

Taxonomic characterization and molecular confirmation of *Eristalinus paria* (Bigot, 1880) collected from *Berberis lycium* Royle in the Northwestern Himalayas, India

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Abstract

Hoverflies (Diptera: Syrphidae) are important pollinators and biological control agents in diverse ecosystems. This study provides an integrated morphological and molecular description of *Eristalinus paria* collected from the flowers of *Berberis lycium* in the northwestern region of India in the state of Himachal Pradesh. Several locations were sampled in April-May 2024, and adult specimens were analysed based on diagnostic morphological characteristics. Wing venation examination revealed a typical syrphid morphology, with a vena spuria and sinuate R₄₊₅ vein. Mitochondrial COI gene sequencing generated a fragment of 621 bp (GenBank accession: PQ329353), which showed 99.84% similarity with the reference sequence (OK444104), confirming species identity. The close floral association of *E. paria* with *B. lycium*, a medicinally important and endangered shrub, highlights the crucial ecological role of syrphid pollinators in sustaining Himalayan biodiversity. This study contributes to the regional DNA barcoding database and supports integrative taxonomy as a vital tool for documenting and conserving important pollinators of mountain habitats.

Keywords: *Eristalinus paria*, *Berberis lycium*, pollinator, mtCOI gene, integrative taxonomy

Introduction

Hoverflies (Diptera: Syrphidae) are among the most ecologically significant groups of insects because they provide dual ecosystem services as effective pollinators and biological control agents (Dunn *et al.*, 2020) ^[1]. Adults are vital for pollinating a wide range of flowering plants, while their larvae exhibit diverse feeding habits, including preying on other insects and consuming agricultural pests. Hoverflies visit 72% of global food crops, and their pollination services are valued at around US\$300 billion annually (Doyle *et al.*, 2020) ^[2]. They also function as efficient bioindicators due to their restricted larval microhabitats (Moquet *et al.*, 2018) ^[3]. The genus *Eristalinus* belongs to the subfamily Eristalinae and tribe Eristalini. India hosts 368 species of syrphid flies across 77 genera, with Eristalinae being the most species-rich subfamily, attributed to diverse larval feeding strategies (Roy *et al.*, 2025) ^[4]. These flies are easily identified by their wing venation patterns and patterned compound eyes. Despite their ecological importance, taxonomic research on *Eristalinus* remains limited in the Himalayan region, which hosts diverse habitats supporting rich and underexplored insect fauna. Traditional morphology-based taxonomy of syrphids can be challenging due to intraspecific variation and phenotypic similarity among closely related species. Therefore, examining the genitalia is essential for accurate identification as it exhibits key morphological diversity helpful in distinguishing different species of dipterans (Sinclair *et al.*, 2013) ^[5]. The use of molecular tools like DNA barcoding, especially mitochondrial cytochrome c oxidase subunit I (COI gene), further improves species delimitation and confirmation (Elyasigorji *et al.*, 2023) ^[6]. The Northwestern Himalayan region, including Himachal Pradesh, is home to a rich diversity of medicinal plants. Among them, *Berberis lycium* is a particularly important medicinal shrub that attracts various insect visitors and is

known for its anti-diabetic, anti-microbial, and anti-cancer activity (Anjum *et al.*, 2023) ^[7]. The plant has been declared endangered in the Northwestern Himalayan region due to overexploitation and anthropogenic activities (Anjum *et al.*, 2025) ^[8]. Dipterans are key pollinators of medicinal plants, making it crucial to understand pollination ecology and plant-pollinator interactions in fragile mountain ecosystems that face ongoing threats from climate change and habitat fragmentation (Mitra *et al.*, 2005 ^[9]; Hoiss *et al.*, 2015 ^[10]). This study aims to provide an integrated taxonomic description of *Eristalinus paria* from flowers of *Berberis lycium* in the northwestern Himalayas. The integration of classical taxonomy and molecular techniques enhances understanding of species identity and distribution. The close association of *E. paria* with *B. lycium* features the ecological importance of syrphid pollinators in Himalayan ecosystems.

Materials and Methods





Fig 1: Adult *Eristalinus paria* visiting the flowers of *Berberis lycium*

Specimen Collection: Adult *E. paria* were collected from *B. lycium* flowers at multiple locations in Himachal Pradesh during April-May 2024 (Fig. 1). Insects were captured with the help of sweeping nets, placed in vials, and preserved, pinned and labelled for morphological examination, and stored at -80°C for molecular analysis.

Taxonomic Studies: Morphological examination was carried out under a stereomicroscope. Genitalia were extracted by detaching the abdomen, clearing in 10% KOH overnight, rinsing in distilled water, and cleaning with needles and brushes. Structures were photographed with a

Nikon SMZ1270 stereomicroscope using NIS-Elements imaging software. Identification followed standard taxonomic references (Brunetti, 1923^[11]; Vockeroth & Thompson, 1987^[12]; Miranda *et al.*, 2013^[13]).

