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Rediscovery of a Rare Hoverfly, *Xanthandrus garhwalensis*, with New Records of the Genus *Xanthandrus* (Diptera: Syrphidae) from Pakistan

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ABSTRACT

In this study, we reported the first confirmed record of the genus *Xanthandrus* Verrall, 1901 from Pakistan, along with the rediscovery of *Xanthandrus garhwalensis* (Kohli, Kapoor & Gupta, 1988) (Diptera: Syrphidae) at three localities: two in Azad Kashmir (Banjosa and Rawalakot), and one in Islamabad Capital Territory (Trail-5). This rediscovery represents the first report of the species since its original description in 1988 from India (Dehradun, Ramgarh). The present study also provides the global distribution map, species diagnosis, adult habitus photographs, and DNA barcoding data of this rare hoverfly.

Keywords: Distribution map, DNA barcoding, new record, Pakistan, Syrphidae, Syrphinae.

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INTRODUCTION

Hover flies or syrphid flies (Diptera: Syrphidae) are one of the most species-rich dipteran families comprising more than 6,300 extant species under 209 genera (Skevington et al., 2019). The distribution of syrphids is global with the exception of some isolated oceanic islands and Antarctica (Thompson, 2019). Hoverflies are renowned for their characteristic flight behaviour, play crucial ecological services primarily through pollination and as natural pest controllers. Several hoverfly species exhibit mimicry, resembles wasps or bees which provides them a defensive mechanisim against predators (Rotheray & Gilbert, 2020). In addition to their pollination services, hoverfly larvae are predators of aphids and other plant damaging pests, making them useful biological control agents (Schmidt, Thewes, Thies, & Tscharntke, 2004; Bugg, Colfer, Chaney, Smith, & Cannon, 2008; Eckberg et al. 2015) or as decomposers of organic matter (Martínez-Falcón, Marcos-García, Moreno, & Rotheray, 2012).

The hoverfly genus *Xanthandrus* Verrall, 1901 (Diptera: Syrphidae) belongs to the tribe Melanostomini within the subfamily Syrphinae (Mengual, 2020; Mengual et al., 2023), and it is closely related to two Afrotropical genera, namely *Pelloloma* Vockeroth, 1973 and *Afroxanthandrus* Kassebeer, 2000 (Mengual et al., 2023; Midgley, Goergen, & Jordaens, 2024). Currently, the genus comprises 23 species in two subgenera: *Androsyrphus* Thompson, 1981 (1 sp.) and *Xanthandrus* (22 spp.). Of these, 11 species are found in the Neotropical region, followed by Oriental (6 spp.), Palaearctic (3 spp.), Australasian (3 spp.), and Nearctic regions (1 spp.) (Mengual, 2020; Borges & Pamplona, 2003; Thompson & Skevington, 2014). The immature stages of *Xanthandrus* species are economically significant as they feed not only on aphids but also on caterpillars from moth families such as Tortricidae and Notodontidae, which occur on both trees and lower-growing plants (Rojo, Gilbert, Marcos-García, Nieto, & Mier, 2003; van Veen, 2010; Santolamazza, Cartea, & Sart, 2011).

Until now, only two species of *Xanthandrus* have been recorded from India: *X. indicus* Curran, 1933 and *X. garhwalensis* (Kohli, Kapoor, & Gupta, 1988) (Mitra, Roy, Imam, & Ghosh, 2015; Sengupta et al., 2016, 2024). Saleem, Arif, & Suhail (2001) listed *X. comtus* (Harris, 1780) from the Peshawar Division in Pakistan, but posterior studies treated this report as a probably misidentification (Ghorpadé & Shehzad, 2013; Ghorpadé, 2015; Shehzad et al., 2017).

In this study, the first confirmed records of the genus *Xanthandrus* from Pakistan are given and *X. garhwalensis* is reported for the first time from Pakistan. We provide a global distribution map, species diagnosis, adult habitus photographs, and DNA barcoding data of this rare hoverfly.

