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# MOLECULAR IDENTIFICATION OF TWENTY SIX MOTH SPECIES BASED ON MITICHONDRIAL COI GENE SEQUENCES

Sijad Islam, Ibnul Saad Sakal, Muhammad Sohel Abedin, Ananna Ghosh[1], Fahmina Sarkar Borsha, Md. Khayrul Hasan, Md. Abdullah Al Mamun, Kawsari Akter, Surma Mohiudden Meem and Md. Monwar Hossain*

*DNA Barcoding Laboratory, Department of Zoology, Jahangirnagar University, Dhaka 1342, Bangladesh,*

**ABSTRACT:** The widespread use of the mitochondrial gene, Cytochrome c oxidase subunit I (COI), for molecular identification of moth species is both reliable and time-efficient. In the present study, a total of 26 moths from various regions of Bangladesh were collected, and their COI gene was sequenced. Subsequently, homology searches of these species at NCBI's GenBank revealed significant similarity across diverse countries of origin except for, *Fodina oriolus* and *Micronia aculeata*. We then submitted all COI gene sequences to Genbank, with the exception of two sequences, *F. oriolus* and *M. aculeata*, which marked the first submission in the Genbank database. We then used BioEdit and MEGA10 to identify a 582 bp COI gene fragment, which included 353 conserved sites, 229 variable sites, and 180 parsimony-informative sites. In the analysis, the average nucleotide composition showed 29.04% A, 41.06% T, 14.43% G, and 15.46% C, respectively. The value of A+T (70.10%) was greater than G+C (29.90%). On the other hand, the Kimura 2-Parameter algorithm calculated the genetic distances between the 26 moth species, revealing variations ranging from 0.051 to 0.197%. This result showed evidence of interspecies hybridization due to the low levels of interspecies divergence (0.051 to 0.197%). However, the DNA barcode dataset serves as a foundation for identifying moth species and establishing a comprehensive barcode reference library for moth pests in Bangladesh and neighbouring countries. Additionally, it supports integrated pest control efforts and contributes to larger ecological studies.

***Key words****:* COI, identification, genetic diversity, moth, Bangladesh

## INTRODUCTION

One of the most diverse and well-known insect order is Lepidoptera, which comprises butterflies and moths (Elanchezhian *et al.*, 2014). There are an estimated 174250 species of moths worldwide, belonging to 46 superfamilies

**Author for correspondence: monwar@juniv.edu; [1] Insect Biotechnology Division, IFRB, Atomic Energy Research Establishment, Dhaka, Bangladesh*
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and 126 families (Elanchezhian *et al.*, 2014). Moth's prime role as a pest is to cause damage to crops and forests. Besides, it is essential for their role in pollination (Hahn and Brühl 2016). The existence of food webs relies on the presence of this insect, which serves as a source of sustenance for birds, animals, and other insects (Mwansat *et al.*, 2015). They damage annual and perennial food and fiber crops, forest products, and stored food the most (Bibi *et al.*, 2022).

Bangladesh is home to a diverse and thriving population of moth species, and so far, over 200 species of moths have been reported (Ahmad *et al.*, 2009, Islam *et al.*, 2013, Neogi *et al.*, 2016, Mahadi *et al.*, 2021, Razzak *et al.*, 2022). The most authentic information comes from the Encyclopedia of Flora and Fauna of Bangladesh, where it mentions 74 species of moths from 53 genera and their geographical distribution in Bangladesh (Ahmad *et al.*, 2009). Moths inhabit all parts of Bangladesh, and the Sylhet and Chittagong divisions' mixed evergreen forest habitats exhibit the highest density and diversity (Neogi *et al.*, 2016). Industrialization and a growing population are creating rapid environmental change in Bangladesh. Invasive species, pesticide use, and urbanisation also affect Lepidopteran populations locally (Dar *et al.*, 2021). We must conduct surveys and studies to assess the current population state and habitats of these species to understand how human activity affects them and create effective management plans. An important limitation in controlling these pests involves species identification (Sethusa *et al.*, 2014). Insect identification relies on traditional taxonomy, which is primarily based on external morphology (Rebijith *et al.*, 2012). The taxonomic study revealed that there exists a huge morphological variation within the species that leads to a huge dilemma in identifying insects, especially moth pests (Ball *et al.*, 2006, Singh *et al.*, 2014). To address the difficulties associated with insect pest identification, a novel technique called DNA barcoding utilising the COI gene may be applicable in this situation (Hebert *et al.*, 2003, Hanner *et al.*, 2009, Rugman-Jones *et al.*, 2009, Quicke *et al.*, 2012, Sethusa *et al.*, 2014). Furthermore, DNA barcoding is gaining broad application in integrated pest management (IPM) programmes as the standard method for species identification (Ball *et al.*, 2006, Etzler *et al.*, 2014).

Several studies have been conducted on the molecular identification of moths from neighbouring countries (Kumar *et al.*, 2019, Yaakop *et al.*, 2020, Kalawate *et al.*, 2022), but Bangladesh has not yet seen any significant research, with the exception of a few instances (Ghosh *et al.*, 2018, Borsha *et al.*, 2019, 2021). This study was therefore aimed at investigating the molecular characterization and identification of 26 moth pests in Bangladesh through the establishment of a DNA barcode dataset.

