

Rediscovery of *Eristalinus (Merodonoides) multifarius* (Walker, 1852) (Diptera, Syrphidae) from Kerala, India and its molecular phylogeny

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ABSTRACT: The hoverfly species *Eristalinus (Merodonoides) multifarius* (Walker, 1852) is rediscovered from Kerala after 45 years. The total number of species of hoverflies in Kerala has been updated to 60, based on the previously omitted first record of the species. Male of the species have been redescribed along with illustrations of male genitalia. The species have been molecular characterised for the mtCO1 gene for the first time. Phylogenetic analysis of the members of the tribe *Eristalini* showed close affinity of *E. (Merodonoides) multifarius* with other members of *Eristalinus* Rondani followed by *Mallota* Meigen. © 2025 Association for Advancement of Entomology

KEY WORDS: Hoverfly species, redescription, male genitalia, molecular characterisation, mtCO1, phylogenetic analysis, *Eristalini*

INTRODUCTION

The hoverflies (Diptera, Syrphidae) are bristle-less, small to medium-sized vividly coloured flies. The primary characteristic is the presence of spurious or false vein between the third and fourth veins of the wings. Many of them mimic bees and wasps. Syrphids play a crucial role in pollination and serve as an important pollinator of many plants (Larson *et al.*, 2001). In India, about 366 species of syrphids

in 77 genera under 13 tribes and four subfamilies have been recorded (Sengupta *et al.*, 2024). In Kerala, 59 species of syrphids were reported (Sankar *et al.*, 2024). The tribe *Eristalini* is represented by 12 species of four genera in Kerala (Sankar *et al.*, 2024). Many species under the tribe *Eristalini* are gaining importance as an effective pollination agent especially in polyhouses due to the ease of mass multiplication, owing to their saprophytic character (Hodgkiss, 2019; Dunn *et al.*,

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2020). There is only very less information on the tribe Eristalini from Kerala. This study reports the rediscovery of an Eristalini from Kerala along with its redescription. The phylogenetic analysis to find the genetic relationship of the species with other members of the tribe is also presented.

MATERIALS AND METHODS

Surveys were conducted in various localities of Thenmala, Kollam district (9°00'25.2"N; 76°59'04.9"E) and Rajakkad, Idukki district (9°58'01.0"N; 77°05'38.2"E) in Kerala; which included open grass land, semievergreen forest patches, and roadside vegetation. The collections were carried out using aerial nets and preserved the specimens in 99 per cent ethanol. Collected flies were later relaxed overnight using a mixture of ethyl alcohol, ethyl acetate and distilled water (1:1:1) and were pinned with entomological pins (No. 1) and dry preserved. The taxonomic identification of specimens and redescription followed Brunetti (1923). The photographs of dorsal, lateral, and ventral habitus, facial profile and male genitalia were captured using Leica M80 stereozoom microscope mounted with Leica MC 170 HD camera. Multiple images of each part of the specimens were taken and combined to a stacked image using Combine ZM software. The stacked images were touched/ digitally optimized using Adobe Photoshop CS6. Terminology used in redescription follows Brunetti (1923).

Preparation of male genitalia: The terminal abdominal segments of the specimen were dissected out and soaked overnight in 10 per cent KOH solution. It was washed with distilled water and then soaked in glacial acetic acid for one to two minutes, then cleaned with distilled water. The digested abdominal parts were transferred to a microscopic slide containing a drop of glycerine using forceps. By observing under stereo zoom binocular microscope, the male genitalia were extracted from abdomen using a No. 0 entomological pin and the attaching tissues were cleaned by using a No. 0 entomological pin with hooked tip.

DNA isolation: DNA was isolated using the 'DNA

Sure Tissue Mini Kit' (Genetix). Total DNA from legs of one side was isolated. The dissected legs were crushed using the pre-autoclaved micro pestle. Standard protocols given in 'DNA Sure Tissue Mini Kit' were immediately followed.