MATERIAL AND METHODS

We collected five female samples between 2018 and 2021 using a hand-sweeping net in the mountainous regions of Islamabad Capital Territory (Margalla Hills, Trail-5) and Poonch District (Banjosa and Rawalakot), Azad Kashmir, Pakistan. The collected specimens were kept in the killing jars containing Potassium Cyanide up-to death.

The killed specimens were transferred in field collection boxes and shifted in the taxonomy laboratory. The insects were relaxed prior to pinning by placing them in a petridish wrapped in a wet cloth. The body parts were spread properly, positioned on the stretching board, and dried for 2 to 3 days. The species was identified by comparing it with the original description provided by Kohli, Kapoor, & Gupta (1988). The terminology used for adult morphology is based on Cumming & Wood (2017) and van Steenis et al. (2023). The examined specimens are deposited in the Entomological Collection at the National Insect Museum (NIM), Islamabad, and the Department of Entomology at The University of Poonch, Rawalakot (UPR), Azad Kashmir, Pakistan.

External body morphology was observed under a Nikon SMZ 745 stereomicroscope, and photographs of adult habitus were taken with a Nikon D800 digital camera equipped with a Nikon MICRO NIKKOR 105 mm lens. A species distribution map was created using QGIS 3.38.2 software.

Barcoding

For molecular sequencing, some specimens were preserve in 95% ethanol and later use for DNA extraction. DNA was extracted using one or more legs from each individual, following the manufacturer's protocol for the QIAGEN QIAamp® DNA Kit. Partial sequence of mitochondrial COI gene was amplified and sequenced. The amplification was done using the universal primers: LCO1490 (forward): 5'- GGTCAACAAATCAT-AAGATATTGG- 3' and HC02198 (reverse): 5' TAAACTTCAGGGTGACCAAAAATCA -3' (Folmer et al., 1994). The PCR reactions consist of 12.5 µl in a reaction volume of 25 µl, which included 1.0 µl DNA templet, 1.0 µl each forward primer and reverse primer and 9.5 µl distilled water. The PCRs involves an initial denaturation set (95°C for 5 min) following by 35 cycles of denaturation at 94°C for 30 s, annealing at 49°C for 30 s, and extension at 72°C for 2 min. A final extension was performed at 72°C for 10 minutes with a holding temperature of 12-16°C. The double-stranded PCR products were visualized by agarose gel electrophoresis on (1.5%).

Molecular Identification

For molecular identification, the COI gene fragment (612 bp) of *Xanthandrus garhwalensis* (PKDIP081-23) was aligned using ClustalW in MEGA 7 (Kumar, Stecher, & Tamura, 2016) with 10 sequences from four species within the genus *Xanthandrus*: *X. palliatus* (CNCDB3506-11), *X. comtus* (CNCDB3504-11, AMTPE1227-15, AMTPD4474-16, UZINS362-23, AMTPE1095-15), *X. callidus* (CNCDB3501-11), and *X. agrolas* (SYRAU017-15, CNCDB3760-11). The sequence of *Eristalis tenax* (OL441830.1) was used as an outgroup. All sequences were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/). Genetic distances were calculated using the Kimura-2-Parameter model in MEGA 7.0 (Kumar et al., 2016). The phylogenetic tree was constructed using the Maximum likelihood (ML) analysis implemented in IQ-TREE using ultrafast bootstrap with 5000 replicates as implemented on the website server (http:// iqtree. cibiv. univie. ac. at) (Nguyen, Schmidt, von Haeseler, & Minh, 2015). Genetic distances were calculated using the Kimura 2 Parameter (K2P, Kimura, 1980) model in MEGA 7.0 (Kumar et al., 2016).

RESULTS

Family Syrphidae Latreille, 1802

Subfamily Syrphinae Latreille, 1802

Genus *Xanthandrus* Verrall, 1901

Xanthandrus Verrall, 1901: 316. Type species: *Musca comtus* Harris, 1780: 47; subsequent designation of Coquillett, 1910: 620.

