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FIRST MOLECULAR CONFIRMATION OF *BACTROCERA SCUTELLATA* AND PHYLOGENETIC ASSESSMENT OF MAJOR FRUIT FLY SPECIES IN BANGLADESH

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ABSTRACT

Fruit flies of the genus *Bactrocera* (Diptera: Tephritidae) are among the most destructive insect pests of fruits and vegetables in tropical and subtropical regions, causing significant economic losses in Bangladesh. Accurate identification of these species remains challenging because of their morphological similarity and the presence of cryptic taxa, while molecular data from Bangladesh are still limited. To fill this gap, extensive field surveys and laboratory analyses were conducted to determine the species composition, host associations, and infestation patterns of fruit flies across major agroecological regions of Bangladesh. Adult flies were collected using pheromone traps and reared from infested fruits and vegetables. Morphological identification followed standard taxonomic keys, and molecular confirmation was performed through cytochrome oxidase subunit I (COI) gene sequencing. Morphological observations revealed *Bactrocera cucurbitae* (melon fly) and *B. dorsalis* (oriental fruit fly) as the predominant species in all surveyed sites. COI sequencing of 27 specimens resolved four major clades: *B. dorsalis*, *B. tau* (pumpkin fruit fly), *B. cucurbitae*, and *B. scutellate* (striped fruit fly), with *B. scutellata* representing the first confirmed record in Bangladesh. A sample initially identified morphologically as *Ceratitis capitata* was molecularly confirmed as *B. tau*, highlighting the limitations of morphology-based identification. These results reaffirm the dominance of *B. dorsalis* and *B. cucurbitae* in Bangladeshi fruit and vegetable ecosystems and highlight the value of integrating molecular diagnostics with traditional taxonomy. The findings provide crucial baseline information for species-specific, sustainable fruit fly management and strengthen the molecular framework for future tephritid research in Bangladesh.

Keywords: Fruit fly, *Bactrocera* spp., molecular identification, DNA barcoding

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INTRODUCTION

Fruit flies (*Bactrocera* spp., Diptera: Tephritidae) are one of the most destructive groups of insect pests affecting fruit and vegetable production in tropical and subtropical regions worldwide. They inflict serious quantitative and qualitative losses in fruit and vegetable production, thereby threatening food security and export potential in many countries, including Bangladesh. The genus *Bactrocera* Macquart is recognized as the most economically important group within Tephritidae, comprising more than 650 described species, of which at least 50 are considered major agricultural pests (White *et al*. 1992, Drew and Romig 2013). These species exhibit wide geographical distribution, broad host ranges, rapid reproductive cycles, and strong adaptability to diverse agroecological conditions across tropical Asia, the Pacific, and Australia. Among the most notorious pest species, *Bactrocera dorsalis* (Hendel) (oriental fruit fly), *B. cucurbitae* (Coquillett) (melon fly), and *B. tryoni* (Froggatt) (Queensland fruit fly) are of major global concern. The *B. dorsalis* complex alone comprises over 85 morphologically similar species (Drew and Romig 2013), including five highly polyphagous pests of commercial importance. The oriental fruit fly, *B. dorsalis*, is native to tropical Asia and infests more than 270 host plants, including mango, guava, citrus, papaya, and other fruit crops (Allwood *et al*. 1999). The melon fly, *B. cucurbitae*, primarily attacks cucurbitaceous vegetables, but also infests papaya and other non-cucurbit hosts, with over 80 known hosts. On the Indian subcontinent, more than 118 fruit fly species have been documented as pests of fruits and vegetables (David and Ramani 2011, Drew and Romig 2013, David *et al*. 2016, and Leblanc *et al*. 2018).

