

Report Of *Tetragonula iridipennis* Pollination On Flowering Plant *Bryophyllum pinnatum* In New Delhi, India

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Abstract

Research was carried out in New Delhi, India, where the discovery of *Tetragonula iridipennis*, also known as the Kelulut or Trigona bee, as a pollinator of *Bryophyllum pinnatum* (Crassulaceae), commonly referred to as "Mother of Thousands," was recorded. *T. iridipennis* has not been previously documented as a pollinator of *B. pinnatum*, a succulent plant known for its distinctive method of vegetative propagation. This finding presents a unique addition to the study of pollination ecology, demonstrating the adaptability of these bees in supporting the reproduction of a plant species that has not been previously documented. The research also emphasises the significance of comprehending the interactions between bees and plants, as well as the importance of safeguarding both bee species and bryophyllum plants in their respective ecosystems. The recent discovery of this connection highlights the wide range of hosts that *T. iridipennis* can infect and its importance in supporting biodiversity and plant reproduction. Additional insights into this distinctive pollination relationship may be obtained through further research, which can enhance our understanding of the dynamics between pollinators and plants.

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Key words: Molecular, first, report, *Tetragonula iridipennis*, bryophyllum, pollinator.

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INTRODUCTION:

Bryophyllum pinnatum, also referred to as the "Mother of Thousands" or "Life Plant," is a fascinating succulent that belongs to the Crassulaceae family. Originally from Madagascar, this species has since expanded its presence to different tropical and subtropical areas across the globe. This distinctive plant is identified by its thick, succulent leaves, which are frequently variegated, and have small plantlets along the edges. As a result, it has earned the nickname "Mother of Thousands."

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The leaves of *B. pinnatum* are frequently used in traditional medicine to treat wounds, burns, and skin conditions by applying them directly to the affected area. This is because they are believed to have anti-inflammatory and wound-healing effects. Additionally, *B. pinnatum* has been used in various traditional medicinal practises to treat digestive issues and respiratory conditions such as asthma and bronchitis (Rout et al., 2012; Ojewole, 2005).

B. pinnatum uses a two-fold approach for pollination in its reproductive strategy. The plant is capable of reproducing through self-pollination by producing plantlets along the edges of its leaves, enabling asexual propagation. Furthermore, the genetic diversity within the plant population is enhanced by insect-facilitated cross-pollination, which is mainly carried out by bees and other pollinators that are attracted to the bell-shaped flowers of the plant. The successful reproduction of *B. pinnatum* is ensured by a combination of self-pollination and cross-pollination. Additionally, the distribution of this plant is further expanded through the dispersal of its small seeds by wind (Rout et al., 2013; Kingsley et al., 2018).

Bees, including honey bees (*Apis mellifera*) and various native species such as bumblebees (*Bombus* spp.), carpenter bees (*Xylocopa* spp.), mason bees (*Osmia* spp.), sweat bees (Halictidae), and solitary mining bees (Andrenidae), play a crucial role in pollinating this succulent plant (Buchmann and Nabhan, 1996; Free, 1993)

Additionally, butterflies, which include various species like the Monarch butterfly (*Danaus plexippus*) and the Painted Lady butterfly (*Vanessa cardui*), are attracted to its bell-shaped flowers and play a crucial role in transferring pollen from one flower to another during their foraging visits, promoting cross-pollination and genetic diversity within the plant population (Rout et al., 2012; Ong, 2017). While ants may also visit the flowers, they are generally less effective as pollinators compared to bees and butterflies.

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To examine the dynamics of pollinators, including their diversity and dispersal within the *B. pinnatum* plant ecosystem, a periodic survey was conducted in various locations across the National Capital Region (NCR) of New Delhi. This survey aimed to gain insights into the reproduction scenarios of this plant, providing valuable information for effective understanding of the role of insects in pollination.

Write about *T. iridipennis* also in introduction.

MATERIALS AND METHOD

Collection, transportation, and preservation of the specimen:

In a 100-m² plot, ten randomly selected *B. pinnatum* plants were sampled for insect collection. The adult bees were challenging to collect due to their active behaviour. To collect the adult bees, a sweep net was used, and they were later transferred to a killing jar. Subsequently, the adults were placed in 30 ml screw-capped vials filled with 70% alcohol and transported to the laboratory (Gouda et al., 2024a). In the lab, the specimens were taken out, and the alcohol was dried using blotting papers. The adult bees were then pinned at the thorax using standard insect pins and left to dry completely for three days. After drying, the dried and pinned specimens were stored in standard insect boxes. And for species identification, we compared reference specimens and pertinent literature.