Hiratana Matsumura & Adachi, 1919: 129. Type species: *Syrphus quadriguttulus* Matsumura, 1911; by original designation. Synonym of *X. comtus* Harris, 1780.

Indosyrphus Kohli, Kapoor & Gupta, 1988: 121. Type species: *Indosyrphus garhwalensis* Kohli, Kapoor & Gupta, 1988: 122; by original designation. Synonymized by Ghorpadé (2014).

Xanthandrus garhwalensis (Kohli, Kapoor & Gupta, 1988)

Indosyrphus garhwalensis Kohli, Kapoor & Gupta, 1988: 122. Type locality: India (Uttarakhand: Dehradun, Ramgarh).

Material examined. Pakistan. Azad Kashmir: 2♀, Poonch District, Banjosa, 4.x.2021, 33.8100°N, 73.8164°E, 1893m, leg. S. Fazal (UPR); 2♀, Poonch District, Rawalakot, 15.x.2021, 33.8584°N, 73.7588°E, 1643m, leg. S. Fazal (UPR). **Islamabad Capital:** 1♀, Margalla Hills, Trail-5, 33.74444°N, 73.04167°E, 1610m, 16.vi.2018, leg. M.A. Hassan (NIM).

Re-description

Female. Head with dichoptic eyes (Fig. 1a-b), eyes bare; ocellar triangle black, with sparse brown hairs; occiput black with dense dark gray pollinosity, hairs mostly yellowish brown except near vertex with black hairs; face black, covered with yellow hairs and whitish-gray pollinosity; facial tubercle round, prominent, and shiny black; gena black with yellow hairs; lunule orange; antennae brown, with scape and pedicel black-haired, basoflagellomere ventrally brown and dorsally dark brown; arista dark brown, bare. Postpronotum bare; mesonotum and scutellum shiny black, with pale yellow hairs; pleuron black (Fig. 1e). Wings hyaline with brownish pterostigma; alula broad and microtrichose; calypter light yellow; halters yellow (Fig. 1d). Legs black; coxa and trochanter black; femur blackish, apically dark brown with yellowish-brown hairs posteriorly; fore tibia brown, covered with short black hairs; mesotibia dark brown to black, slender at the base; hind tibiae black with short black hairs. Abdomen black, wide, unmargined, and flat; tergite 1 and 2 black with black hairs, tergite 2 anteriorly grey; tergite 3 with confluent spots, and tergite 4 with narrowly separated, slightly confluent spots; tergite 5 small, black, and difficult to see dorsally (Fig. 1c). Sternite 1 black; sternite 2 with yellow margins on the posterior 1/5; sternite 3 with a round spot on the anterior 1/3; sternite 4 with yellow spots on anterolateral and lateral margins; sternite 5 black (Fig. 1f).

Host plant. *Xanthandrus* species are found in deciduous and coniferous forests, particularly those with a thick understory of shrubs (van Veen, 2010). In this study,

the adult specimens *X. garhwalensis* were collected from a dense pine forest on the flowers of *Strobilanthes attenuata* in Azad Kashmir (Fig. 4a-c) and near stagnant water at Margalla Hills in the Islamabad Capital Territory (Fig. 4d-f).