## MATERIAL AND METHODS

*Field Collection and Preliminary Identification of Specimens***:** 26 moth specimens were collected from various parts of Bangladesh during the years 2017 to 2023 (Table 1). Detailed information on these collected moth specimens, along with the geographical coordinates of the different locations depicted in Table 1. Using an insect net, the insects were gently taken away from their wild environment and then preserved by desiccation in a small box. Then prepared voucher specimens were stored in the museum collection at DNA Barcoding Laboratory in the Department of Zoology at Jahangirnagar University campus. Further, we excised the hind legs of each specimen and subsequently preserved them in ethanol at -20 ◦C for molecular laboratory analyses at the DNA Barcoding Laboratory. Preliminary identification of moth species was carried out with the help of faunal reference works and online taxonomy resources (Hampson 1892-1896, Bell and Scott 1937, Robinson *et al.*, 1994, Kononenko and Pinratana 2005, Shubhalaxmi 2018 and Moths of India (https://www.mothsofindia.org/).

*DNA extraction, amplification and* sequencing: Genomic DNA from these moths was isolated from the legs of adult moths' specimens according to the procedure of the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Using the primers LCO 1490 (5'- GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5- TAAACTTCAGGGTGACCAAAAAATCA-3'), the mitochondrial cytochrome c oxidase 1 (COI) gene area was amplified using PCR in 20 µl of Q2 Green PCR Master Mix (Promega, Madison, WI, USA) in a thermal cycler (Veriti™, USA). The cycle parameters are as follows: an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, primer annealing at 49 °C for 30 sec, primer extension at 72 °C for 45 sec, and a final extension at 72 °C for 5 min. Electrophoresis on 1% agarose gel in the presence of UV light was used to determine whether the amplification was successful (BioDoc Analyze). The final PCR product was then cleanup by the Wizard SV Gel and PCR Clean-Up Kit (Promega, Madison, WI, USA). The amplification result was sequenced using an ABI 3500 sequencer. All sequences generated in this study are now available on GenBank (Table 1).

## RESULTS AND DISCUSSION

The current study involved the generation and submission of mitochondrial COI gene sequences (mtCOI) for 26 moth species belonging to 10 different families. The scientific names, geographical origin, accession number obtained from GenBank, and additional relevant information are detailed in

Table 1. The very first inclusion of *Fodina oriolus* and *Micronia aculeata* in the NCBI's GenBank was highlighted by the submission of these sequences. The 26 species belonged to 10 different Lepidopteran families. The Erebidae family comprises 14 species, the Crambidae, Saturniidae, and Sphingidae families each consist of two species, and the Noctuidae, Uraniidae, Geometridae, Nolidae, Eupterotidae, and Drepanidae families each comprise 1 species (Table 1).

**Table. 1: List of 26 moth taxa and their relevant information**

| Scientific name | Voucher | Geo Location | Accession No. | AT Content (%) | GC Content (%) | Base pair (bp) |
|---|---|---|---|---|---|---|
| **Family: Erebidae** | | | | | | |
| *Achaea janata* | MBV 0012 | 21°50'50.4"N 89°49'27.0"E | MN186874 | 70.1% | 29.9% | 658 bp |
| *Achaea serva* | MBV 0016 | 23°52'57.6"N 90°15'51.3"E | OQ660464 | 70.6% | 29.4% | 677 bp |
| *Plecoptera reflexa* | MBV 0015 | 23°52'39.4"N 90°15'54.9"E | OQ660462 | 70.1% | 29.9% | 668 bp |
| *Spirama retorta* | MBV 0014 | 23°52'31.9"N 90°15'55.2"E | OQ660319 | 70.9% | 29.1% | 684 bp |
| *Eudocima materna* | MBV 0013 | 23°48'18.5"N 90°22'32.9"E | MT626859 | 70.6% | 29.4% | 632 bp |
| *Creatonotos transiens* | MBV 0007 | 23°50'47.3"N 90°14'37.3"E | MK348954 | 69.5% | 30.5% | 649 bp |
| *Nyctemera coleta* | MBV 0009 | 25°17'06.0"N 89°55'22.1"E | MK719692 | 66.4% | 33.6% | 601 bp |
| *Erebus macrops* | MBV 0001 | 23°54'24.0"N 90°15'53.0"E | MH019973 | 71.5% | 28.5% | 618 bp |
| *Fodina oriolus* | MBV0008 | 24°19'40.1"N 91°46'59.9"E | PP101020 | 69.4% | 30.6% | 624 bp |
| *Lymantria atemeles* | MBV0024 | 23°52'32.9"N 90°15'54.0"E | PP100124 | 67.3% | 32.7% | 648 bp |
| *Argina astrea* | MBV0025 | 21°51'18.7"N 89°46'54.5"E | PP100148 | 69.7% | 30.3% | 657 bp |
| *Neochera dominia* | MBV0028 | 25°18'01.0"N 89°55'20.1"E | PP104398 | 68.5% | 31.5% | 660 bp |
| *Episparis tortuosalis* | MBV0026 | 22°30'34.3"N 92°12'30.0"E | PP100529 | 70.3% | 29.7% | 666 bp |
| *Ophiusa coronata* | MBV0021 | 23°53'07.6"N 90°16'09.6"E | OR915447 | 70.3% | 29.7% | 650 bp |
| **Family: Noctuidae** | | | | | | |
| *Episteme* sp. | MBV 0010 | 24°19'31.1"N 91°47'13.6"E | MK719693 | 69.8% | 30.2% | 646 bp |
| **Family: Uraniidae** | | | | | | |
| *Micronia aculeata* | MBV0023 | 22°41'24.1"N 91°38'20.4"E | PP106428 | 69.5% | 30.5% | 660 bp |
| **Family: Geometridae** | | | | | | |
| *Cleora* sp. | MBV0019 | 23°52'39.4"N 90°15'54.9"E | OQ672753 | 68.3% | 31.7% | 659 bp |
| **Family: Crambidae** | | | | | | |