In Bangladesh, *B. cucurbitae* and *B. dorsalis* are the two most widely distributed and economically damaging species, infesting over 16 cucurbit vegetables and a range of fruit crops including mango, guava, citrus, lemon, ber, and mandarin (Alim *et al*. 2012). Recent surveys have demonstrated the remarkable diversity of fruit flies in the country. A comprehensive six-year survey (2013-2018) identified 29 species, including 13 pest and 16 non-pest species, from both cultivated and forest habitats, with *B. dorsalis*, *B. zonata* (peach fruit fly), *Zeugodacus tau* (pumpkin fruit fly), and *Z. cucurbitae* emerging as dominant species (Leblanc *et al*. 2019). Earlier studies also recorded *B. nigrofemoralis*, *B. scutellata*, and several *Dacus* species, including eight new country records (Leblanc *et al*. 2013 & 2014),

indicating expanding species diversity and possible species invasions. However, these findings were based primarily on morphological identification, which can be unreliable due to phenotypic variability and overlapping morphological traits within the *Bactrocera* species complex.

Traditional morphology-based taxonomy, though foundational in insect systematics, requires expert knowledge and specialized skills that are increasingly scarce. Morphological identification becomes particularly challenging in cryptic species complexes, immature life stages (larvae and pupae), and specimens with damaged diagnostic characters. These limitations can result in misidentification, misinterpretation of pest status, and ultimately, ineffective management interventions. Such taxonomic uncertainty has serious implications for species-specific pest management strategies, especially pheromone-based and baiting systems, since their efficacy depends on correct species identification. The introduction or emergence of new or cryptic fruit fly species could undermine existing Integrated Pest Management (IPM) programs developed for *B. cucurbitae* or *B. dorsalis* and lead to control failures.

Molecular techniques, particularly DNA barcoding, have revolutionized insect taxonomy by providing a rapid, reliable, and universal method for species identification. The mitochondrial cytochrome c oxidase I (COI) gene has proven to be an efficient and standardized marker for DNA barcoding in fruit flies (Hebert *et al*. 2003 and Blaxter 2003). Several molecular diagnostic techniques such as PCR-RFLP (Restriction Fragment Length Polymorphism), real-time PCR, and loop-mediated isothermal amplification (LAMP) have been successfully applied to discriminate closely related *Bactrocera* species (Sultana *et al*. 2022). These molecular tools overcome the limitations of traditional taxonomy and facilitate early detection of invasive species, monitoring of species complexes, and accurate pest diagnosis for biosecurity and quarantine purposes.

Despite the global advancement in molecular diagnostics, molecular-level studies on fruit fly taxonomy and population composition remain very limited in Bangladesh. Most existing information relies solely on morphological identification, and comprehensive molecular evidence on species diversity, distribution, and host association are lacking. Consequently, the understanding of *Bactrocera* species

complexes and their ecological significance in the country remains incomplete. Therefore, the present study was undertaken to document the species composition, host association, and damage intensity of fruit flies infesting major fruits and vegetables across different agro-ecological zones of Bangladesh. Importantly, the study applied molecular characterization using mitochondrial COI gene-based DNA barcoding to identify economically important *Bactrocera* species. This research provides the first integrated morphological and molecular assessment of fruit fly species diversity in Bangladesh, offering novel insights into their taxonomy, ecology, and distribution. The findings will contribute to the refinement of species-specific IPM strategies, strengthen national fruit fly surveillance, and support sustainable pest management and biosecurity initiatives.

MATERIALS AND METHODS

Study sites and sampling design: Field surveys were conducted across eight agro-ecological regions of Bangladesh, encompassing diverse fruit and vegetable production systems. Sampling locations included BARI regional research stations and Multi-Location Testing Sites (MLTS) situated in Gazipur, Jamalpur, Ishwardi, Bogura, Rangpur, Jashore, Barishal, and Khagrachari (Table 1). Each site was selected to represent distinct agro-ecological conditions, cropping patterns, and host diversity.

