

First Record of Parasitoid, *Psytalia fletcheri* (Silvestri, 1916) Reared from Fruit Fly Infested Cucurbits in Nepal

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ABSTRACT

The parasitoid, *Psytalia fletcheri* (Silvestri, 1916) [Hymenoptera: Braconidae: Opiinae] is known for parasitizing tephritid fruit flies. Fruit fly infested cucurbit flowers and fruits (365 samples) were collected from cucurbit growing regions of the terai and mid-hills of Nepal and cultured separately in plastic containers. A parasitoid, *P. fletcheri* emerged from 27 samples. The parasitoid was identified after detailed morphological examination and molecular analysis. This is the first report of *P. fletcheri* from Nepal. This parasitoid has potential for inclusion in integrated pest management targeting fruit fly in cucurbit crops.

Keywords: Biological control, Braconidae, Opiinae, Parasitoid, Rearing, Tephritid.

Sapkota, R., Thapa, R. B., & Dahal, K. C. (2026). First Record of Parasitoid, *Psytalia fletcheri* (Silvestri, 1916) Reared from Fruit Fly Infested Cucurbits in Nepal. *Journal of the Entomological Research Society*, 28(2), 177-182.

Received: January 30, 2026

Accepted: July 17, 2026

INTRODUCTION

The braconid subfamily Opiinae comprises over 2,000 valid species in 39 genera (Yu, van Achterberg, & Horstmann, 2016). This subfamily is distributed worldwide and comprises small (3-5 mm in length), commonly occurring parasitoid wasps that mainly attack fruit-infesting dipterous larvae. *Psytalia* is a moderately large genus within Opiinae, comprising 82 valid species (Wharton, 2009; Wu, van Achterberg, Tan, & Chen, 2016). Of them, about 40% of *Psytalia* species have known hosts of Tephritidae. Notably, *Psytalia fletcheri*, *P. incisi*, and *P. makii* have been introduced as biocontrol agents against fruit flies (Li, van Achterberg, & Tan, 2013). These species were originally described under the genus *Opius*. The *Opius fletcheri* was first described in India by Silvestri (1916) and was later placed in the subgenus *Psytalia* by Fischer (1987).

Several species that parasitize fruit-infesting tephritids including *Bactrocera cucurbitae*, *B. oleae*, and *Ceratitis capitata*, have played significant roles in biological control (Vargas et al., 2004; Mohamed, Overhold, Lux, Wharton, & Eltoum, 2007; Daane et al., 2008). Initially, *P. fletcheri* was considered as a strict solitary larval parasitoid of the melon fly; however, later studies demonstrated that it functions as both an egg and larval parasitoid of fruit flies (Bautista, Harris, Vargas, & Jang, 2004). It has also been collected from several *Bactrocera* species in Thailand and Malaysia (Chinajariyawong et al., 2000). New fruit fly species have been identified across countries around the globe (Yaran, Gormez, Koyuncu, & Tarla, 2023) and more than 30 fruit fly species have been reviewed and reported from Nepal (Sapkota et al., 2024). However, their parasitoids have not yet been reported. The present study contributes to the knowledge of biological control of fruit flies offering a new record of *P. fletcheri* reared from fruit fly infested cucurbits from Nepal.

MATERIALS AND METHODS

Sample and sampling sites

Samples were collected based on fruit fly infestation in the flowers and fruits of cucurbits from six geographically isolated locations; viz., Bhairahawa, Rupandehi (27°48'N, 83°45'E); Rampur, Chitwan (27°65'N, 84°35'E); Nawalpur, Sarlahi (26°06'N, 85°61'E), representing the terai region; Kirtipur, Kathmandu (27°67', 85°29'E); Sindhuligadi, Sindhuli (27°21'N, 85°90'E) and Tansen, Palpa (27°86'N, 83°54'E), representing the mid-hill region of Nepal. Samples of cucurbit crops (cucumber, squash/pumpkin, bitter gourd, and sponge gourd) were collected based on their availability during March-November in 2022. The flowers and fruits were collected, placed individually into zip plastic bag and transported to laboratory for rearing. A total of 365 samples were studied throughout the period. The rearing continued in room condition of Kirtipur, Kathmandu. Infested individual fruit was placed in plastic container (13×12.3×12.3 cm³) with 5 cm moist sandy soil at the bottom to facilitate pupation. The container was covered with muslin cloth of mesh size 0.15 mm tightly tied with rubber band to prevent escape of newly emerged fly. Each container was sprayed with water at every two days' interval to maintain moisture.