| Scientific name | Voucher | Geo Location | Accession No. | AT Content (%) | GC Content (%) | Base pair (bp) |
|---|---|---|---|---|---|---|
| *Parotis atlitalis* | MBV0017 | 23°52'57.6"N 90°15'51.3"E | OQ660436 | 69.4% | 30.6% | 677 bp |
| *Glyphodes bivitralis* | MBV0027 | 22°30'34.3"N 92°12'30.0"E | PP100720 | 69% | 31% | 664 bp |
| **Family: Saturniidae** | | | | | | |
| *Attacus atlas* | MBV0002 | 21°51'18.0"N 89°46'54.8"E | MK014747 | 67.3% | 32.7% | 640 bp |
| *Actias maenas* | MBV0004 | 22°57'00.0"N 92°09'00.1"E | MH807560 | 67.1% | 32.9% | 630 bp |
| **Family: Sphingidae** | | | | | | |
| *Pergesa acteus* | MBV0020 | 23°53'07.9"N 90°16'11.9"E | OR915444 | 68.2% | 31.8% | 635 bp |
| *Eupanacra poulardi* | MBV0022 | 22°30'34.2"N 92°12'30.4"E | PP099868 | 68.9% | 31.1% | 666 bp |
| **Family: Nolidae** | | | | | | |
| *Westermannia superba* | MBV0018 | 23°52'39.4"N 90°15'54.9"E | OQ660441 | 71.1% | 28.9% | 619 bp |
| **Family: Eupterotidae** | | | | | | |
| *Eupterote undata* | MBV0006 | 23°50'47.1"N 90°14'37.0"E | MK348955 | 65.4% | 34.6% | 654 bp |
| **Family: Drepanidae** | | | | | | |
| *Cyclidia substigmaria* | MBV0011 | 24°19'37.9"N 91°47'05.6"E | MK719691 | 69.6% | 30.4% | 626 bp |

The species of an unknown moth specimen can be identified by comparing its COI gene sequences to a database of known moth COI sequences. This can be particularly useful in cases where the morphological features of the moth are not sufficient for identification, or when dealing with species complexes that are difficult to distinguish based on morphology alone (Liu *et al.*, 2014). In the present study, 26 moth species were submitted to NCBI's Genbank where two new sequences *Fodina oriolus* and *Micronia aculeata* will enhance the repository of NCBI's GenBank and will be valuable data for other researchers (Table.1, 3). We previously identified *F. oriolus* and *M. aculeata* based on their morphology and, after generating their COI genes, submitted them to Genbank (Fig. 1A,B). The morpho-taxonomical characteristics of these two species are as follows:

*F. oriolus*: The colour of the head and thorax is black; the shaft of the antennae and a band between their bases is white; abdomen orange. The black forewing possesses broad oblique medial white band, and the outer margin and cilia are grey. The hind wing is orange, the apical area is black, and there is an

elongate black patch near anal angle, as well as a black streak at anal angle. The wing expanse is 48 mm (Hampson, 1894).

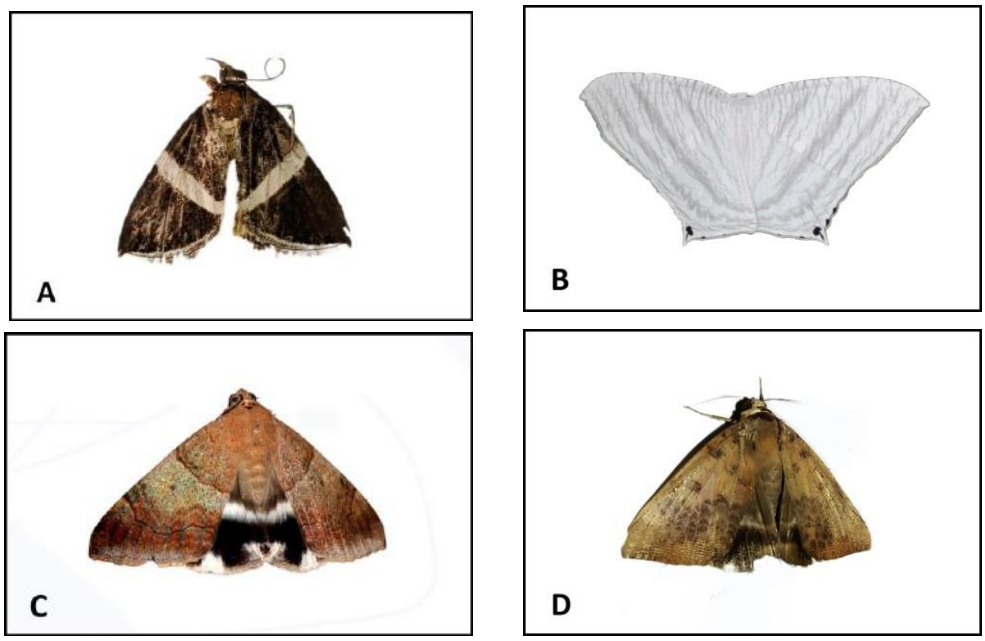


Fig. 1. GenBank received the first COI gene sequences data from *F. oriolus* (A) and *M. aculeata* (B); *A. janata* (C) and *A. serva* (D) show very close genetic makeup (0.051%).