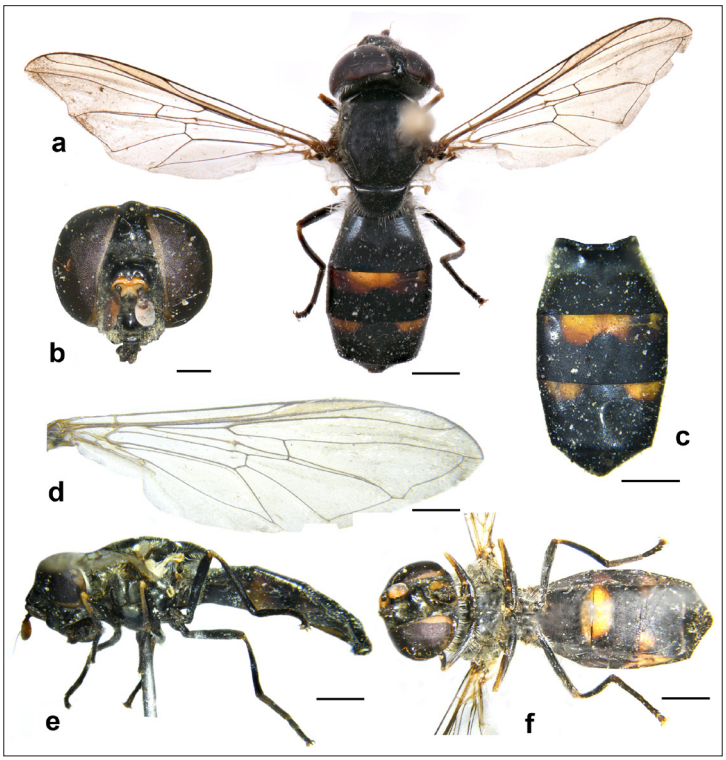

Figure 1. *Xanthandrus garhwalensis*. Female: a) dorsal habitus, b) head, frontal view, c) abdomen dorsal view, d) right forewing, e) lateral view, f) ventral view. Scale bar: 1.0 mm.

Distribution. Pakistan: Azad Kashmir: Poonch District, Rawalakot; **Islamabad Capital:** Margalla Hills, Trail-5 (present study - new country record); **India**: Uttarakhand (Kohli et al., 1988).

Genetics. Phylogenetic analysis reveals that *X. garhwalensis* is closely related to two new species from Thailand and Malaysia, and *X. comtus*, with a genetic distance ranging from 0.005 to 0.006 (Fig. 2-3).

Remarks. Morphologically, *X. garhwalensis* resembles *X. comtus* (Harris, 1780), particularly in the external body morphology, but they can be distinguished by specific abdominal markings. In *X. comtus*, the male exhibits round spots on tergite 2 and large yellow spots on tergites 3 and 4 usually extending onto the apical half of the tergite (van Veen, 2010: fig. 787), while the female has oval yellow spots on tergite 2 and square spots on tergites 3 and 4, usually also extending beyond the apical half of the tergite. In contrast, *X. garhwalensis* have no markings on tergite 2, confluent spots on tergite 3, and male with slightly confluent spots on tergite 4 but separated spots in female,

limited to the basal half of the tergite in both sexes (Kohli et al., 1988: fig. 36; Fig. 1C).