Morphological identification of species

Species of *Psytalia* are distinguished from other opiines by morphological characteristics, particularly metasomal features, wing venation patterns, and propodeal sculpture. The adult parasitoid emerged from each sample were trapped, counted and recorded separately. Each parasitoid was magnified under trinocular stereo microscope (BS 3040T, Bestscope, China) and identification was done based on the morphological key suggested by Fischer (1987), Wharton (2009) and Oanh, Long, Xuan, van Tinh, & Thi (2024). The genus *Psytalia* was characterized by short clypeus, broadly exposed labrum and the species *fletcheri* was recognized with occipital carina present laterally.

DNA based species identification

The morphologically identified specimen was preserved in 99.99% ethanol in Eppendorf tube and stored at -20°C for molecular tests. The sample was subjected to partial mitochondrial cytochrome COI gene for DNA barcoding as suggested by Hebert, Cywinska, Ball, & DeWaard (2003). The hind legs were lysed with Lysis buffer and Proteinase K (56°C for 10 hours). DNA extraction was done using GeneAll® Exgene™ Tissue SV kit in line with protocol. A fragment (~650 bp) of the COI gene was amplified by using primers; LCO-1490 (5' GGTCAACAAATCATAAAGATATTG-3') and HCO-2198 (5' TAAACTTCAGGGTGACCAAAAAATCA-3'). Polymerase chain reaction (PCR) was performed in 25 µL containing 12.5 µL Multiplex Master Mix (Qiagen, Germany); 5.0 µL Q solution, 3 µL of Nuclease Free water, 1.0 µL of each forward and reverse primer, and 2.5 µL of undiluted extracted DNA. The PCR thermocycler settings initiated with denaturation at 95°C for 15 min. followed by 5 cycles of denaturation at 94°C for 1 min., annealing at 45°C for 90 sec. and extension at 72°C for 90 sec., followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 50°C for 90 sec. and extension at 72°C for 1 min., then a final extension at 72°C for 5 min. and cooled down at 4°C forever by one cycle. The PCR amplification result was purified using HiMedia Gel/PCR and assessed by electrophoresis with 1.5% agarose gel for DNA visualization and bromophenol loading dye (Fig. 1). The running agarose took place at 80-150°V for 1-1.5 hours.

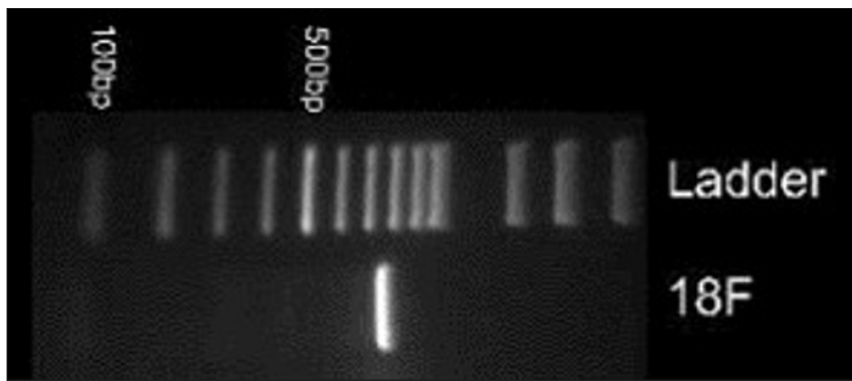


Figure 1. Agarose gel (1.5%) represents amplified mitochondrial COI gene region of parasitoid. Sample ID (18F): *Psytalia fletcheri* (Silvestri).

A 100 bp DNA ladder from Solis Biodyne was used as reference as suggested by Hebert et al. (2013). The purified PCR amplification product was sequenced with both directions forward and reverse primers using Sanger Sequencer in AB1310 Genetic Analyzer at the laboratory of Intrepid Nepal, Kathmandu, Nepal. The sequence was evaluated using the BLAST program at the National Center for Biotechnology Information (NCBI) website. The sequence was interpreted by comparing the parasitoid COI sequence with the NCBI database and a unique accession number was obtained for the sequence.