PCR amplification: The mitochondrial DNA cytochrome oxidase subunit-I (CO-I) region of total DNA was amplified using the SimpliAmp Thermal cycler of Thermo Fisher Scientific. The universal primer designed by Foler *et al.*, 1994, was used. The name and 5'-3' sequence of the forward and reverse primers are given below;

LCO1490

GGTCAACAAATCATAAAGATATTGG

HCO2198

TAAACTTCAGGGTGACCAAAAAATCA

The primers were diluted to 10µM using DNAase and RNAase-free molecular water. The PCR master mix used was the EmeraldAmp GT PCR Master mix (TAKARA). The master mix included an optimised buffer, PCR enzyme, dNTP mixture, gel loading dye (emerald green), and a density reagent in a 2x premix format. Gradient PCR was carried out for standardising the annealing temperature. The best annealing temperature for this reaction was recorded as 51°C.

For PCR amplification of mitochondrial cytochrome oxidase subunit I locus, a reaction mixture of 40µL was prepared. The composition of the reaction mixture was as follows: 20µL PCR master mix, 2µL template DNA, 1µL forward, 1µL reverse primer, and 16µL molecular water. The PCR product obtained was stored at 4°C.

DNA sequencing: Sanger sequencing of PCR product was done by sending PCR product to Eurofin Genomics India Pvt. Ltd., Bengaluru. For each sample, bidirectional sequencing was done. Data were received in FASTA, ab1, and pdf formats.

Bioinformatic analysis: The forward and reverse sequence were trimmed and contig was generated using bioedit software. The sequence was submitted to NCBI through Bankit portal and accession number was obtained. Molecular studies were done

to understand the phylogenetic affinity of the species, for which genetic sequences for mtCO1 gene of representative genera under the tribe Eristalini was retrieved from NCBI. They were aligned and the phylogenetic tree was constructed in MEGA 11, by Neighbour joining method with Jukes- Cantor model and a bootstrap value of 1000.

RESULTS AND DISCUSSION

Eristalinus (Merodonoides) multifarius (Walker, 1852), is rediscovered after 45 years from Kerala. The male specimens of the species have been redescribed along with illustrations of male genitalia.

Systematic account

Eristalinus (Merodonoides) multifarius (Walker, 1852)

