHIPS MACHLOPIS* (MEYRICK) ON SWEET ORANGE IN BANGLADESH

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ABSTRACT

The occurrence of *Archips machlopis* (Meyrick) (Tortricidae: Lepidoptera) for the first time on sweet orange (*Citrus sinensis*) at the Regional Agricultural Research Station, Burirhat, Rangpur, Bangladesh during 2021-22 is reported herein. Larva bears a brown prothoracic shield and a thin white line which delineates the reddish-brown head capsule. Moths are dark grey, 8-10 cm long, while pupa are dark brown to black with elongate cremaster. DNA barcoding with mtCOI gene confirmed it as *A. machlopis*. Larvae are observed attacking young leaves, and being voracious cause severe foliage damage. It folds leaf around them and feeds inside the folded leaf. Approximately 21.5% young leaves were infested. More investigations are suggested to assess the pest status, hosts, biology and other aspects.

Key words: *Archips machlopis*, new record, Bangladesh, citrus, leaf roller, sweet orange, pest, DNA barcoding, mtCOI, morphological, molecular characterization

Archips machlopis (Meyrick) is a polyphagous lepidopteran pest belonging to the family Tortricidae (Razowski, 2009). It is reported as a serious pest to several important crops and generally roll or tie the leaves of host plant (Soumia et al., 2019). Meyrick (1912) had first recorded this insect pest from Assam. Recently, it has been extensively disseminated in southern Asia containing Thailand, Vietnam, Malaysia, Indonesia, Sumatra, China, Pakistan, India, Nepal and Myanmar. It was detected to feed on different hosts such as *Acacia nilotica*, *Araucaria cunninghamii*, *Ipomoea batatas*, *Cassia fistula*, *Medicago sativa*, *Litsea monopetala*, *Stylosanthes guianensis*, *Cedrela toona*, *Lansium domesticum*, *Toona ciliate*, *Morus indica*, *Coffea liberica*, *Citrus sinensis*, *Citrus medica*, *Santalum album*, *Litchi chinensis*, *Nephelium lappaceum*, *Theobroma cacao*, *Camellia sinensis*, *Lantana camara*, *Toona ciliate*, *Daucus* sp., *Psophocarpus tetragonolobus*, *Vigna unguiculata*, *Persea americana*, *Allium sativum*, *Gossypium herbaceum*, *Donax* sp., *Jasminum sambac*, *Averrhoa carambola*, *Cedrela* sp., *Aglaia* sp., *Araucaria cunninghamii*, *Durio zibethinus* and *Gloriosa superba* (Bhumannavar et al., 1991; Fletcher 1932; Robinson et al., 2010; Soumia et al., 2019; Tuck, 1990; Kuznetsov 2000; Pathania et al., 2020).

Citrus including sweet orange, mandarin, lemon, lime and pummelo are widely cultivated in Bangladesh. Citrus cultivation in Bangladesh is plagued with various problems including high incidence of insect pests (Bhuiyan et al., 2016). Several researchers reported that different insect pests such as citrus leaf miner (*Phyllocnistis citrella*), Asian citrus psyllid (*Diaphorina citri*), lemon butterfly (*Papilio demoleus*), lemon butterfly (*Papilio polytes*), spined citrus bug (*Biprorulus bibax*), citrus red mite (*Panonychus citri*), thrips (*Scirtothrips citri*), citrus red scale (*Aonidiella aurantii*), citrus leaf roller (*Platynota stultana*), citrus leaf folder (*Psorosticha zizyphi*) red fire ant (*Solenopsis invicta*), citrus mealy bug (*Pseudococcus filamentosus*), citrus stem borer (*Chelidonium cinctum*), citrus aphid (*Toxoptera aurantii*), bark and stem borer (*Indarbela quadrinotata*), citrus red scale (*Aonidiella aurantii*), citrus yellow scale (*Aonidiella citrina*), citrus blackfly (*Aleurocanthus woglumi*), citrus whitefly (*Dialeurodes citri*), termite (*Odontotermes obesus*) and oriental fruit fly (*Bactrocera dorsalis*) had been infested different citrus crops in Bangladesh (Bhuiyan et al., 2016; Uddin et al., 2014). Recently, citrus leaf roller (*Archips rosanus*) (Lep.: Tortricidae) has become a serious insect pest of citrus crops in Iran (Toorani and Abbasipour,