Trapping and collection of fruit flies: The monitoring program employed ten pheromone lures specific to different fruit fly species, all of which were procured from Russell IPM Ltd., UK (Plate 1). BARI-designed water traps were placed in bottle gourd, bitter gourd, pumpkin, guava, mango, papaya, and citrus fields. Traps were installed depend upon the crops and maintained throughout the fruiting period. Traps were inspected every three days, and captured adult fruit flies were collected using fine forceps, washed, and preserved in 100% ethanol for laboratory examination. Specimens were labeled with location, date, host crop, and lure type and stored at -20°C for molecular analysis.

Morphological characterization and laboratory rearing: Infested fruits and vegetables were collected from the field and transported to the IPM Laboratory, Entomology Division, BARI, Gazipur, for rearing and identification. Infested samples were placed in plastic trays containing a 5-cm layer of sterilized sand to

Table 1. Sampling locations of fruit fly species with corresponding latitude and longitude

Sl.	Species	Location	Latitude	Longitude
1.	*Bactrocera cucurbitae*	Entomology Research Field, BARI, Gazipur	23.99941	90.42027
2.	*B. tau*	Regional Agricultural Research Station, BARI, Jamalpur	24.92302	89.95011
3.	*B. triyoni*	Regional Agricultural Research Station, BARI, Ishwardi, Pabna	24.12011	89.07770
4.	*B. zonata*	Multi Location Test Site (MLTS), OFRD, Sherpur, Bogura	24.85104	89.36972
5.	*B. papayae*	Regional Agricultural Research Station, BARI, Rangpur	25.92660	89.23426
6.	*B. dorsalis*	Regional Agricultural Research Station, BARI, Jashore	23.18535	89.18720
7.	*B. nigrofema*	Regional Agricultural Research Station, BARI, Barishal	22.78736	89.29257
8.	*Ceratitis capitata*	Hill Agricultural Research Station, BARI, Khagrachari	23.14167	92.00229
9.	*C. rosa*	Do		
10.	*C. cosyra*	Do		

facilitate pupation. Emerging pupae were collected daily and maintained in rearing cages (30 × 30 × 30 cm) under controlled laboratory conditions (25 ± 2°C, 60 ± 5% RH, and 12:12 h light: dark photoperiod). Adults emerging from pupae were morphologically identified based on diagnostic features including coloration and patterns of the head, thorax, abdomen, scutum, and wing venation. Identification was followed as *Bactrocera* based features following the descriptions given by Leblanc *et al*. 2013, 2014 & 2019, Hossain *et al*. 2019, Drew and Hancock (1994) and White and Elson-Harris (1992). Morphometric variations among species were also documented and compared.

DNA extraction, PCR amplification and phylogenetic analysis: Molecular confirmation of morphologically identified fruit fly species was conducted through DNA barcoding of the mitochondrial cytochrome oxidase I (COI) gene. Total genomic DNA was extracted from the thoracic tissue of individual adult flies using a commercial extraction kit (Promega USA) following the manufacturer's protocol with minor modifications to optimize yield and purity.

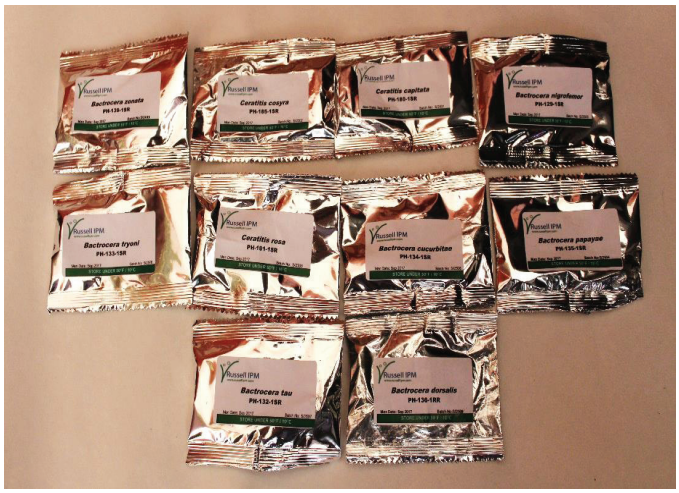

Plate 1. Ten pheromone lure types from Russell IPM, UK, were used for monitoring fruit fly species in the study

