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Report of (*Tetragonula iridipennis)* Smith, 1854 Pollination on Flowering Plant [*Bryophyllum pinnatum* (Lam.)] in New Delhi, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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* (Lam.) (Crassulaceae), commonly referred to as "Mother of Thousands," was recorded and species

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identification was mainly carried out by molecular method. *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.

Keywords: Molecular; first; report; Tetragonula iridipennis; bryophyllum; pollinator.

1. 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" [1].

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 [2,3].

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 [4,5].

Bees, including western 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 [6,7].

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 [2,8]. While ants may also visit the flowers, they are generally less effective as pollinators compared to bees and butterflies [2].

Stingless bees (Apidae: Meliponini) hold significant economic, ecological, and cultural value. These bees, known for their complex social organization, live in perennial colonies and are commonly found in tropical and subtropical regions. They play a crucial role as primary pollinators for both native and cultivated plants, including key global crops such as coffee. Approximately one-fourth of the more than 1000 plant species cultivated in tropical regions for food, beverages, fiber, spices, and medicines are likely pollinated by stingless bees. However, human activities are contributing to a decline in their diversity and abundance, which could have serious implications for global food security, crop-based economies, and livelihoods [9].

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.

2. MATERIALS AND METHODS

2.1 Study Area

A periodic survey was conducted across various locations in the NCR region (Pusa Farm, IARI, Najafgarh Farm, Talkotora Garden, Indraprastha Park, Amrit Udyan Park, and Budha Jayanthi Park) to evaluate the dynamics of pollinators during the period of November 2022 to February 2023. This survey resulted in the documentation of *T. irrdipennis* bee insects infesting chamomile at Amrit Udyan, New Delhi, India (28.6156 latitude, 77.1984 longitude), on February 24, 2023.

2.2 Collection, Transportation, and Preservation of the Specimen

In a 100-m^2 plot, ten randomly selected *B. pinnatum* flowering stage 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 [10]. 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 [11]. 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-µl reaction mixture containing 12.5 µl of PCR master mix (Promega M750A), 7.5 µl of nuclease-free water, 1 µl of forward and reverse primers, and 3 µl of DNA template [12]. The PCR-amplified product (3 µl) 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) [13]. 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 [14]. 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 [15].

3. RESULTS AND DISCUSSION

During the survey, on March 26, 2023, at Amrit Udyan in New Delhi, India (latitude: 28.6156, longitude: 77.1984), the report of *T. irrdipennis* (Stingless bee) 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).

Fig. 1. Adult of *Tetragonula irdipennis*

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. irridipennis* 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 *Tetragonula* 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).