2019). However, there is no previous report of its presence from Bangladesh. This study, therefore, made a survey to determine the presence of *A. rosanus* from Bangladesh and first occurrence of citrus leaf roller *A. machlopi*s infesting sweet orange (*Citrus sinensis*) leaf is documented at the Regional Agricultural Research Station, Burirhat, Rangpur, Bangladesh confirmed by morphological and molecular characterization.

MATERIALS AND METHODS

Regular survey and monitoring was done on sweet orange and the infestation was noticed on leaf at the Regional Agricultural Research Station, Burirhat, Rangpur, Bangladesh during rabi, 2021-22. For morphological and molecular diagnostics, a large number of larvae were collected from infested leaf and reared on petri dishes at the laboratory feeding the leaves of host plant. The total DNA was extracted from adult using GeneJET Genomic DNA Purification Kit (Catalog number: K0721, <https://www.thermofisher.com/>) following the manufacturer's protocol and 50 µg/ µl stock DNA solution was prepared and stored. Study was targeted to amplify approximately 411 bp long fragment at the 3' end of the barcoding region by using the forward primer BF2 (5'-GCHCCHGAYATRGCHTTTCC-3') and the reverse primer BR2 (5'-TCDGGRTGNCCRAARAAYCA-3') (Elbrecht and Leese, 2017). The PCR reaction conditions were as follows: 94°C for 180 s, 35 cycles of 94°C for 30 s, 50°C for 30 s, and 65°C for 150 s, and then a final incubation at 65°C for 5 min. The amplified PCR DNA was visualized on 1.5% agarose gel. The purified DNA was sent for sequence determination (BTSeq™ Sequencing Service, <https://www.celemics.com/>, Korea). After sequencing of CO1 gene amplicons, homology of the obtained sequence was evaluated with Basic Local Alignment Search Tool (BLAST) tool of National Center for Biotechnology Information (NCBI) for comparison and identification of the sample. BioEdit software was used to check the quality of sequenced data. Sequence was submitted to GenBank. MEGA (Molecular Evolutionary Genetic Analysis) tools (version 11) software was used for constructing phylogenetic tree (Tamura et al. 2021). Maximum likelihood (ML) and neighbor-joining (NJ) methods were used for reconstruction of phylogeny using bootstrap with 1000 times of repetition. Some relevant sequences were downloaded from NCBI GenBank for phylogenetic tree analysis.

RESULTS AND DISCUSSION

Citrus leaf roller *Archips machlopi*s extensively infests sweet orange young leaves causing approximately 21.5% damage. The larva wraps the young leaves and continues to feed insight into the wrapped leaves (Fig. 1A, B). This insect's attack causes the infested leaves to drop off hampering the fruit production severely. Larva were collected from infested sweet orange leaves and reared artificially to adults in the laboratory. The developed adults were identified *A. machlopi*s according to Soumia et al. (2019) description. The larva is greenish grey to whitish grey and 10-12 cm long (Fig. 1C). Head reddish brown which is demarcated from the prothorax by a narrow white line. Pupa are dark brown to black and 12-14 cm long (Fig. 1D, E). It has cremaster elongate and becomes adult inside the wrapped leaf. The adult is 8-10 cm long (Fig. 1F). Head and thorax are dark purplish-fuscous. Wings are held like a flattened roof similar to an arrowhead. The identity of *A. machlopi*s was also confirmed by DNA sequencing. DNA was extracted from adult and oligonucleotide primer pair BF2 and BR2 were used for partial (~411bp) mitochondrial cytochrome c oxidase subunit I (mtCOI) gene amplification (Fig. 2)