*M. aculeata*: The adult is whitish, with darker striae on its wings. Forewing with three oblique bands. The hindwing is characterized by a tail on vein M3, a dark spot at its base, and a black margin dotted with small spots along the marginal line. The male genitalia have a strongly bifurcated uncus, a slightly bulbous tegumen at the base of the uncus, a vinculum that forms a small flat saccus, a very long juxta, valvae with a triangular costal edge, and a long, narrow aedeagus (Guenee 1857, Hampson, 1895).

Researchers can effectively use this dataset to identify moth pests that are currently significant in cultivated land and commercial food markets**.** COI gene can help in identifying pests at any stage of life, making it easier to control them and saving farmers from cost of billion dollars from pest damage (Kaur 2015, Sarvananda 2018). In addition to species identification, DNA barcoding can also be used for revealing cryptic species (i.e., species that are morphologically similar but genetically distinct), tracking the spread of invasive species, and monitoring changes in biodiversity over time (Lopez-Vaamonde *et al.*, 2021).

*Nucleotide analysis of COI gene*: The COI gene sequences were analyzed using BioEdit and MEGA10, and the result showed a 582 bp COI gene fragment

with 229 variable sites and 180 parsimony informative sites (Table 2). Most variations occurred at the second and third codon positions. Among informative sites, 48 were in the first position, 67 were in the second, and 65 were in the third position (Table 2). The mean base compositions of the COI sequences were 41.06% T, 15.46% C, 29.04% A, and 14.43% G, respectively. There was a strong AT bias (70.10%). The AT content of the first, second, and third codon positions of the COI fragment was 58.23%, 95.35% and 56.69% respectively (Table 2). Like other genes that code for proteins, most of the alterations were found at the third position of the codon, which is not supported by our current results (Table 2) (Win *et al.,* 2015). Nonetheless, the most of the alterations at the third position of the codon are not universal and hence, in the present study, most of the alterations were found at 2nd codon position (Phillips and Penny 2003, Simmons *et al.*, 2006). These findings highlight the intricate relationship between nucleotide composition, codon usage, protein function, and evolutionary dynamics in mitochondrial genomes. However, the parsimony informative is employed in molecular phylogenetic and evolutionary studies to elucidate diverse relationships among organisms.

**Table 2. Basic statistics of the COI gene sequences among 26 moth species**

| Position | No. of sites | No. of variable | Parsimony-informative sites | No. Conserved sites | Average base frequencies (%) | | | | AT % |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | T | C | A | G | |
| All Positions | 582 | 229 | 180 | 353 | 41.06 | 15.46 | 29.04 | 14.43 | 70.10 |
| First Position | 194 | 73 | 48 | 121 | 43.29 | 25.25 | 14.94 | 16.49 | 58.23 |
| Second Position | 194 | 76 | 67 | 118 | 55.15 | 4.63 | 40.20 | 00.00 | 95.35 |
| Third Position | 194 | 80 | 65 | 114 | 24.74 | 16.49 | 31.95 | 26.80 | 56.69 |

*Analysis of genetic divergence:* Using the Kimura-2 parameter (K2-P) model in MEGA, pairwise gene distances among 26 species of moths based on COI nucleotide sequences were estimated (Table. 3). After evaluating the sequences to determine genetic divergence and phylogenetic affinities, the interspecific genetic divergence among 26 species of moth ranged from 0.051 to 0.197%. The current research reveals a genetic difference of 0.051% between *Achaea janata* and *Achaea serva*, suggesting a close genetic makeup between these two species (Fig. 1 C,D). On the other hand, *Eupterote undata* and *Glyphodes bivitralis* had the greatest distance, 0.197%, and may have separated from a common ancestor further in the past (Table 3). This study found evidence of interspecies hybridization due to the low levels of interspecies divergence (0.051 to 0.197%)