Molecular Analysis: Genomic DNA was isolated from leg and thorax tissue using the Qiagen DNeasy kit. DNA quality and quantity were assessed using a NanoDrop spectrophotometer and stored at -20°C . The mitochondrial COI gene was amplified using Folmer primers (LCO1490/HCO2198) in a 25 μL PCR reaction on a VeritiPro Thermal Cycler (Folmer *et al.*, 1994^[14]). Products were visualised on a 1.5% agarose gel, purified using a Qiagen kit, and sequenced via Sanger sequencing (ABI 3500xL). Sequences were BLAST-verified, edited in BioEdit, submitted to GenBank, and nucleotide composition was analysed in MEGA12 software.

Results and Discussion

Eristalinus (Eristalodes) paria (Bigot, 1880)

1880. *Eristalomyia paria* Bigot, *Annls Soc. Ent. Fr.*, (5)10: 218.

Material Examined: 2ex., 5.iv.24, Darlaghat (31.2359°N, 76.9271°E); 4ex., 14.iv.24, Rajgarh (30.8500°N, 77.2994°E); 3ex., 16.iv.24, Namhol (31.2541°N, 76.8592°E); 6ex., 22.iv.24, Dharamshala (32.2065°N, 76.3653°E); 3ex., 24.iv.24, Anni (31.4030°N, 77.4023°E); 5ex., 29.iv.24, Chail (30.9676°N, 77.1916°E); 3ex., 2.v.24, Taradevi (31.0490°N, 77.1480°E); 3ex., 3.v.24, Naldehra (31.1733°N, 77.2048°E); 6ex., 4.v.24, Theog (31.1270°N, 77.3430°E); 5ex., 7.v.24, Churag (31.3581°N, 77.1575°E)
Coll. Monika Kaundal.

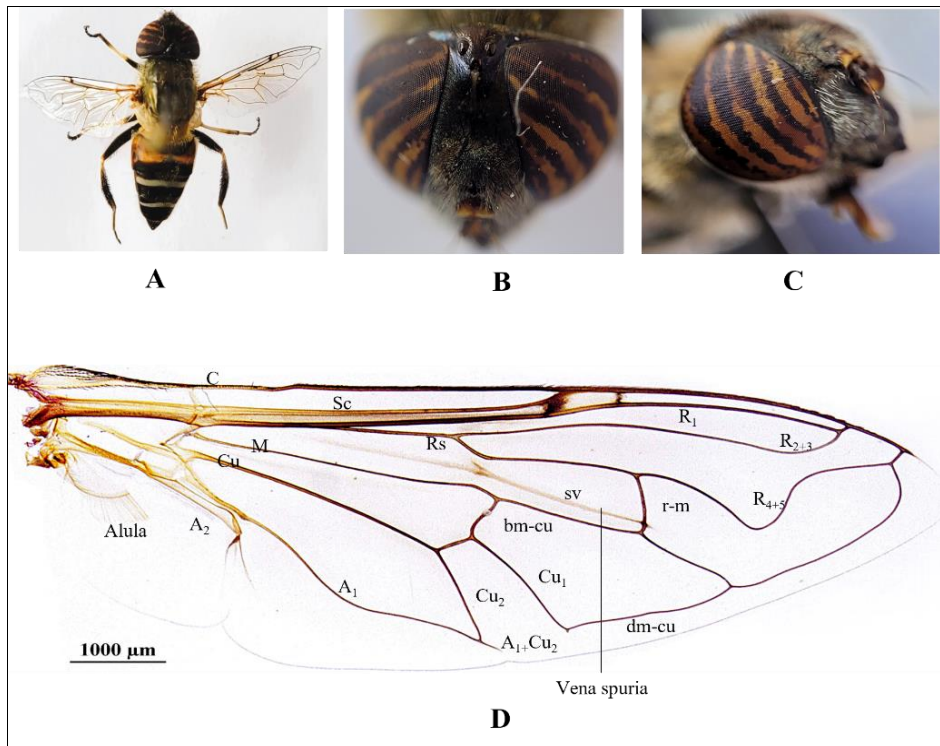


Fig 2: *Eristalinus paria*: (A) Adult; (B) Head; (C) Face; (D) Forewing

Diagnostic Features: Face bearing two broad longitudinal median stripes flanking the central tubercle; eyes marked with six narrow, parallel longitudinal dark stripes. Antennae dark brown. Thorax yellowish-grey with four evenly dull

black longitudinal stripes; scutellum translucent, yellowish-brown with matching pubescence. Wings hyaline, nearly transparent, articulated with thorax. Abdomen black and shining; first segment yellow, second brownish-yellow with

black posterior margin. Legs predominantly black, with partly yellow segments (Fig. 2A-C). Similar observations were made by Sengupta *et al.* (2019) ^[15], who documented this species as a new record in the Renuka Wetland Area, Himachal Pradesh.

