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Morphological and molecular characterization of rat tailed maggot Syrphid fly *Eristalis* (*Eoseristalis*) *cereal* Fabricius, 1805 (Syrphidae: Diptera)

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Abstract
Rat tailed maggot syrphid fly *Eristalis* (*Eoseristalis*) *cerealis* (Syrphidae: Diptera) is copious on major crops in the area of Pantnagar farms (Uttrarkhand). Apart from being dominant in nature, Rat tailed maggot is excellent bio indicator. Current study of morphological identification of *Eristalis* (*Eoseristalis*) *cerealis* was conducted in X-ray laboratory, Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). Major character of the species is triangular spot on 3rd abdominal tergite in male which extend up to end of abdomen in female.

Keywords: Syrphidae, Morpholgy, Mitochondrial genome

Introduction
Syrphid flies belong to the family Syrphidae, which is one of the largest families of the order Diptera and popularly called as hover flies or flower flies or sun flies or drone flies. The syrphid fly family consists of small to medium flies about 6 to 18 mm long most of which are brightly coloured having black and yellow striped bodies resembling wasps and bees. The members of family Syrphidae have a characteristics feature having vena spuria, it is a vein like thickening in the membrane of wing (Khan *et al*., 2016) [9]. They often found hovering over the flowers on bright sunny days.

The pollination is best known ecosystem services provided by the insects. Among insects syrphid flies play vital role in pollination besides bees and butterflies. *Eristalis* (*Eoseristalis*) *cerealis* (Syrphidae: Diptera) belongs to the sub family Eristalinae and tribe Eristalini is found to pollinate many of the agricultural, horticultural, medicinal plants. Widespread distribution of members of family syrphidae (Joshi *et al*., 2010) [7]. Availability of excellent taxonomic keys for species identification and differences in environmental requirements of larvae are features that promote Syrphidae as potentially good bioindicators. The Syrphidae is an important group of resources and natural enemy insects, which not only could be used for controlling aphids and pollination, but also as important experimental materials for bionics (Wenbin *et al*., 2009) [16].

When discussing dipteran pollination, the function of it is constantly emphasised how effective and charismatic hover flies (Insecta: Diptera: Syrphidae) are in pollination. It is a collection of pollinating animals (Chambers *et al*., 1983; Bhagat, 2012) [3, 1]. It comprises up 4.90% of the known Dipteran fauna from India and is one of the most speciose groups composed of morphologically and physiologically diverse organisms (Sengupta *et al*., 2016) [12]. They are both cosmopolitan and omnipresent (Evenhuis & Pape, 2019) [5]. The present analysis is therefore intended to consolidate current knowledge while also identifying areas that most require additional exploration.

Materials and Methods
Specimens of *Eristalis* (*Eoseristalis*) *cerealis* were collected from various farms of Pantnagar, U.S. Nagar Uttarakhand. The specimens were collected using insect sweep net of 15 diameters. Total of 17 species are collected and subjected for morphological identification. The specimens collected were pinned and preserved in insect boxes. Identification of the adults followed the keys of Miranda *et al*., 2013, Vockeroth 1987, Thompson *et al*., 1982, Teskey *et al*., 1981 and Brunetti, 1907 [11, 15, 14, 13] keeping in mind the recent nomenclatural changes (Evenhuis & Pape, 2019) [5]. The images of the key identification features were captured from Nikon SMZ742 microscope (with camera).

The *Eristalis cerealis* specimen caught in sweep net was transferred to effendorf tubes of 2ml capacity containing 70% ethanol. The collected samples are preserved at -20 ºC till further analysis. Legs were used to isolate DNA. Each legs were thoroughly cleaned in autoclaved double distil water. The washed legs were then homogenized with a hand-held homogenizer (Sigma Aldrich) and DNA was extracted using the DNASure Tissue mini kit (Qiagen#NP-61305) according to the manufacturer's instructions. The extracted DNA was treated with RNase (0.1g/L) for 45 min at 37 °C. The DNA was quantified using NanoDrop ND-1000® spectrophotometer and used for further PCR analysis.