A total of 32 specimens from eight locations were subjected to PCR amplification using the universal COI primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). PCR reactions were performed in 25 µL volumes containing 12.5 µL of 2× PCR Master Mix, 1 µL of each primer (10 µM), 2 µL of template DNA, and 8.5 µL of nuclease-free water. Thermal cycling conditions were: initial denaturation at 94°C for 3 min; 39 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; followed by a final extension at 72°C for 10 min. PCR products were visualized on 1% agarose gel electrophoresis in 0.5× Tris-borate-EDTA (TBE) buffer and stained with ethidium bromide. Amplicons showing clear and specific bands (~650 bp) were purified using a commercial PCR purification kit and sent to 1st BASE Laboratory (Malaysia) for Sanger sequencing. Obtained sequences were edited and aligned using BioEdit software, and homology searches were conducted using BLASTn (NCBI database) to confirm species identity. Phylogenetic analyses were performed in MEGA 7 software using the neighbor-joining (NJ) method with the Kimura 2-parameter (K2P) model. Confidence levels of tree nodes were assessed by 1,000 bootstrap replications. Reference COI sequences of closely related *Bactrocera* and *Ceratitis* species were retrieved from GenBank for comparative analysis.

RESULTS AND DISCUSSION

Morphological identification and variation: Morphological differentiation among adult *Bactrocera* species remains challenging due to high intraspecific variability, cryptic species complexes, and overlapping diagnostic characters (White *et al*. 1997, Drew and Romig 2013). In the present study, adult fruit flies reared from infested fruits and vegetables at the laboratory, revealed that the species *Bactrocera cucurbitae* (melon fly) and *B. dorsalis* (oriental fruit fly) were the most prevalent across the eight surveyed locations. Quantitative assessment indicated that *B. cucurbitae* was most abundant, followed by *B. dorsalis* and *B. zonata* (peach fruit fly).

Pheromone trap data confirmed these findings, showing that *B. cucurbitae* and *B. dorsalis* were widely distributed across all study sites. *Bactrocera cucurbitae* predominated in cucurbit vegetables, whereas *B. dorsalis* primarily infested fruit crops including guava (*Psidium guajava*), mango (*Mangifera indica*), and citrus (*Citrus* spp.). *Bactrocera zonata* was detected in traps but at lower abundance. Other species including *B. tau* (pumpkin fruit fly), *B. papayae* (papaya fruit fly), *B. tryoni* (Queensland fruit fly), and *B. nigrofemoralis* were occasionally captured, but their adult emergence from infested fruits was inconsistent, likely due to subtle morphological differences complicating species identification (Leblanc *et al*. 2013, Hossain *et al*. 2019). The Mediterranean fruit fly *Ceratitis capitata* was recorded once from Barishal; however, molecular analysis identified this specimen as *B. tau*, demonstrating the limitations of morphology-based identification within closely related tephritid species. No adults were captured in *Ceratitis rosa* and *Ceratitis cosyra (Walker)* traps during the survey works.

These results align with previous surveys in Bangladesh where *Bactrocera dorsalis*, *Zeugodacus cucurbitae* (*B. cucurbitae*), *Zeugodacus tau*, and *B. zonata* were consistently dominant (Leblanc *et al*. 2013, 2014 & 2019, and Sultana *et al*. 2022). Similarly, Rahman (2021) reported five major pest species including *B. dorsalis*, *Z. cucurbitae*, *Z. tau*, *B. zonata*, and *Dacus longicornis*, with *B. dorsalis* showing the highest abundance in Bandarban and Chapainawabganj district. These findings confirm the persistent dominance of *B. dorsalis* and *B. cucurbitae (Z. cucurbitae)* in fruit and vegetable agro-ecosystems in Bangladesh.