Eristalis multifarius Walker, 1852

Merodonoides circularis Curran, 1931

M. minutus Hull, 1944

M. czernyi Hull, 1944

Eristalis (Merodonoides) kandyensis Keiser, 1958

Eristalis yamunanagarensis Awtar Singh, Sodhi & Gupta, 1986

Pseudomeromacrus setipenitus Li, 1994

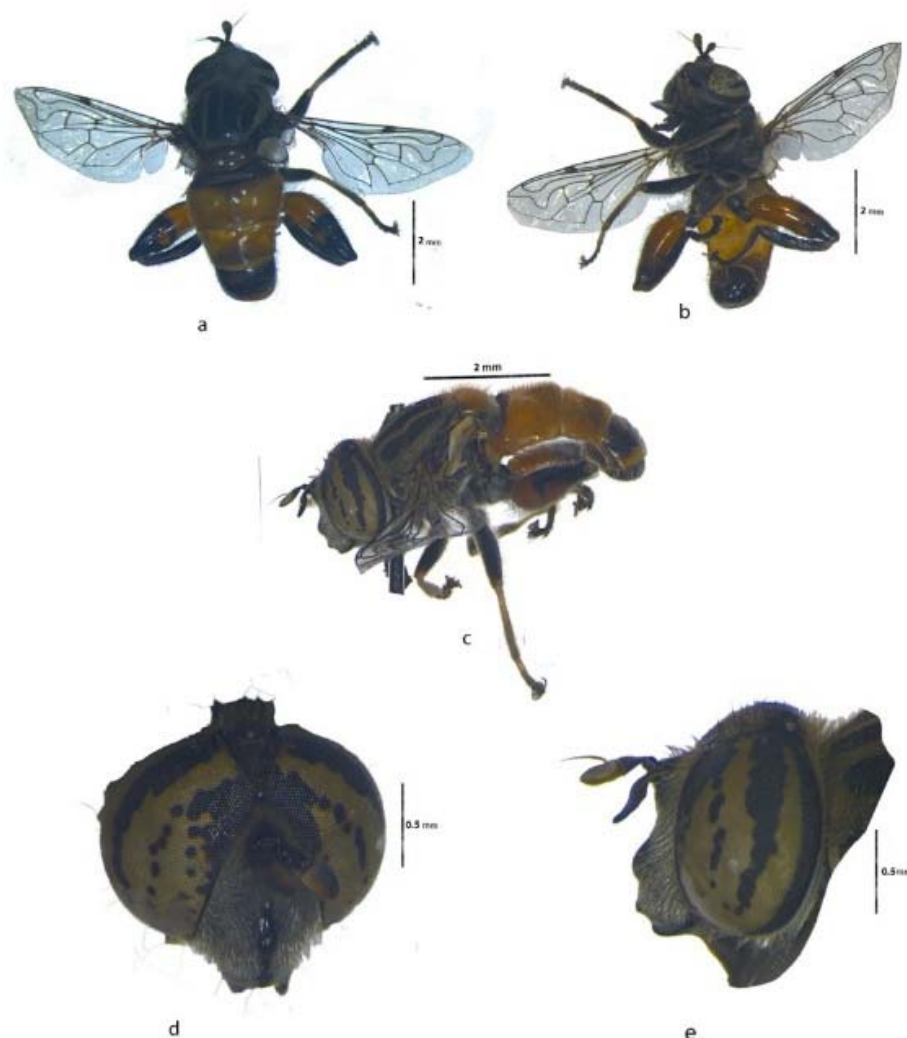


Fig. 1 *Eristalinus (Merodonoides) multifarius* (Walker); a-c. Habitus a. dorsal, b. ventral, c. lateral, d & e. head d. frontal, e. lateral

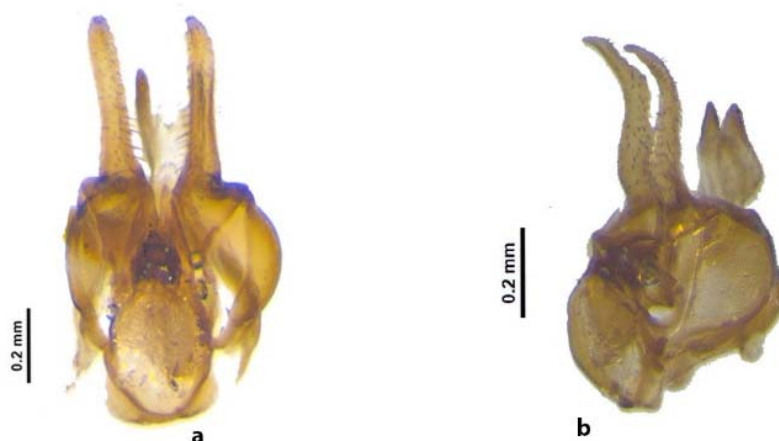


Fig. 2 Male genitalia of *Eristalinus (Merodonoides) multifarius* (Walker): (a) Ventral view: surstyli is more elongate and narrower; rows of fine bristles are present at the inner edge; (b) Lateral view: basoventral lobe is elongated and sickle shaped; cercus broad and narrowed towards the tip

Description:

Size: 9 mm in length

Male:

Head: Eyes light reddish-brown, bare, featuring two distinct black stripes on the disc, alongside dark inner and hind borders; frons and face are moderately shining black, primarily covered with greyish-white dust, leaving the vertex broadly black and a transverse space across the middle of the frons; face gradually widens and is somewhat prominent, with a short, narrow median shining black stripe that points at the upper end; lower part of the head is bare, with only whitish dust and shining black stripes on each side; occiput is dusted with ash-grey and has whitish pubescence on the lower part (Fig. 1d, e). **Thorax:** entirely ash-grey, with four shining black stripes in the usual pattern, positioned well away from the anterior and hind margins, and the outer stripes are pointed behind; hind corners of the dorsum form a narrow shining black ridge with stiff black hairs, and the pleurae are ash-grey; scutellum is entirely orange, with yellowish pubescence covering the whole thoracic dorsum and scutellum, while the pleurae have whitish pubescence (Fig. 1a, c). **Abdomen:** 1st segment is pale grey with whitish pubescence; 2nd segment is