**Table 3: Percentage pairwise distances among 26 moth species**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Spirama retorta* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Achaea serva* | 0.105 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Plecoptera reflexa* | 0.135 | 0.117 | | | | | | | | | | | | | | | | | | | | | | | |
| *Achaea janata* | 0.105 | 0.051 | 0.113 | | | | | | | | | | | | | | | | | | | | | | |
| *Eudocima materna* | 0.123 | 0.099 | 0.116 | 0.108 | | | | | | | | | | | | | | | | | | | | | |
| *Creatonotos transiens* | 0.135 | 0.115 | 0.123 | 0.111 | 0.147 | | | | | | | | | | | | | | | | | | | | |
| *Nyctemera coleta* | 0.152 | 0.131 | 0.130 | 0.139 | 0.145 | 0.141 | | | | | | | | | | | | | | | | | | | |
| *Erebus macrops* | 0.105 | 0.083 | 0.113 | 0.091 | 0.101 | 0.119 | 0.143 | | | | | | | | | | | | | | | | | | |
| *Fodina oriolus* | 0.133 | 0.108 | 0.117 | 0.119 | 0.117 | 0.133 | 0.150 | 0.127 | | | | | | | | | | | | | | | | | |
| *Lymantria atemeles* | 0.158 | 0.131 | 0.141 | 0.135 | 0.139 | 0.152 | 0.156 | 0.146 | 0.148 | | | | | | | | | | | | | | | | |
| *Argina astrea* | 0.164 | 0.127 | 0.127 | 0.133 | 0.127 | 0.143 | 0.135 | 0.125 | 0.141 | 0.137 | | | | | | | | | | | | | | | |
| *Neochera dominia* | 0.131 | 0.125 | 0.115 | 0.129 | 0.115 | 0.117 | 0.123 | 0.119 | 0.119 | 0.145 | 0.113 | | | | | | | | | | | | | | |
| *Episteme* sp. | 0.137 | 0.147 | 0.103 | 0.135 | 0.116 | 0.121 | 0.131 | 0.121 | 0.117 | 0.135 | 0.131 | 0.117 | | | | | | | | | | | | | |
| *Pergesa acteus* | 0.169 | 0.156 | 0.139 | 0.147 | 0.149 | 0.167 | 0.171 | 0.141 | 0.147 | 0.164 | 0.164 | 0.154 | 0.139 | | | | | | | | | | | | |
| *Episparis tortuosalis* | 0.112 | 0.123 | 0.121 | 0.120 | 0.112 | 0.133 | 0.145 | 0.114 | 0.129 | 0.133 | 0.135 | 0.111 | 0.095 | 0.156 | | | | | | | | | | | |
| *Cleora* sp. | 0.147 | 0.117 | 0.123 | 0.129 | 0.123 | 0.123 | 0.150 | 0.127 | 0.135 | 0.139 | 0.141 | 0.116 | 0.139 | 0.145 | 0.147 | | | | | | | | | | |
| *Micronia aculeata* | 0.147 | 0.129 | 0.135 | 0.131 | 0.154 | 0.169 | 0.169 | 0.121 | 0.160 | 0.189 | 0.141 | 0.162 | 0.162 | 0.173 | 0.158 | 0.177 | | | | | | | | | |
| *Parotis atlitalis* | 0.151 | 0.141 | 0.111 | 0.151 | 0.139 | 0.135 | 0.152 | 0.139 | 0.137 | 0.148 | 0.171 | 0.141 | 0.141 | 0.139 | 0.141 | 0.141 | 0.164 | | | | | | | | |
| *Glyphodes bivitralis* | 0.141 | 0.143 | 0.162 | 0.156 | 0.127 | 0.156 | 0.169 | 0.147 | 0.160 | 0.184 | 0.156 | 0.131 | 0.154 | 0.184 | 0.141 | 0.158 | 0.195 | 0.137 | | | | | | | |
| *Attacus atlas* | 0.127 | 0.131 | 0.106 | 0.117 | 0.133 | 0.129 | 0.150 | 0.121 | 0.131 | 0.158 | 0.156 | 0.129 | 0.113 | 0.139 | 0.111 | 0.143 | 0.169 | 0.145 | 0.158 | | | | | | |
| *Actias maenas* | 0.137 | 0.118 | 0.123 | 0.127 | 0.141 | 0.141 | 0.139 | 0.152 | 0.123 | 0.147 | 0.131 | 0.154 | 0.143 | 0.154 | 0.156 | 0.147 | 0.171 | 0.164 | 0.165 | 0.133 | | | | | |
| *Ophiusa coronata* | 0.105 | 0.089 | 0.108 | 0.083 | 0.109 | 0.127 | 0.156 | 0.101 | 0.118 | 0.154 | 0.141 | 0.113 | 0.139 | 0.141 | 0.116 | 0.118 | 0.127 | 0.139 | 0.150 | 0.115 | 0.145 | | | | |
| *Eupanacra poulardi* | 0.145 | 0.139 | 0.137 | 0.137 | 0.143 | 0.162 | 0.160 | 0.131 | 0.149 | 0.173 | 0.171 | 0.158 | 0.141 | 0.113 | 0.141 | 0.127 | 0.188 | 0.152 | 0.169 | 0.137 | 0.156 | 0.131 | | | |
| *Westermannia superba* | 0.131 | 0.112 | 0.111 | 0.119 | 0.107 | 0.129 | 0.165 | 0.103 | 0.115 | 0.141 | 0.129 | 0.127 | 0.111 | 0.131 | 0.115 | 0.119 | 0.133 | 0.129 | 0.141 | 0.117 | 0.123 | 0.109 | 0.149 | | |
| *Euptorote undata* | 0.164 | 0.152 | 0.182 | 0.173 | 0.162 | 0.158 | 0.165 | 0.180 | 0.184 | 0.182 | 0.173 | 0.175 | 0.171 | 0.204 | 0.168 | 0.184 | 0.164 | 0.195 | 0.197 | 0.169 | 0.174 | 0.162 | 0.188 | 0.164 | |
| *Cyclidia substigmaria* | 0.137 | 0.125 | 0.111 | 0.131 | 0.127 | 0.129 | 0.143 | 0.123 | 0.125 | 0.160 | 0.131 | 0.127 | 0.115 | 0.162 | 0.129 | 0.145 | 0.135 | 0.143 | 0.166 | 0.145 | 0.160 | 0.129 | 0.154 | 0.131 | 0.184 |

(Win *et al.*, 2015). Many insects are known to undergo interspecies hybridization (Zhao *et al.*, 2005, Wu *et al.*, 2021). In this connection, two Danaied species, *D. chrysippus* and *D. genutia* are reported for interspecific mating (Baidya *et al.*, 2018). However, it's important to note that successful mating and production of viable offspring may not always happen, as there can be genetic and behavioral barriers to interbreeding (Singh *et al.*, 2020).