Molecular identification: Molecular characterization using mitochondrial cytochrome oxidase I (COI) gene sequencing validated the morphological identifications and provided additional insight into species diversity. Out of 32 samples, 27 produced high-quality COI sequences suitable for phylogenetic analysis. Neighbor-joining analysis grouped the sequences into four major *Bactrocera* clades: *B. dorsalis* (13 samples), *B. tau* (8 samples), *B. cucurbitae* (4 samples), and *B. scutellata* (2 samples) (Figs. 1-3). The *B. dorsalis* clade was clearly distinct from *B. tau* and *B. cucurbitae*, confirming the genetic separation reported in previous phylogenetic studies (Drew and Romig 2013, Schutze *et al*. 2015). Interestingly, two samples identified as *B. scutellate* (striped fruit fly) represent the first confirmed molecular records of this species in the agro-ecological regions of Bangladesh. This detection suggests a possible range expansion or introduction event, warranting continued monitoring. One specimen (LCO11) initially presumed to be *C. capitata* through morphological observation was later identified as *B. tau* based on COI sequence similarity (>99%) with GenBank accessions. Similar misidentifications have been reported in other studies, where *B. tau* was frequently confused with *B. cucurbitae* or *B. dorsalis* due to subtle morphological differences (San Jose *et al*. 2018, Hossain *et al*. 2019).

Earlier records from Bangladesh reported 10-15 tephritid fruit fly species (Alam 1967, Kapoor *et al*. 1980). More recent studies have expanded the known diversity to 29 species, including both pest and non-pest taxa (Leblanc *et al*. 2019). The present molecular results are consistent with these findings and further confirm that *B. dorsalis*, *B. tau*, and *B. cucurbitae* remain the predominant pest species affecting major fruits and vegetables across the country.

The integration of molecular methods alongside morphological identification proved essential for resolving cryptic species and confirming pest status. COI barcoding provides a rapid, reliable, and universally applicable tool, overcoming the limitations of traditional taxonomic approaches and supporting accurate species-level identification for effective pest management (Hebert *et al*. 2003a, b, Greenstone 2006). Overall, the findings confirm that the fruit fly fauna of Bangladesh is dominated by *B. dorsalis*, *Z. tau*, and *Z. cucurbitae*, with emerging records of *B. scutellata*. Accurate identification of these species using DNA barcoding is crucial for updating species distribution maps, refining pheromone lure deployment, and developing sustainable fruit fly management strategies suited to local agro-ecological conditions.