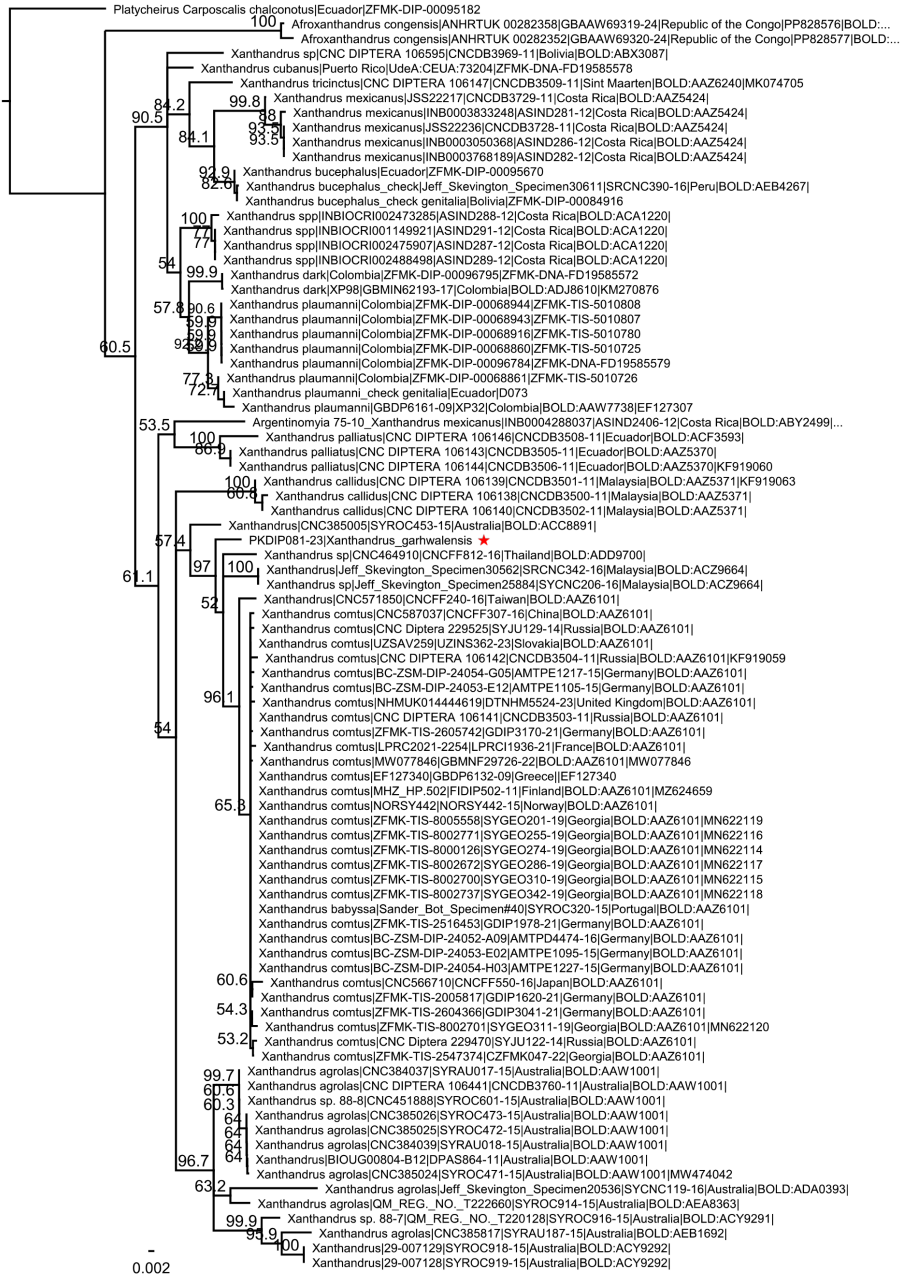

Figure 2. Phylogenetic relationships among the species of *Xanthandrus* Verrall, 1901, based on the 5'-end fragment of the COI gene. The number at each node shows the posterior probability in the ML analysis.

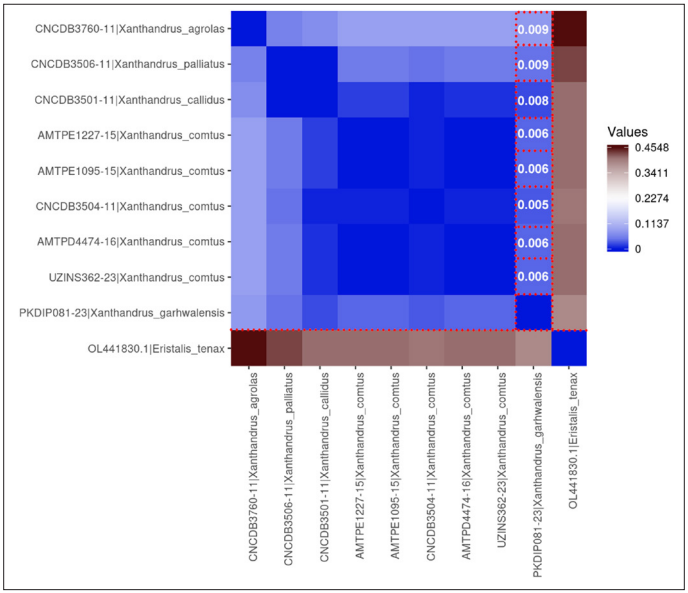

Figure 3. Heatmap of pair-wise K2P genetic distances. This heatmap depicts the genetic distances between each sampled individual based on the mitochondrial COI gene.