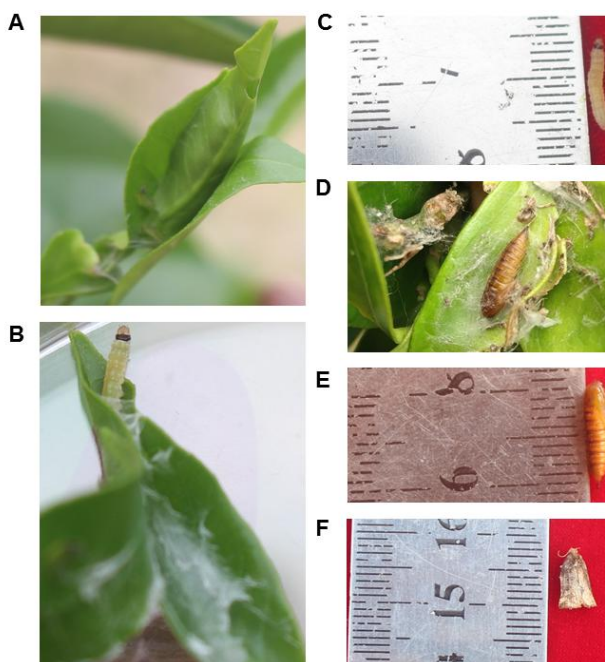


Fig. 1. Infested leaf of sweet orange and life stages of *A. machlopi*s. A. Folded leaf of sweet orange. B. *A. machlopi*s infesting sweet orange leaf. C. *A. machlopi*s larvae. D. *A. machlopi*s pupa at infested sweet orange leaf. E. *A. machlopi*s pupa. F. *A. machlopi*s adult

(Elbrecht and Leese, 2017). The obtained partial COI gene sequence was submitted to the NCBI-GenBank with the accession number was OP574698 and also to BOLD SYSTEMS, DNA barcode with Process ID “ARMBD001-23” was successfully generated (Fig. 3). Phylogenetic analyses were conducted using the MEGA 11 program. The phylogenetic tree was generated using the most identical sequences of *A. machlopi*s available in the GenBank database (Fig. 4). Phylogenetic analysis comparing with publicly available COI sequences revealed that our isolate was identified as *A. machlopi*s.

Thus, it is confirmed the presence of *A. machlopi*s as the new insect pest of sweet orange from Rangpur, Bangladesh. Since, sweet orange is an important fruit in Bangladesh, a comprehensive study covering biology, host range, identification of parasitoids, insecticide

efficacy, etc. should be undertaken to develop sustainable management options.

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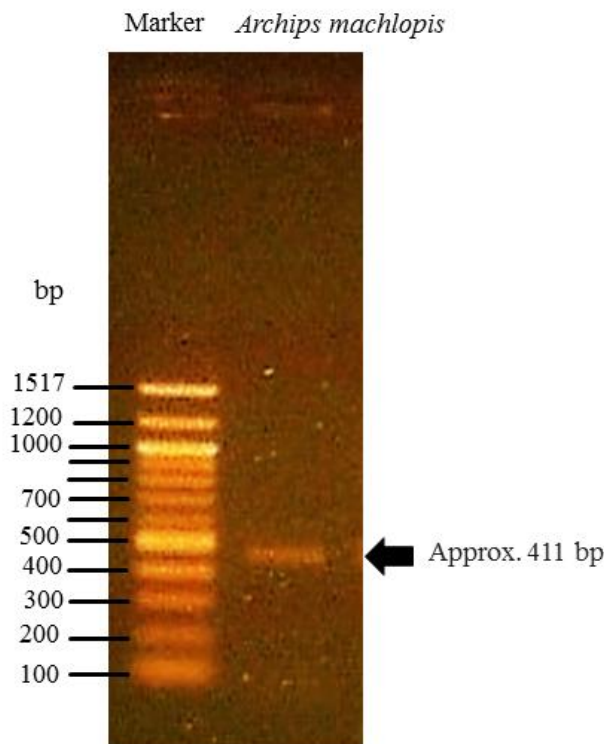