mainly orange, with a narrowly black anterior margin (except at the corners) and a transverse blackish band near the hind margin (not reaching the sides). 3rd segment is predominantly orange, featuring a large semicircular blackish spot on the hinder half that is clear of the hind margin and corners, not extending to the anterior margin. 4th segment is entirely black, except for narrowly orange anterior corners, while 5th segment is wholly blackish. 2nd, 3rd, and 4th segments have pale yellow extreme hind margins and a fringe of pale-yellow hairs; pubescence on the dorsum and sides is whitish, while the venter is mainly orange in the basal half and black in the apical half (Fig. 1a). **Legs:** coxae and anterior legs are black, with the tips of the femora and the basal half of the fore tibiae being yellowish; middle tibiae mostly yellowish but brownish at the apical part, and the anterior tarsi are blackish-brown, often orange at the base, particularly in the middle pair; hind femora are significantly thickened, brownish-orange with broadly black tips, featuring an incomplete black subapical ring on the outer side; hind tibiae and tarsi are black or blackish-brown, and the pubescence of the legs is whitish, with orange-yellow on the underside of the hind tarsi (Fig. 1a, b, c). **Wings:** very pale grey and vitreous, with the 2nd longitudinal vein distinctly curved downward in a loop near the

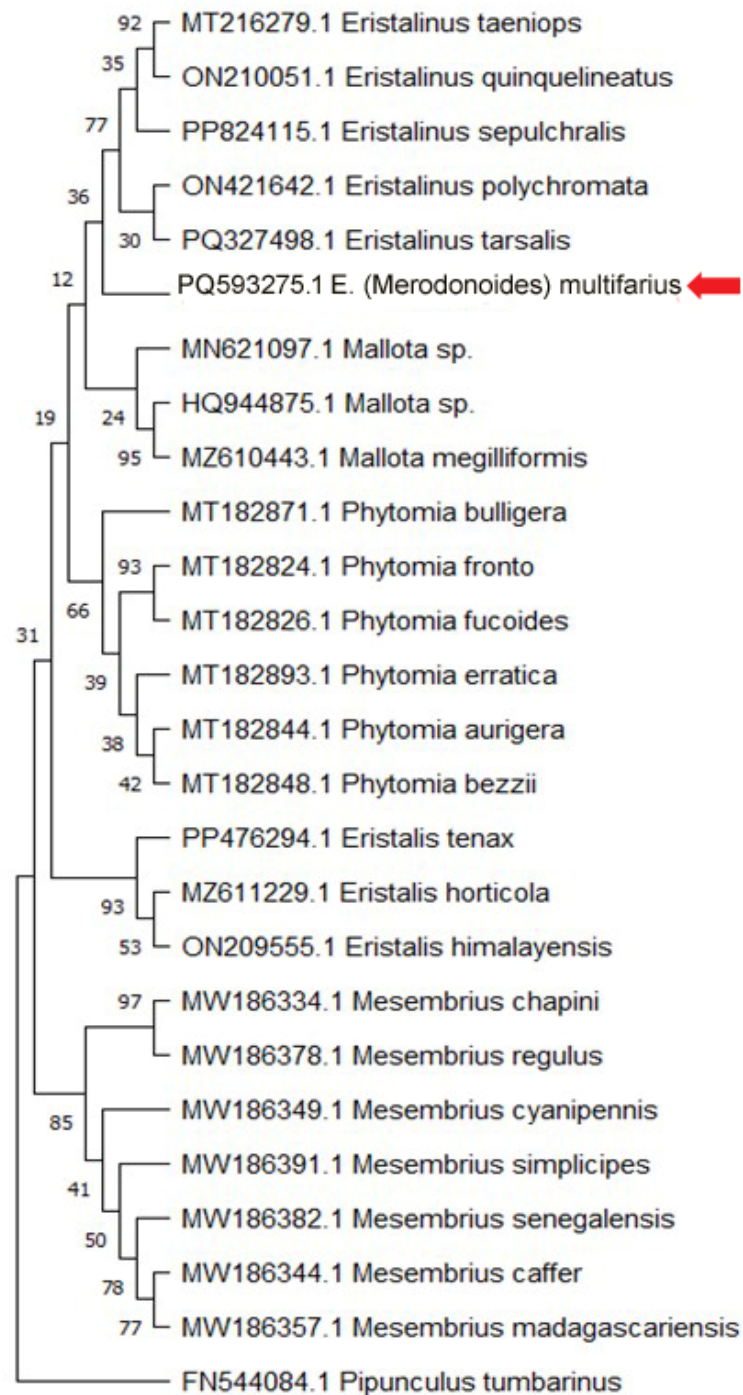


Fig. 3 The phylogenetic tree presenting the relationship of the *Eristalinus (Merodonoides) multifarius* (Walker) with other species of tribe Eristalini, constructed in MEGA 11, by neighbour joining method, with a booter trap value of 1000

tip; 3rd vein is deeply looped, and the anterior cross-vein is longer than usual, narrowing the basal part of the submarginal cell; squamae are grey with brown margins; halteres are orange.

Male genitalia: *Ventral view:* surstyli is more elongate and narrower; rows of fine bristles are present at the inner edge (Fig. 2a). *Lateral view:* basiventral lobe is elongated and appears to be sickle shaped; cercus broad and narrowed towards the tip (Fig. 2b).

Material examined: 3, labelled 'India: Kerala: 1 ♂, 1 ♀ Rajakkad (9°58'01.0"N; 77°05'38.2"E) 28.i.2024; 1 ♂, Thenmala (9°00'25.2"N; 76°59'04.9"E) 19.ii.2024 'Aerial net', 28.i.2024. Coll. Aiswarya Joy.