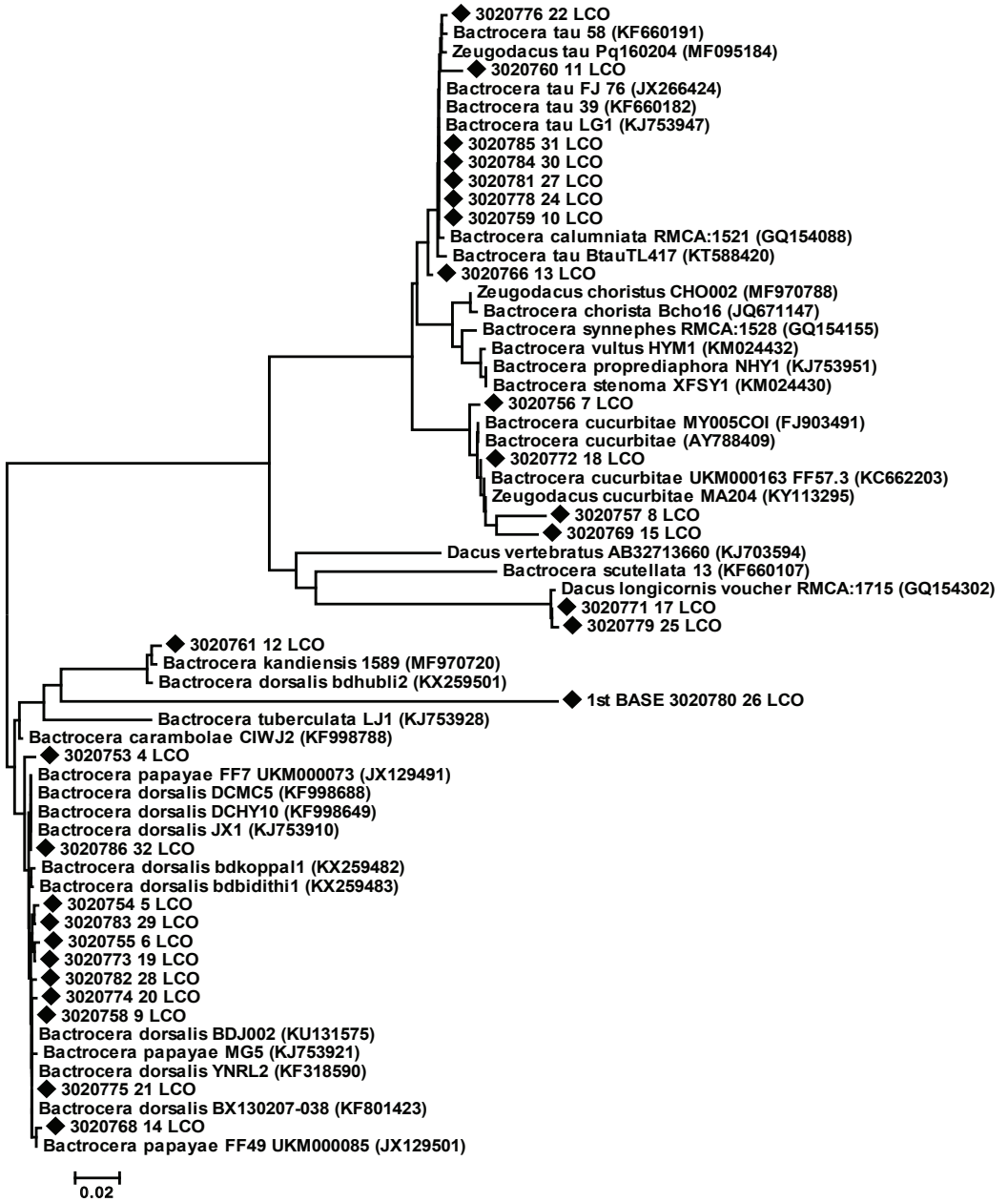

Fig. 1. Phylogenetic tree (traditional) based on mitochondrial Cytochrome Oxidase I (COI) gene sequences illustrating genetic relationships among *Bactrocera* species. The 27 isolates from this study are indicated by black diamonds. Bootstrap values from 1000 replicates are shown at the nodes.