For molecular identification, bee samples were collected and stored in 70% ethanol at -20 degrees Celsius until DNA extraction. DNA extraction was performed using the DNA Sure Tissue Mini Kit (Qiagen #NP-61305) following the manufacturer's instructions. The extracted DNA was examined on a 0.8% agarose gel containing 0.5 g/ml of ethidium bromide, and the quantified DNA was used for further PCR analysis (Gouda et al., 2024b). A partial mtCOI gene fragment was amplified using the universal primers LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAACTTCAGGGTGACCAAAAAATCA-3'). PCR amplification was carried out in a 25- μ l reaction mixture containing 12.5 μ l of PCR master mix (Promega M750A), 7.5 μ l of nuclease-free water, 1 μ l of forward and reverse primers, and 3 μ l of DNA template (Gouda et al., 2024c). The PCR-amplified product (3 μ l) was electrophoresed at 100 V for 45 minutes on a 1.2% agarose gel in 1X TAE buffer. The purification and sequencing of the PCR-amplified products were outsourced. BLAST analysis was performed to search for homologous sequences using the National Centre for Biotechnology Information (NCBI) database (<http://ncbi.nlm.nih.gov/BLAST>) (Gouda et al., 2024d). The obtained sequence was submitted to NCBI GenBank to obtain accession numbers. Multiple alignments for homology search were conducted using the Clustal W algorithm software, with the default gap penalty values being gap opening 10 and extension 0.2 with a p-distance model and pairwise gap deletion selected (Gouda and Subramanian, 2024). Dendrograms by the neighbour joining method were generated using MEGA11 software, incorporating the isolate from the current study and reference strain sequences obtained from GenBank (Gouda et al., 2024e).

RESULT AND DISCUSSION

During the survey, on March 26, 2023, at Amrit Udyan in New Delhi, India (latitude: 28.6156, longitude: 77.1984), the first report of *T. irrdipennis* as a pollinator of *B. pinnatum* was documented. Adult individuals typically measure around 3 to 4 mm in body length and are characterised by their predominantly black coloration. Their body structure includes a compact and slender shape with well-defined head, thorax, and abdomen regions, and they have two pairs of wings that lock together during flight. These bees possess a long proboscis for nectar and pollen collection, segmented antennae, compound eyes, and hind legs adapted for pollen transport, featuring pollen baskets or corbiculae. Unlike some bee species, *T. iridipennis* lacks a stinger for defence (Fig. 1).

Comment [DN7]: Add a separate section as study area and give the time period of study, study location details along with its latitude and longitude.

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Comment [DN8]: Apart from random selection of plants, please mention if any other criteria for plant selection used (eg., plants in flowering stage etc).

Comment [DN9]: Before identification of species, was there any study on the no. of visits to the plants inflorescence.

Comment [DN10]: These details should come under study area section.

Comment [DN11]: It is also known as stingless bee, and no where in the article, this name is used. Nesting or habitat of the bee is also not mentioned. This is important because the author says they are reporting for the first and should look in the study area for nests of stingless bees.



Fig. 1 Adult of *Tetrasonula iridipennis*

Comment [DN12]: Photo credits are missing.

The DNA was amplified by mtco1 universal primer and amplification was confirmed by running gel (**Fig.2**). Furthermore, molecular identification using the NCBI BLAST algorithm indicated a 100% identity with *T. iridipennis* and we obtained accession number OR793172 and subsequently, a comprehensive phenogram construction was constructed along with reference sequences unveiled the formation of two distinct clades one for *Tetrasonula* sps. and other with *Melipona* sps. Notably, our isolate *T. iridipennis* species clustered harmoniously with the reference sequences of its respective species. This insightful molecular scrutiny further fortified the case for the accurate identification of the *T. iridipennis* (**Fig. 3**).