Fig. 2. Agarose gel electrophoresis (1.5%) of amplified COI (~411 bp) PCR products of *Archips machlopi*s sample

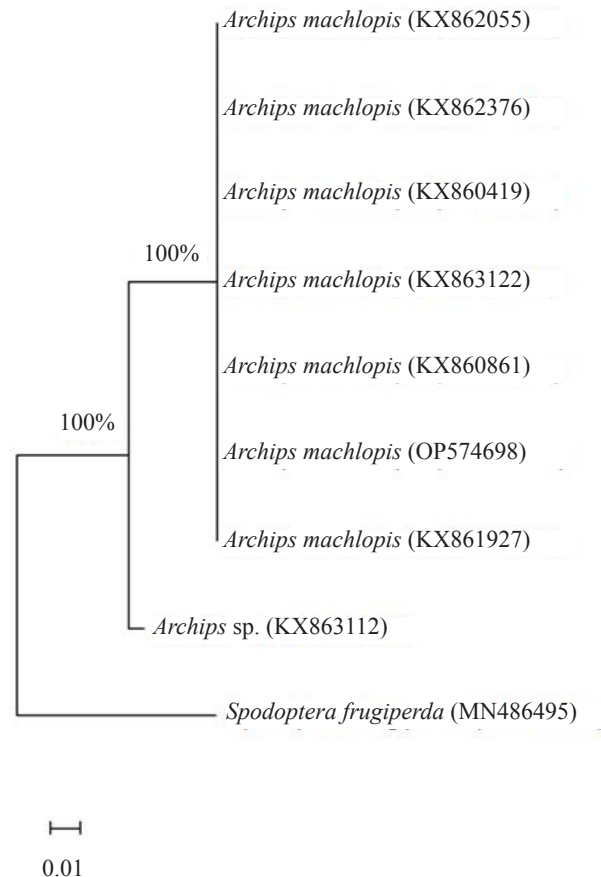


Fig. 4. The phylogenetic relationship of the *Archips machlopi*s according to the Neighbor-Joining method using gene sequences of mitochondrial cytochrome oxidase I (COI) regions of the rDNA. Numbers in parentheses indicated the accession numbers in GenBank. Our deposited the NCBI-GenBank accession number is OP574698. The bar represents sequence divergence

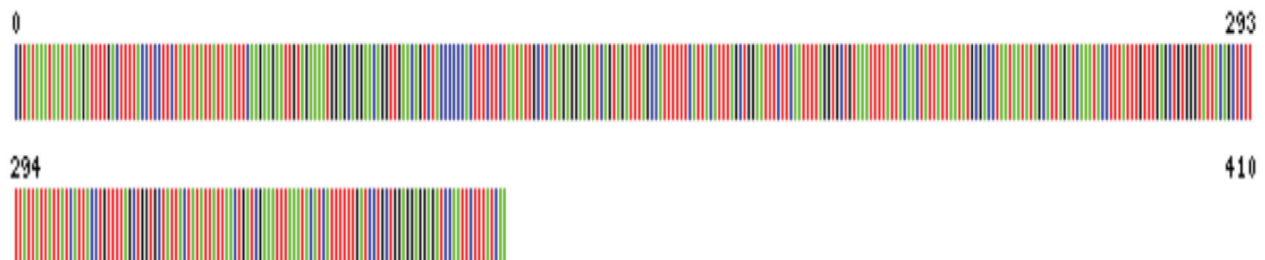


Fig. 3. DNA Barcode of *Archips machlopi*s with Process ID: ARMBD001-23

AUTHOR CONTRIBUTION STATEMENT

MHR planned and designed this study. MTHH performed molecular diagnosis. MHR analyzed data. MAS, NKD and AKS revised the draft. MHR and MTHH drafted and revised original manuscript.

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CONFLICT OF INTEREST

The authors declare that the research work was carried out without any financial or commercial dealings that could be raised as a probable conflict of interest.

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