Wing venation: The forewing is hyaline, with the costa (C) arising from the wing margin and extending to the wing apex. Beneath the costa, two veins emerge: the subcosta (Sc), which runs forward and joins the costa at mid-wing, and the radius (R), which divides into branches R₁ and R_s, with R_s further splitting into R₂₊₃ and R₄₊₅. The R₄₊₅ vein forms a deep sinus in r₄₊₅ cell; it connects to the media (M) via the radial-medial (r-m) cross-vein. A reduced, transparent vena spuria also exists, extending up to the r-m cross vein. From the mid-axillary region, both the media and cubitus veins originate. The media runs straight, while the cubitus divides into Cu₁ and Cu₂, with Cu₂ bending downward toward the posterior wing margin. The CuP vein is absent. The media and cubitus are connected by the basal-medial cubital (bm-cu) and discal-medial cubital (dm-cu) cross veins. In the posterior region, Cu₂ fuses with the anal vein (A₁+Cu₂), and a small A₂ vein also arises. Hindwings are modified into halteres (Fig. 2D).

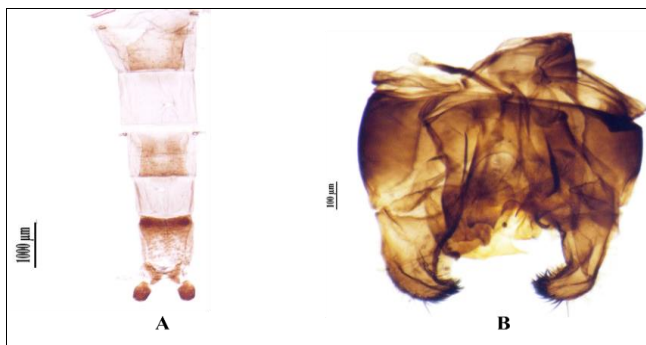


Fig 3: *Eristalinus paria*: (A) Female genitalia; (B) Male genitalia

Genitalia studies: Female tergite 8 is well developed and sclerotized. Internal genital apodemes are well developed. Epiproct located at the base of cerci, pilose; apodeme short and sclerotized. Cerci paired, oval and moderately setose, present apico-laterally to the epiproct. (Fig. 3A). Male genitalia is short, broad, and dark brown. Epandrium is large, with a pointed basal margin and a blunt apical tip. Surstylus is rounded, bearing dense pubescence at both its base and apex. Between the surstyli, inner lobes are present, light brown, oval in shape. Aedeagus is relatively small, with its distal portion short and curved downward (Fig. 3B). In a similar study, Rana *et al.* (2024) ^[16] analysed the morphological traits, wing venation, and external genitalia of *Eristalis tenax* and *Eristalis cerealis* in the Shimla hills of Western Himalayas. Rana and Thakur (2025) ^[17] studied the diagnostic features of *Episyrphus balteatus*, including wing and genital characters critical for accurate species identification in hoverflies. Mielczarek & Tofilski (2018) ^[18] highlighted the importance of wing venation studies combined with morphometric statistical analysis in identifying hoverfly species. Miranda and Moran (2017) ^[19] provided a comparative framework for female abdominal traits across Syrphidae tribes with similar observations. Cumming and Wood (2009) ^[20] investigated the comparative morphology of male genitalia across Dipteran groups, highlighting their critical role in phylogenetic

systematics. In regional studies, Sengupta *et al.* (2016) ^[21] conducted a comprehensive survey on hoverflies in Himachal Pradesh, including detailed taxonomic accounts.

Molecular Analysis and GenBank Accession Number:

The mitochondrial cytochrome c oxidase subunit I (COI) gene was successfully amplified and sequenced from an insect specimen collected in Churag, Mandi, Himachal Pradesh. After editing, a 621 bp partial fragment was submitted to the NCBI GenBank under accession number PQ329353. BLAST analysis revealed 99.84% sequence similarity to the previously published GenBank sequence (OK444104) of *E. paria*, thereby confirming the species identity. Similarly, Kumari and Thakur (2025) ^[22] performed molecular characterisation of insect pollinators of *Punica granatum*, including *E. paria* from Himachal Pradesh. Kar *et al.* (2024) ^[23] used mtCOI-based DNA barcoding to identify 13 species of ecologically important hoverflies, including two species in the genus *Eristalinus*. The hoverfly species *Eristalinus (Merodonoides) multifarius* was rediscovered in Kerala and molecularly characterised for the mtCOI gene for the first time, contributing to the regional DNA database and facilitating future biodiversity studies (Joy *et al.*, 2025) ^[24]. The final edited sequence of *E. paria* exhibited a GC and AT content of 30.9% and 69.1%, respectively, indicating a clear AT bias. Li & Li (2022) ^[25] studied the complete mitogenomes of four hoverflies of the genus *Eristalinus* and revealed a strong AT base composition, with positive AT skew and negative GC skew across the complete mitogenome, including the COI gene.

Conclusion

The integration of taxonomic and molecular techniques successfully confirmed the identity of *Eristalinus paria* collected from *Berberis lycium* flowers in the northwestern Himalayas. Morphological study and genitalia examination combined with COI gene-based molecular analysis proved to be effective tools for taxonomic studies. The close association of *E. paria* with *B. lycium* highlights its ecological significance as a pollinator of an endangered medicinal plant. The present study contributes valuable reference data for future syrphid biodiversity and phylogenetic studies. Integrative taxonomic studies help in understanding pollinator networks, distribution and population structure, which further aid in developing conservation strategies in the backdrop of climate change in Himalayan ecosystems.

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