Distribution in India: Gujarat, Haryana, Karnataka, Meghalaya, Assam, Madhya Pradesh, Rajasthan, Tamil Nadu, Kerala (Ghorpade, 2014, 2019).

The DNA sequence generated for the species using mtCO1 marker furnished below is the first representation of molecular characterization of the species.

ATCATAAAGATATTGGAACATTATATTTTG
TATTTGGTACATGAGCCGGAATAGTAGG
AACTTCCCTTAGTATCCTAATTCGAGCTG
AATTAG GTCATCCTGGTGCATTAATTGGA
GATGATCAAATTTATAATGTTATTGT AACA
GCTCATGCTTTTGTAATAATTTTCTTTATA
GTAATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTTCCTTTAATATTAGGAG
CACCTGATATAGCTTTTCCTCGAATAAATA
ATATAAGTTTTTGATTACTTCCTCCTTCTT
TAACCTTTATTATTAGTAAGAAGTATAG
TAGAAAATGGGGCTGGGACTGGATGAAC
AGTTTATCCCCCTTTATCAAGAAATATTGC
TCACGGTGGGGCTTCAGTTGATTTAGCTA
TTTTTCCCT TCATTTATCCGGTATATCTT
CAATTTTAGGAGCAGTAAATTTTATTAC
AACAGTAATTAATATACGTTTCGACAGG
AATTACATATGATCGAATACCTTTATTTGT
TTGATCAGTTGCTATTACAGCATTA
TTACTTTTATTATCATTACCAGTACTAGCCG
GAGCTATTACAATATTATTAACAGATC

GAAATCTTAATACTTCTTTTTTTTGACCC
TGCAGGAGGAGGTGACCCTATTTTATA
TCAACATTTATTCTGA

GenBank accession number of *E. (Merodonoides) multifarius*: PQ593275.

E. (Merodonoides) multifarius was described as *Eristalis multifarius* Walker 1854 from the type female in the British Museum from India and another female from the lower ranges of North Khasi Hills, Assam. The types were deposited in British Museum from India. Brunetti, 1923 had then suggested to place it outside *Eristalis* Latreille owing to characters like the greatly thickened hind femora and the dipped second vein. Later the genus *Merodonoides* was erected by Curran, 1931 based on characters *viz.*, thickened hind femora and distinctive eye pattern with four conspicuous brown stripes.

The males of the species was described by Singh *et al.*, 1986, as a new species *Eristalis yamunanagarensis* Awtar Singh, Sodhi and Gupta, 1986, which was later synonymized with *E. (Merodonoides) multifarius* (Ghorpade, 2014). The most recent synonymization of the species was done by Thomson, 2003, wherein he synonymised *Pseudomeromaerus setiprinitus* Li, 1994; which was described as a new genus species from China. The colour plate of the male specimen of *E. (Merodonoides) multifarius* along with the male genitalia had been illustrated here.