There has not been much research done on moth species in Bangladesh, and so far 200 species have been documented by researchers (Ahmad *et al.*, 2009, Islam *et al.*, 2013, Neogi *et al.*, 2016, Mahadi *et al.*, 2021, Razzak *et al.*, 2022). In Bangladesh, the process of COI gene-based identification of butterflies has already commenced, resulting in the collection of more than 100 species of gene sequences (Ghosh *et al.*, 2018, Hossain *et al*, 2022, Akter *et al*,. 2023, Meem *et al.*, 2023). However, the identification of moths using the COI gene is still relatively limited (Borsha *et al.*, 2019, 2021). In the present study, we collected moths during 2017-2023 from different areas of Bangladesh to identify moth species based on COI gene sequences. Among them, *Attacus atlas* and *Achaea janata* were collected from the Sundarbans mangrove forest, which is a popular Ramsar site of Bangladesh. The *A. atlas* is one of the largest

Lepidopteran with a wingspan measuring up to 24 cm and a wing surface area of about 160 $cm^2$ (Holloway 1987). Moreover, the silks that were produced by *A. atlas* larvae were alternatively severed for commercial silk production (Reddy *et al.*, 2013). These species share a number of traits, seasonal variations and numerous abundances. Because of this, it may be difficult and time-consuming to identify them morphologically, and hence COI gene based identification is more precise in this case.

The moth species, their larval stage, and in some cases adults are destructive to forest plants, causing damage by boring stems, fruits, seeds, roots and defoliating and mining leaves of forest trees (Sathe and Pandharbale 2006). To effectively manage pests in the forest and minimize crop damage, it is essential to identify possible pest species. Moths and other pests destroy millions of tons of crops every year (Jacobs and DeJong 2014). The present studies could aid in the creation of more efficient pest management techniques by precisely identifying moth species using DNA barcoding. Also, identifying beneficial moth species can help guide conservation initiatives designed to maintain biodiversity and the health of ecosystems. This study demonstrates the importance of utilizing molecular techniques for identifying moths and emphasizes the need to comprehend the range of moth species in Bangladesh for effective pest control and conservation initiatives. Additionally, it establishes baseline molecular data for the COI gene for future research, which is an important step in this field.

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## CONCLUSION

This study generated and submitted 26 COI gene sequences from 26 moth species to Genbank, with the exception of *F. oriolus* and *M. aculeata*, which were the first submissions to Genbank. BIOEdit and MEGA10 were used to find a 582 bp COI gene fragment and describe its nucleotide composition. As for the genetic distances between the 26 moth species, the K2-P analysis found variances from 0.0517 to 0.1979%. This low genetic divergence suggests that these species are closely related and can interbreed, leading to interspecies hybridization. This hybridization results in genetic exchange and affects the evolutionary dynamics and classification of these moth species. Finally, the DNA barcode information from the current research assists in identifying moth species and establishing a reference library for moth pests in Bangladesh.

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## LITERATURE CITED

AHMAD, M., KABIR, S.M.H., AHMED, A.T.A., RAHMAN, A.K.A., AHMED, Z.U., BEGUM, Z.N.T., HASSAN, M.A. and KHONDKER, M. (eds.). 2009. Encyclopedia of Flora and Fauna of Bangladesh, Vol. 21. Pterygota (Part III). Asiatic Society of Bangladesh, Dhaka. 460 pp.

AKTER, K., MEEM, S.M., ISLAM, S., MAMUN, M.A.A., ABEDIN, M.S., BORSHA, F.S., GHOSH, A and HOSSAIN, M.M. 2023. Utilizing COI gene for the identification of thirteen Hesperiidae butterflies and determining their genetic relationship. *J. Biodivers. Conserv. Bioresour. Manag.* **9**(2): 21-28.

BAIDYA, S., BASU, D.N., ROY, S. and ROY, A.B. 2018. Occurrence of Interspecific Mating between Two Species of Danaus Kluk, 1780 (Lepidoptera: Nymphalidae) in Nature. Psyche. **3059017**: 1-5 pp.

BALL, S.L. and ARMSTRONG, K.F. 2006. DNA barcodes for insect pest identification. Canadian *J. Forest Res.* **36**(2): 337-350.

BELL, T.R.D. and SCOTT, F.B. 1937. The Fauna of British India, including Ceylon and Burma, Moths. Vol. V. Sphingidae, Taylor & Francis Ltd., London. 1-537 pp.

BIBI, M., BIBI, S., AKHTAR, N., ULLAH, Z., KHAN, F.M. and QURESHI, I.Z. 2022. Butterfly (Order: Lepidoptera) species Richness, diversity and distribution in different localities of Battagram, Pakistan. *Saudi Journal of Biological Sciences*. **29**(3): 1853–1857.

BORSHA, F.S., HASAN, M.K., GHOSH, A., HOWLADER, A.J. and HOSSAIN, M.M. 2019. DNA barcoding of *Achaea janata* (Lepidoptera: Erebidae), a moth pest from the Sundarbans mangrove forest, Bangladesh. 21st International Biennial Conference and AGM of the Zoological Society of Bangladesh. 101 pp.

BORSHA, F.S., HASAN, M.K., GHOSH, A., HOWLADER A.J. and HOSSAIN, M.M. 2021. Molecular identification and phylogenetic relationship of a moth, *Eudocima materna* from Bangladesh. 22nd National Conference and AGM 2020 of the Zoological Society of Bangladesh. 77 pp.