RESULTS

Host association and parasitoid emergence

The parasitoids emerged in association with fruit fly species viz. *Zeugodacus cucurbitae*, *Z. tau*, *Z. scutellaris*, and *Z. diversus*. The parasitoid emergence was recorded from 27 of the 365 samples examined. One to three parasitoids per sample emerged after 20-25 days of rearing during August to November. The parasitoids emerged from two bitter melon (*Momordica charantia*) samples collected from Chitwan and from 25 pumpkin (*Cucurbita* spp.) samples collected from Kathmandu.

Morphological characterization

The morphological features of adult *P. fletcheri* are presented in fig. 2. Adults possessed a slender, yellowish brown body measuring approximately 3-5 mm in length. The antennae were filiform, multi-segmented, bristly setose and longer than the body, a key diagnostic character. The head was transverse with large oval compound eyes, and the mesosoma was smooth and shiny, indicating weak sculpturing. Wing morphology was distinctive: the forewings were hyaline with light brown venation and a well-developed brownish pterostigma; the radial vein arose from the middle of the pterostigma, with an elongate second submarginal cell and an antefurcal m-cu vein. The hind wings showed narrow, reduced venation with a sub-basal cell, a distinct basal vein, and a straight to weakly convex vannal lobe. Legs were pale yellowish-brown, with a moderately swollen hind femur and five-segmented tarsi. The metasoma was elongate and lanceolate. Females were distinguished by a prominent exerted ovipositor, approximately equal to or longer than the body length, indicating adaptation for parasitism in concealed hosts.

Molecular characterization

The parasitoid was confirmed to *P. fletcheri* species with 99.7% identity and 675 bp query length in its NCBI genebank database with its accession number was PP949383.

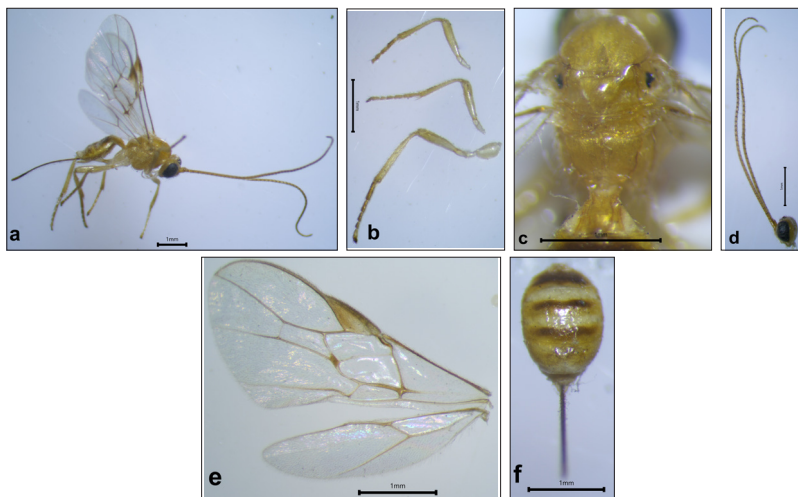


Figure 2. Morphological parts of the parasitoid, *P. fletcheri* reared from fruit fly infested cucurbits. a) adult female, b) fore, mid and hind legs, c) thorax, d) antenna, e) fore and hind wings, and f) abdomen with ovipositor (Scale bar- 1mm).

CONCLUSIONS AND DISCUSSION

Psytalia fletcheri (Silvestri, 1916) was recorded for the first time from Nepal with morphological and molecular analysis. The morphological features of the parasitoid were similar with descriptions provided by Fischer (1987) and Wharton (2009).

The present study confirmed the emergence of *P. fletcheri* from fruit fly infested cucurbit crops, in association with different fruit fly species. The emergence of this parasitoid in association with fruit flies has also been reported in other countries. In Kenya, two Opiinae species (*P. masneri* and *P. halidayi*) were reared from fruit fly infested fruits (Wharton, 2009). Similarly, *P. fletcheri* was recovered from infested snake gourd fruits in Bangladesh, in association with *Z. cucurbitae* and *Z. tau* (Lablanc et al., 2019), and from bitter gourd in Reunion Island (Quilici et al., 2002). Additionally, *P. fletcheri* has been reported to emerge from rotting fruits of cucumber, zucchini, and tomato in association with *B. cucurbitae* (Purcell & Messing, 1996). This finding indicates the potential for widening the scope of integrating this parasitoid into biological control programs.

ACKNOWLEDGEMENTS

Authors are thankful to the National Plant Protection Organization (NPPO), Nepal, and Dr. Samundra Lal Joshi for his guidance and assistance to the species identification.

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