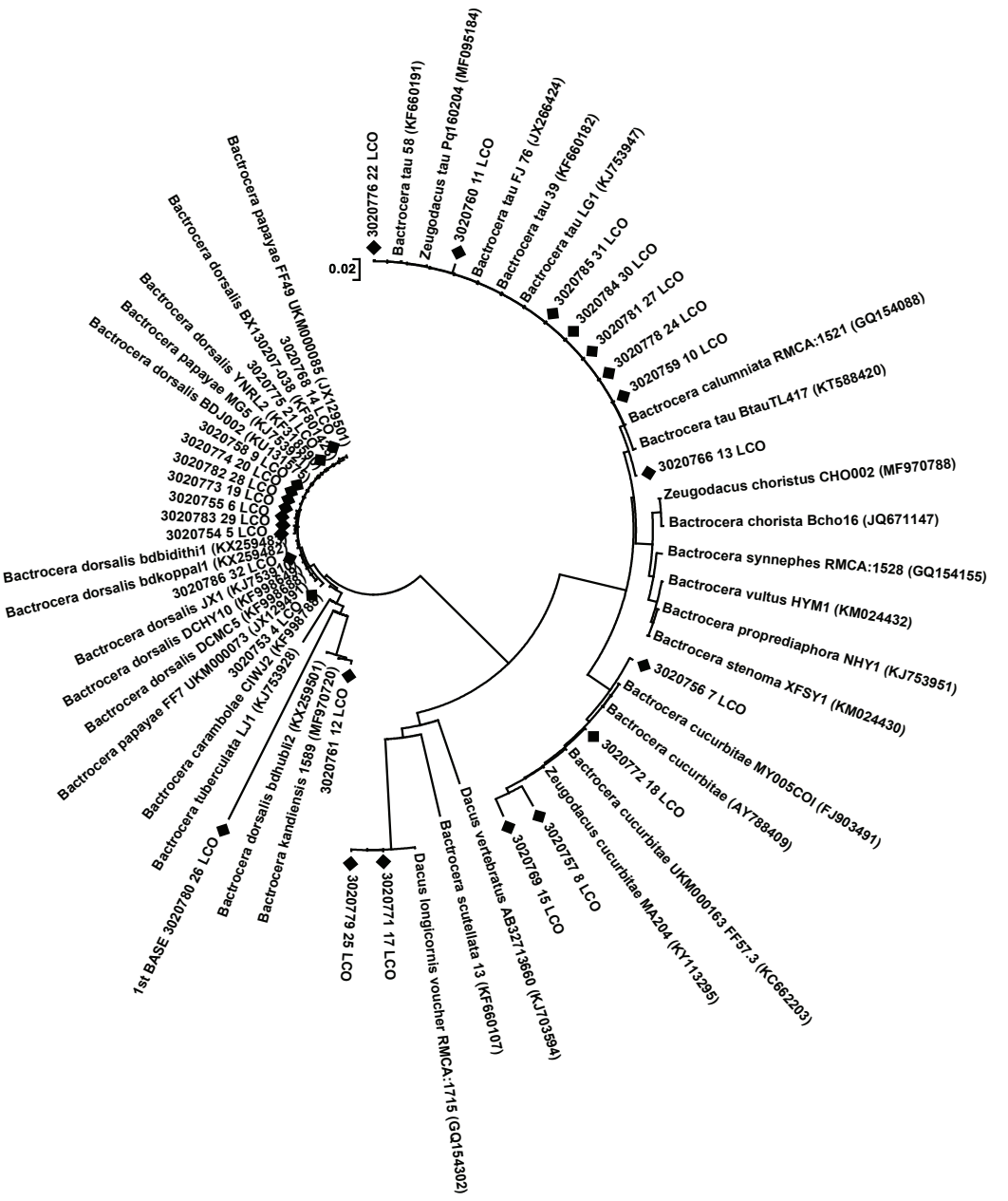

Fig. 2. Circular phylogenetic tree based on mitochondrial Cytochrome Oxidase I (COI) gene sequences showing the evolutionary relationships among *Bactrocera* species. The 27 isolates analyzed in this study are marked with black diamonds. Node support is indicated by bootstrap values from 1000 replicates.