DAR, S.A., ANSARI, M.J., NAGGAR, Y.A., HASSAN, S., NIGHAT, S., ZEHRA, S.B., RASHID, R., HASSAN, M. and HUSSAIN, B. 2021. Causes and Reasons of Insect Decline and the Way Forward. Intech Open eBooks. 1-22 pp.

ELANCHEZHIAN, M., GUNASEKARAN, C. and DEEPA A.A. 2014. A Study on Moth Diversity in Three Different Habitats of Maruthamalai Hill, Western Ghats, South India. **3**(12): 136-138.

ETZLER, F.E., WANNER, K.W., MORALES, R.A. and IVIE, M.A. 2014. Barcoding to improve the species-level management of wireworms (Coleoptera: Elateridae). *J. Econ. Entomol.* **107**(4): 1476- 1485.

GHOSH, A., SULTANA, S., HOSSAIN, M.M. and HOWLADER, A.J. 2018. Molecular phylogenetic relationship of a moth, *Actias maenas* (Lepidoptera: Saturinidae) from Rangamati, Bangladesh. 21st National Conference and AGM of the Zoological Society of Bangladesh. 81 pp.

HANNER, R.H., LIMA, J. and FLOYD, R. 2009. DNA barcoding and its relevance to pests, plants and biological control. *Acta Hortic.* **823**: 41-48.

HAHN, M. and BRÜHL, C.A. 2016. The importance of moths for biodiversity and ecosystem functioning. Insects, **7**(4): 30.

HAMPSON, G.H. 1892-1896. Fauna of British India including Ceylon and Burma: Moths, Vols. I-IV, Taylor & Francis, London.

HEBERT, P.D.N., CYWINSKA, A., BALL, S.L. and. deWAARD, J.R. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B. Biol. Sci.* **270**: 313-321.

HOLLOWAY, J.D. 1987. The moths of Borneo, Part 3*: Lasiocampidae, Eupteroptidae, Bombycidae, Brahmaeidae, Saturniidae, Sphingidae*. Malayan Nature Society: Southdene Sdn. Bhd, Kuala Lumpur. 199 pp.

HOSSAIN, M.M., GHOSH, A., BORSHA, F.S., HASAN, M.K., ABEDIN, M.S. and HOWLADER, A.J. 2022. Molecular phylogeny of swallowtail butterflies (Lepidoptera: Papilionidae) based on mitochondrial cytochrome c oxidase I (COI) gene from Bangladesh. *Nepalese Journal of Zoology*. **6**(S1):1–6.

ISLAM, A.T.M.F., ISLAM, M.H., SAIFULLAH, A.S.M. and YAMANAKA, A. 2013. A preliminary report of moth's fauna in the campus of Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh. *International Journal of Fauna and Biological Studies.* **1**(1): 56-62.

JACOBS, B.S. and DEJONG, T.M. 2014. Tree Fruits and Nuts. Elsevier eBooks. 303-314.

KALAWATE, A.S., SHABNAM, A. and DINESH, K.P. 2022. First Indian DNA barcode record for the moth species Pygospila tyres (Cramer, 1780) (Lepidoptera: Crambidae: Spilomelinae) distributed in Asia and Australia. *Journal of Threatened Taxa.* **14**(2): 20637–20642.

KAUR, S. 2015. DNA barcoding and its applications. *Int. J. of Eng. Res. and Gen. Sci.* **3**(2): 602-604.

KONONENKO, V.S. and PINRATANA, A. 2005. Moths of Thailand 3: Noctuidae (Part 1). Brothers of St. Gabriel in Thailand, Bangkok. 261 pp.

LIU, X.F., YANG, C.H., HAN, H.L., WARD, R.D. and ZHANG, A. 2014. Identifying species of moths (Lepidoptera) from Baihua Mountain, Beijing, China, using DNA barcodes. Ecology and Evolution. **4**(12): 2472– 2487.

LOPEZ-VAAMONDE, C., KIRICHENKO, N., CAMA, A., DOORENWEERD, C., GODFRAY, H.C.J., GUIGUET, A., GOMBOC, S., HUEMER, P., LANDRY, J-F., LAŠT ˚UVKA, A., LAŠT ˚UVKA, Z., LEE, K.M., LEES, D.C., MUTANEN, M., VAN NIEUKERKEN, E.J., SEGERER, A.H., TRIBERTI, P., WIESER, C. and ROUGERIE, R. 2021. Evaluating DNA Barcoding for Species Identification and Discovery in European Gracillariid Moths. *Front. Ecol. Evol.* **9**: 626752.

MAHDI, S.H.A., FERDOUS, M., KHALED, S.S., YESMIN, F., LOTIFUNNESA. and RAHIM, M.A. 2021. First report on checklist, species abundance, seasonal distribution and diversity of moth fauna (Lepidoptera: Heterocera) in Rajshahi University Campus (Ruc), Bangladesh. *International Journal of Entomology Research.* **6** (1): 51-57.

MEEM, S.M., AKTER, K., MAMUN, M.A.A., ISLAM, S., BORSHA, F.S., ABEDIN, M.S and HOSSAIN, M.M. 2023. DNA barcoding and phylogenetic relationships of ten butterfly caterpillars. *Asian Australas. J. Biosci. Biotechnol.* **8** (3): 49-55.

MOTHS OF INDIA. (https://www.mothsofindia.org, Accessed on 22.03.2024).

MWANSAT, G.S., TURSHAK, L.G. and OKOLIE, M.O. 2015. Insects as important delicacy for birds: Expanding our knowledge of insect food ecology of birds in the tropics. *Journal of International Scientific Publications*. **9**: 434-441.