The universal primers LCO 1190 (5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO 2198 (5'TAACTTCAGGGTGACCAAAAAATCA -3') were used to amplify a partial mtCOI gene fragment of nearly 700 bp (Simon *et al*., 1994). A 25 µl reaction mixture comprising 12.5 µl of Ready to use PCR master mix (Promega M750A), 7.5 µl of nuclease free water, 1 µl each of forward and reverse primer, and 3 µl of DNA template was used for PCR amplification. A 3 µl amplified PCR product was run for 45 min on a 1.2 per cent agarose gel in 1X TAE at 100V (Jordan Scientific). The amplified PCR products were outsourced to Green Genome India Pvt. Limited, New Delhi for purification and sequencing.

A phylogeny analysis based on the mtCOI was conducted for *Eristalis cerealis*. 10 sequences of various species from syrphid family was retreived from NCBI. All 11 syrphid sequences, including the sequence of *E. cerealis* sequenced in this study were aligned using ClustalW, which was implemented in BioEdit v7.2.5, before being analysed with Mega X for phylogeny (Kimura 1980; Kumar *et al*., 2018) [8, 10]. The phylogenetic tree was constructed using maximum likelihood approach and the Kimura 2-parameter model. A bootstrap replication of 1000 was run to test the phylogenies (Felsenstein, 1985) [6]. The syrphid sequences were assigned to species based on pair-wise sequence divergence greater than 3.5 per cent upon clade formation **(**Dinsdale *et al*., 2010) [4].

Results

The current studies on *Eristalis (Eoseristalis) cerealis* was collected from Pantnagar and morphological identification was done using following keys.

Key to the Families syrphidae

1. Presence of spurious vein……Syrphidae
Absence of spurious vein…….others

Key to the Sub families syrphidae

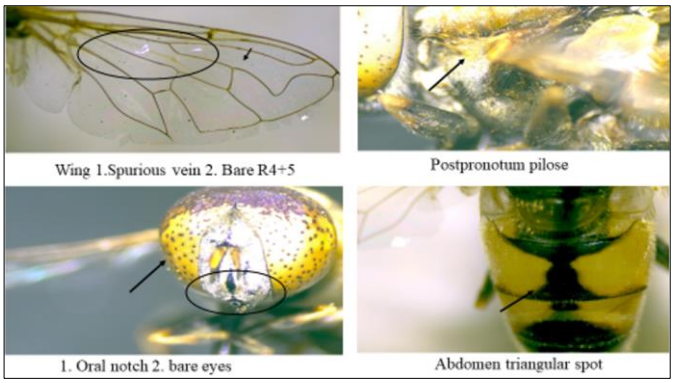
1. Postpronotum pilose……..2
Postpronotum bare……..…Syrphinae
2. R_{4+5} with spur, Oral margin not notched…...Microdontinae
R_{4+5} without spur, Oral margin notched…..……. Eristalinae

Key to genera *Eristalis* (*Eosristalis*)

1. Postalar tuft absent, non-metallic flies, eye bare in appearance…… ……. ***Eristalis* (*Eosristalis*)**
Postalar tuft absent, metallic green to purple flies……. others

Key to species *cerealis*

1. Abdominal tergite 3 within male with a triangular or oblique spot on anterior margin reaching laterally in female spot smaller, narrower in the same position……..... cerealis Fabricius, 1805



Good quality DNA was isolated from E. cerealis species which are members of syrphidae family using DNA Sure Tissue mini kit (Qiagen#NP-61305) according to the manufacturer's instructions. Using UV spectrophotometer (NanoDrop ND-1000®) the quality and quantity of the isolated DNA was calculated. TheA_{260}/A_{280} ratio was 1.85 indicating good quality and the quantity was measured to be 983 ngµl^{-1}. The sample was diluted to 40 ngµl^{-1} and PCR was carried out. PCR was carried out at annealing temperature 48 ºC. Following the reaction, four microliters of PCR products were run in a 1.8% agarose gel for confirmation of amplification. A single band of expected size (710 bp) was found present (Fig. 1).