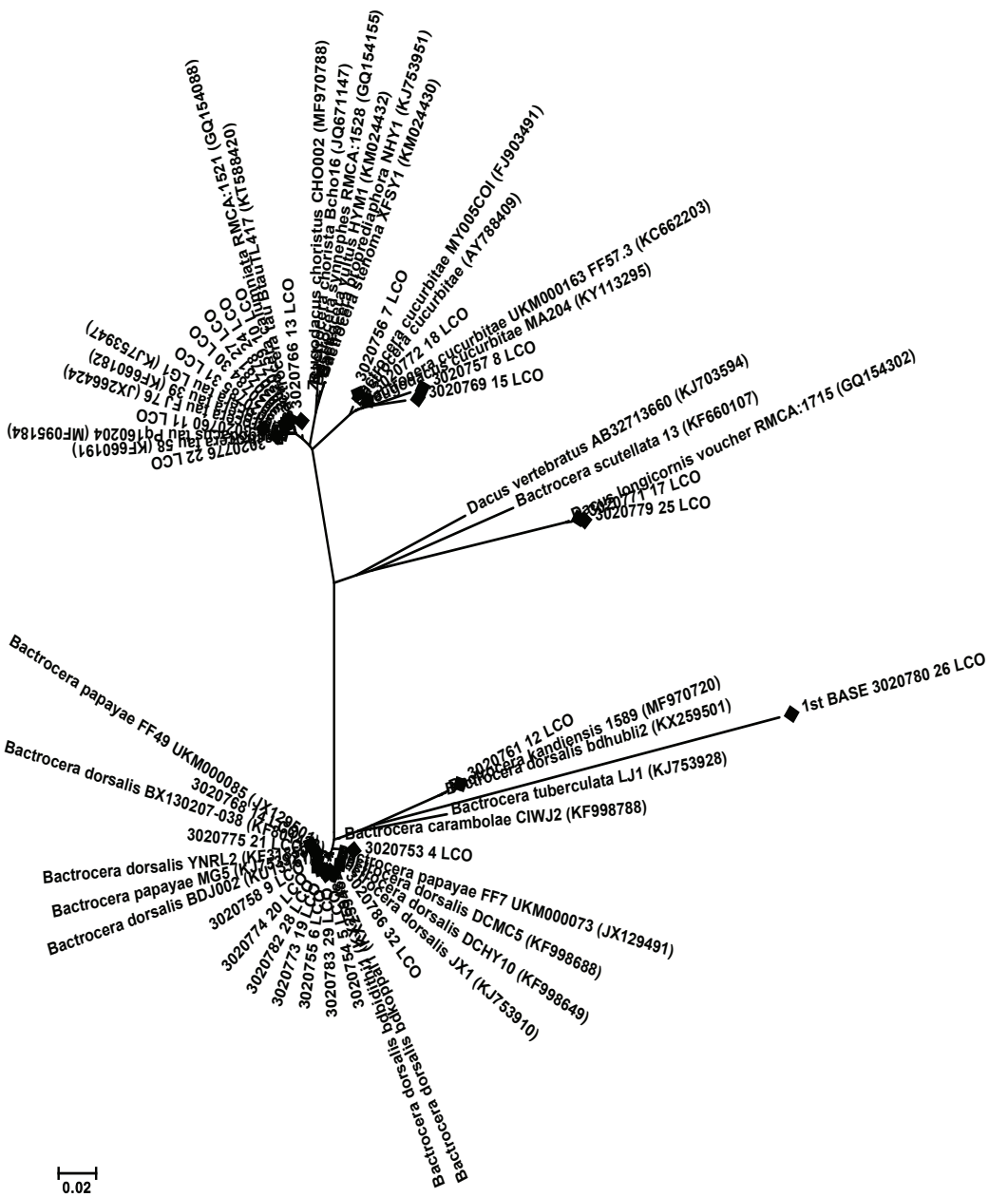

Fig. 3. Radial phylogenetic tree based on mitochondrial Cytochrome Oxidase I (COI) gene sequences depicting the phylogenetic relationships among *Bactrocera* species. The 27 isolates from this study are highlighted with black diamonds. Bootstrap support values from 1000 replicates are provided at each node.

CONCLUSION

This study provides a comprehensive assessment of fruit fly diversity, distribution, and host associations in Bangladesh. Morphological and molecular analyses confirmed that *Bactrocera dorsalis* and *B. cucurbitae (Zeugodacus cucurbitae)* remain the dominant pests in fruit and vegetable crops. The detection of *Bactrocera scutellata* represents the first confirmed record of this species in the country, indicating potential changes in fruit fly fauna and the need for ongoing monitoring. Misidentifications based on morphology underscore the importance of molecular identification methods for precise species determination. These findings offer essential insights for designing targeted and effective pest management strategies, including species specific interventions, and provide a foundation for future research on fruit fly ecology, host range, and integrated pest management in Bangladesh.

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