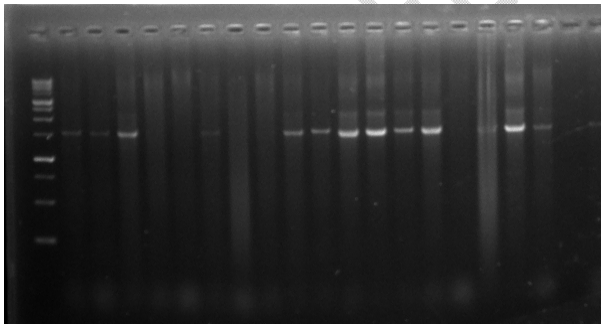


Fig. 2 Gel picture of DNA amplified by mtco1 primer

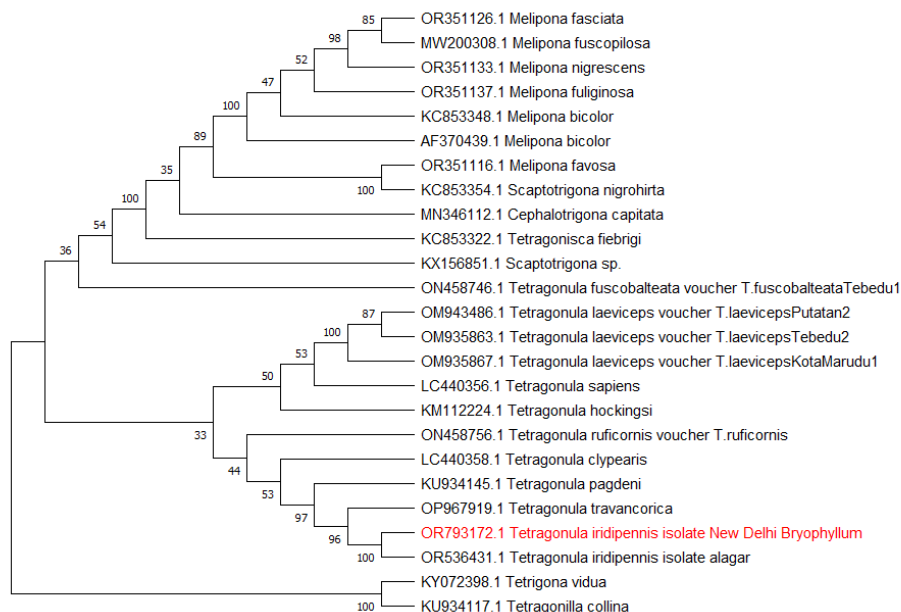


Fig. 3 Molecular identifications of *Tetragonula iridipennis*. Red colour indicates sequences generated in this study.

During our observations, we found that this insect species was highly active during the daytime, especially while pollinating (Fig. 4). While they were capable of flying for up to 50–60-meter distance. Additionally, a pollen basket was seen in the hind leg.

Comment [DN13]: Mention the time range (early mornings or evenings).

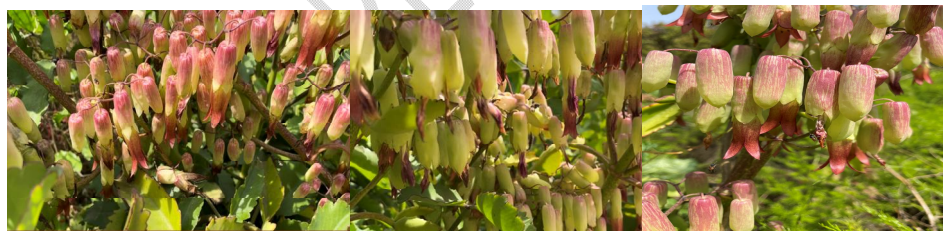


Fig. 4 Pollination of bryophyllum by *Tetragonula iridipennis*

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T. iridipennis, commonly known as the Kelulut or Trigona bee, serves as a valuable and versatile pollinator with a host range that extends across various plant families. These bees are known to visit and pollinate a wide array of plant species, contributing significantly to pollination in various ecosystems and agricultural settings. Notable plant families benefiting from their pollination services include the Rosaceae family, where they efficiently pollinate fruit trees like apples, pears, and cherries, enhancing fruit production. In the Rutaceae family, *T. iridipennis* plays a vital role in pollinating citrus fruits, such as oranges, lemons, and limes, which are crucial for the citrus industry (Bueno et al., 2023). Additionally, cucurbits in the Cucurbitaceae family, including cucumbers, pumpkins, and squash, rely on these bees for effective pollination (Chauhan et al., 2019; Kishan et al., 2017). They also visit herbs like basil, oregano, and thyme in the Lamiaceae family, supporting culinary and medicinal herb production (Bueno et al., 2023). Furthermore, they are recognised pollinators of economically significant crops like oil palm and rubber in the Palmaceae family (Bueno et al., 2023). *T. iridipennis* plays a

pivotal role in enhancing the diversity and health of natural ecosystems by pollinating a wide range of wildflowers, often spanning multiple plant families. Their adaptability and efficiency as generalist pollinators underscore their importance in sustaining both agricultural productivity and biodiversity, making them a key contributor to plant reproduction and ecosystem health.

As of now there is no prior report on the pollination of bryophyllum plants belonging to crassulaceae by *T. iridipennis*, this represents a significant and novel discovery in the field of pollination ecology. Bryophyllum plants, which are known for their unique method of vegetative propagation through plantlets on their leaves, have now been observed to receive pollination services from *T. iridipennis*. This discovery highlights the adaptability and broad foraging behavior of these bees, shedding light on their role in facilitating the reproduction of an entirely new plant species. Such findings contribute to our understanding of both bee-plant interactions and the plant species that rely on *T. iridipennis* for their reproductive success. This newly reported association underscores the importance of protecting both the bee species and the bryophyllum plants within their ecosystem. Further studies and research may provide deeper insights into the intricacies of this unique pollination relationship.

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