NEOGI, A.K., ABBAS, S., BRUNO, A., ISLAM, M.S., SMETACEK, P., ALIM, M.A. and MONDAL, A.C. 2016. Inventory of moth fauna (Lepidoptera) from five districts of Bangladesh. 20th National Conference and Annual General Meeting. Zoological Society of Bangladesh. Dhaka, Bangladesh. 54 pp.

PHILLIPS, M.J. and PENNY, D. 2003. The root of the mammalian tree inferred from whole mitochondrial genomes. Mol. *Phylogenet. Evol.* **28**: 171-185.

QUICKE, D.L., ALEX S.M., JANZEN, D.H., HALLWACHS, W., FERNANDEZ-TRIANA, J., LAURENNE, N.M., ZALDIVAR-RIVERON, A., SHAW, M.R., BROAD, G.R. and KLOPFSTEIN, S. 2012. Utility of the DNA barcoding gene fragment for parasitic wasp phylogeny (Hymenoptera: Ichneumonoidea): Data release and new measure of taxonomic congruence. *Mol. Ecol. Resour*. **12**: 676-685.

RAZZAK, M.A., SHAMIM, M.I.A., ISLAM, R., ISLAM, R., RAHMAN, K.M.Z., BASHAR K. and ISLAM A.T.M.F. 2022. A preliminary checklist of moths (Lepidoptera: Heterocera) of Jahangirnagar University campus, Savar, Dhaka, Bangladesh. *Journal of Entomology and Zoology Studies*. **10**(5): 397-404

REBIJITH, K.B., ASOKAN, R., KRISHNA, V.N.K., KRISHNA, K. and RAMAMURTHY, V.V. 2012. DNA Barcoding and development of species-specific markers for the identification of tea mosquito bugs (Miridae: Heteroptera) in India. *Fla Entomol.* **95**(3): 674-682.

REDDY, N., ZHAO, Y. and YANG, Y. 2013. Structure and properties of cocoons and silk fibers produced by *Attacus atlas*. *J. Polym Environ*. **21**(1): 16–23.

ROBINSON, G.S., TUCK, K.R. and SHAFFER, M. 1994. A Field Guide to the Smaller Moths of South-east Asia. Malaysian Nature Society, Kuala Lumpur & The Natural History Museum, London. 309 pp.

RUGMAN-JONES, P.F., ROBERT, W., TOM, van NOORT and RICHARD, S. 2009. Molecular differentiation of the Psyttaliaconcolor (Szépligeti) species complex (Hymenoptera: Braconidae) associated with olive fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae), in *Africa. J. Biol. Control*. **49**(1): 17-26.

SATHE, T.V. and PANDHARBALE, A.R. 2006. Forest Pest Lepidoptera. Manglam Publishers and Distributors, Delhi, India. 186 pp.

SIMMONS, M.P., ZHANG, L., WEBB C.T. and REEVES, A. 2006. How Can Third Codon Positions Outperform First and Second Codon Positions in Phylogenetic Inference? An Empirical Example from the Seed Plants. *Syst. Biol.* **55**(2): 1245-258.

SETHUSA, M.T., MILLAR, I.M., YESSOUFOU, K., JACOBS, A., VAN DER B.M. and VAN DER B.H. 2014. DNA barcode efficacy for the identification of economically important scale insects (Hemiptera: Coccoidea) in South Africa. *African J. Zool.* **22**(1): 257-267.

SHUBHALAXMI, V. 2018. Birdwing Field Guide to Indian Moths. Birdwing Publishers. 474 pp.

SINGH, B.B. and SINGH, R. 2014. Major rice insect pests in north eastern UP. Int. *J. Life Sc. Bt & Pharm.* **3**(1): 124-143.

SINGH, P., BALLMER, D., LAUBSCHER, M. and SCHÄRER, L. 2020. Successful mating and hybridisation in two closely related flatworm species despite significant differences in reproductive morphology and behaviour. Scientific Reports. **12830**: 1-16.

SARVANANDA, L. 2018. Short introduction of DNA barcoding. *Int. J. Res.* **5**(4): 673-685.

WIN, N.Z., CHOI, E.Y., JANG, D.J., PARK, J. and PARK, J.K. 2015. Molecular comparison of the genus *Junonia* (Lepidoptera:Nymphalidae) in Myanmar. *Journal of Asia-Pacific Biodiversity* **8**(4): 287-294.

WU, H., CAO, L., HE, M., HAN, R. and DE CLERCQ, P. 2021. Interspecific Hybridization and Complete Mitochondrial Genome Analysis of Two Ghost Moth Species. Insects, **12**: 1046.

YAAKOP, S., DAVID-DASS, A., SHAHARUDDIN, U.S., SABRI, S., BADRULISHAM A.S. and CHE-RADZIAH, C.M.Z. 2020. Species Richness of Leaf Roller and Stem Borers (Lepidoptera) Associated with Different Paddy Growth and First Documentation of Its DNA Barcode. *Pertanika J. Trop. Agric. Sci.* **43** (4): 523–535.

ZHAO, X.C., DONG, J.F., TANG, Q.B., YAN, Y.H., GELIBIC, I., VAN LOON, J.J.A. and WANG, C.Z. 2005. Hybridization between *Helicoverpa armigera* and *Helicoverpa assulta* (Lepidoptera: Noctuidae): Development and morphological characterization of F1 hybrids. *Bull. Entomol. Res.* **95**: 409–416.

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