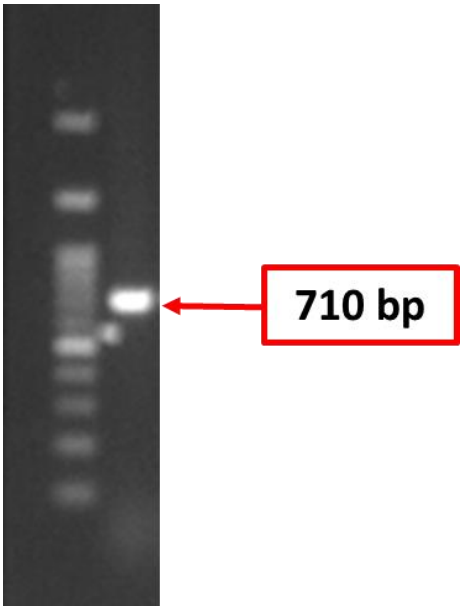


Fig 1: PCR amplicon 710 bp in size of mtCOI primers

Phylogenetic analysis was carried out using 11 mtCOI sequences (including *E. cerealis* sequenced in this study. List of mtCOI sequences of carious species amd their accession number retrieved from NBCI is given in table 1. The tree sepeareated into 2 major clusters A and B. Cluster A further split into 2 sub clusters A1 and A2. *Ersitalis abusive* was the closest relative of E. cerealis, were present in sub cluster A2. The other species closer to *E. cerealis* are *Eristalinus surcoufi Eristalinus fuscicornis Eristalinus arvorum* and *Eristalis cryptarum* which belong to the same clade. The phylogenetic tree is represented in Fig.1.

Table 1: List of mtCOI sequences of carious species and their accession number retrieved from NCBI

S. No.	Species	Accession number
1	Eristalis cerealis (Present study)	OP006110
2	Eristalinus surcoufi	KR831015.1
3	Eristalinus fuscicornis	KR830993.1
4	Eristalinus arvorum	MK751023.1
5	Eristalis cryptarum	MT132162.1
6	Eristalis abusiva	MT132165.1
7	Episyrphus balteatus	MN973969.1
8	Syritta orientalis	HQ561044.1
9	Sphaerophoria scripta	KU752537.1
10	Melanostoma mellarium	KJ848079.1
11	Serratoparagus crenulatus	AY476863.1

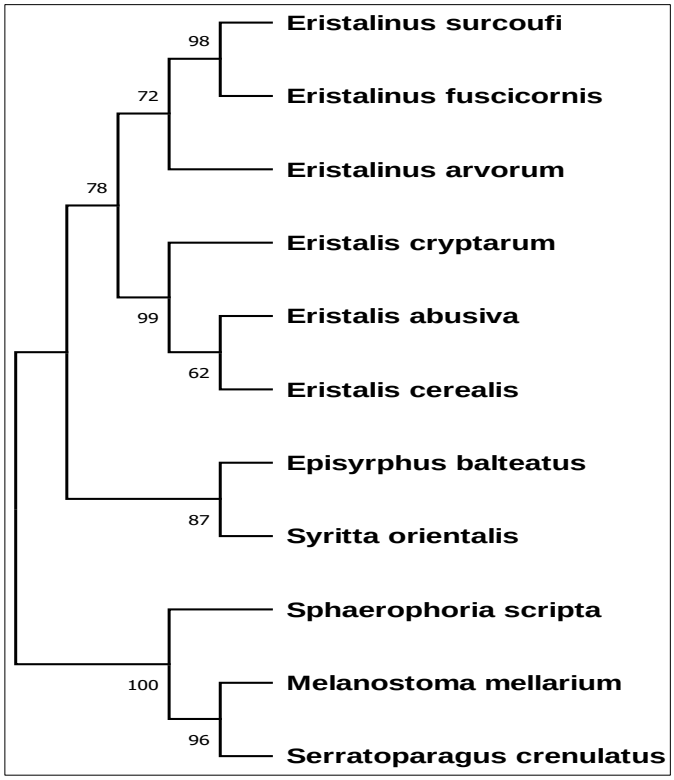


Fig 2: Phylogentic tree of various members of syrphid family based on mtCOI seqiuence

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