E. (Merodonoides) multifarius is a wide spread species distributed across a wide range in the Indian subcontinent, Southeast Asia, and southern China, with records from diverse ecological regions like the Western Ghats, Eastern Droogs, and the Indochinese Peninsula (Ghorpade, 2019). *E. (Merodonoides) multifarius* had been reported from Gujarat, Haryana, Karnataka, Meghalaya, Madhya Pradesh, Rajasthan, Tamil Nadu, (Ghorpade, 2014), other than its type locality, Assam. Though the species occur wide spread in India, it was reported from Kerala only from a single female specimen collected by A.N.T. Joseph in 1979 from Parambikulam, Palghat district (Datta and Chakraborti (1986). Subsequent workers who

had prepared the checklist of hoverflies of India (Mitra *et al.*, 2015; Sengupta *et al.*, 2024) and Kerala (Sankar *et al.*, 2024) have omitted the record and have presented that the species as not recorded in Kerala. The recent checklist of hoverflies of Kerala by Sankar *et al.* (2024) had omitted the aforesaid study and had presented the total number of syrphids reported from Kerala as 59, whereas it should have been 60. The record of the species from Thenmala, Kollam district and Rajakad, Idukki district, furnished here, presents the rediscovery of the species from Kerala after 45 years, ever since it was first collected by A.N.T. Joseph in 1979 and also the first confirmed report of males of the species from, Kerala.

The mitochondrial cytochrome-c oxidase subunit I (*mtCO1*) gene has proven to be a powerful tool for species identification, primarily due to its strong phylogenetic signal, which enables clear discrimination among species (Hebert *et al.*, 2003; Hebert and Gregory, 2005). Previous studies on hoverflies have demonstrated the utility of this approach, successfully identifying and distinguishing various species (Pérez-Bañón *et al.*, 2003; Ståhls *et al.*, 2009; Gibson *et al.*, 2011). In this study also the *mtCO1* marker was found to be very reliable. The DNA sequence of *E. (Merodonoides) multifarius* for *mtCO1* marker furnished here is the first ever sequence for the species and the sequence submitted to the NCBI GenBank database will help in understanding the phylogenetic affinities of the subgenus *Merodonoides*. The subgenus closely resembles the *Eristalinus* Rondani 1845 on overall similarity, but shares a few characters similar to that of *Mallota* Meigen, 1822 like the swollen hind femur and colouration. Phylogenetic studies are necessary to find out the actual affinity of the subgenus with its related genera.

Merodonoides is a poorly studied taxa phylogenetically, with not even a single DNA sequence of the subgenus available in the GenBank previously. The molecular characterisation and the phylogenetic studies presented here will aid in addressing the gaps regarding phylogenetic affinities within the genus *Eristalinus*. The phylogenetic analysis of *E. (Merodonoides) multifarius* with

other members in tribe *Eristalini* is presented (Fig. 3). The phylogenetic tree revealed that the subgenus *Merodonoides* was closely related to *Eristalinus* Rondani. *E. (Merodonoides) multifarius* was found basal to all other members of *Eristalinus*. The genus *Mallota* Meigen, appeared as basal to *Eristalinus*. *Eristalis* Latreille was found be basal to the clade which consisted of *Eristalinus*, *Mallota* and *Phytomia* Guérin-Méneville. *Mesembrius* Rondani was found basal to all other genera analysed. The outgroup *Pipunculus tumbarinus* Kehlmaier got separated out in a different branch outside of main tree which confirmed the validity of the generated phylogenetic tree.

E. (Merodonoides) multifarius shares habitat almost similar to that of most other *Eristalinus* and possess both morphological and genetic similarity with other members of *Eristalinus*, but it is an uncommon species in Kerala. The phylogenetic study reveals that *Mallota* is the next closely related taxa to the subgenus *Merodonoides*, after the clade which encompasses other *Eristalini*. Further studies are needed on the distribution and biology of this uncommon member of *Eristalini*, for any kind of exploration of the species as a pollinating agent locally.

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