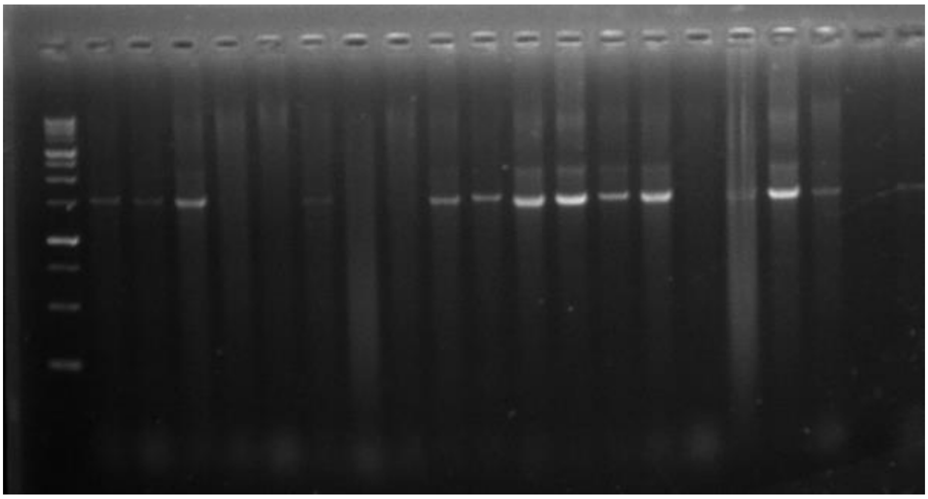

Fig. 2. Gel picture of DNA amplified by mtco1 primer

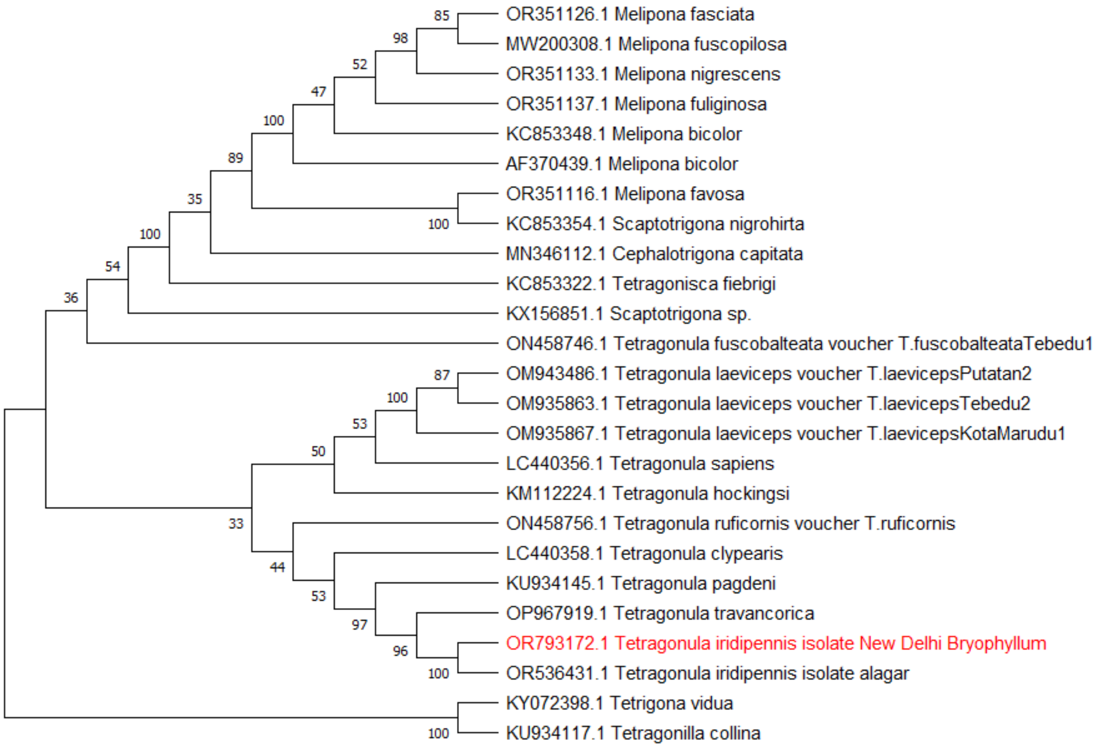

Fig. 3. Molecular identifications of *Tetragonula iridipennis*. Red colour indicates sequences generated in this study

Fig. 4. Pollination of bryophyllum by *Tetragonula iridipennis*

During our observations, we found that this insect species was highly active during the daytime (8:00 am to 5:00 pm), especially while pollinating (Fig. 4). They were capable of flying for up to 50–60-meter distance. Additionally, a pollen basket was seen in the hind leg.

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 [16]. Additionally, cucurbits in the Cucurbitaceae family, including cucumbers, pumpkins, and squash, rely on these bees for effective pollination [17,18]. They also visit herbs like basil, oregano, and thyme in the Lamiaceae family, supporting culinary and medicinal herb production [16]. Furthermore, they are recognised pollinators of economically significant crops like oil palm and rubber in the Palmaceae family [16]. *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.

4. CONCLUSION

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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during writing or editing of manuscripts. The generative AI used is ChatGPT, based on the GPT-4 architecture, provided by OpenAI. Various user queries and instructions were given as input prompts for generating and editing text.

Details of the AI usage is given below:

1. Chat GPT-Open AI

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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