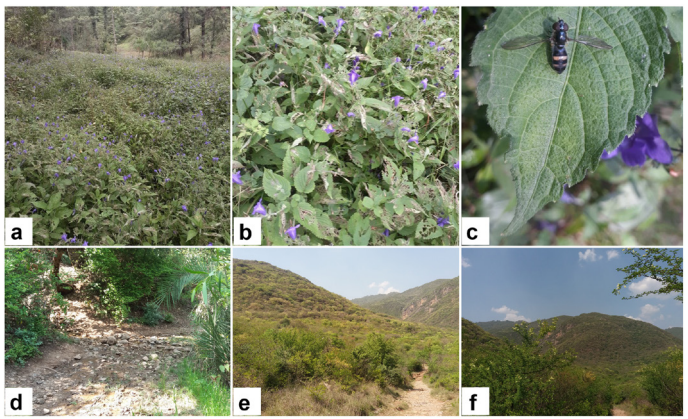

Figure 4. Banjosa Lake, Rawalakot. a) habitat photograph of *Xanthandrus garhwalensis*, b) host plant: *Strobilanthes attenuata*, c) live habitus of female *X. garhwalensis*; d-f) Margalla Hills National Park, Islamabad Capital Territory.

DISCUSSION

Before this study, *X. garhwalensis* was only known from its type locality in Ramgarh, Dehradun, Uttarakhand in India, which was originally part of Uttar Pradesh before becoming part of Uttarakhand State. Ghorpadé (2014, 2015), Shah,Jan, & Wachkoo (2014), Mitra et al. (2015), and Sengupta et al. (2016) listed this species

from Uttarakhand State. Sengupta et al. (2016) also listed this species from Uttar Pradesh, but upon review of the original literature, it was confirmed that the type locality, Ramgarh, was indeed within Uttar Pradesh at the time and later became part of Uttarakhand. The present record from Pakistan, however, suggests that this species has a broader geographical distribution along the Himalayan foothills (Fig. 5).

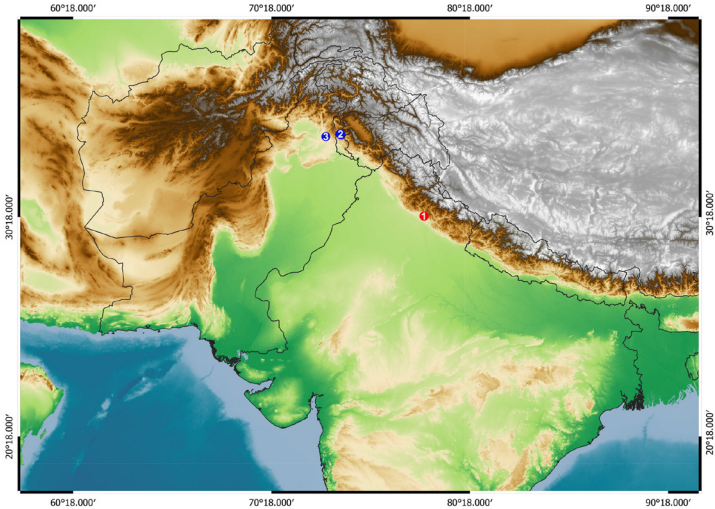

Figure 5. Global distribution map of *Xanthandrus garhwalensis* (red dot with number 1 represents the type locality; blue dots represent new locality records).

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CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding publication of this article.

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