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Molecular Identification of Dysphania Militaris, Tirumala Septentrionis and Euploea Core based on Mitochondrial COI Gene

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Abstract

Butterflies and moths belong to the second largest insect order, Lepidoptera, enclosing 46 super families and 126 families. Out of 15000 species of butterflies recorded globally, India harbours 1500 species, of which 316 species are found in Kerala. The morphological studies of *Dysphania militaris* (Family: Geometridae), *Tirumala septentrionis* (Family: Nymphalidae), *Euploea core* (Family: Nymphalidae) enabled the identification of the specimens up to species level and molecular confirmation was done by the analysis of mitochondrial CO1 gene. Genomic DNA, isolated from leg tissue extracts using Genomic DNA extraction kit was amplified using PCR specifically for the Mitochondrial encoded cytochrome c oxidase I gene sequences (MT- CO I). The amplified product was undergone Agarose gel electrophoresis, visualized under UV-trans-illuminator and later gel elution was conducted. The sequences were analysed using COI gene specific forward and reverse primers of insect. The BLAST results

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confirmed similarity with *Dysphania militaris*, *Tirumala septentrionis* and *Euploea core*.

Keywords: Molecular identification, mitochondrial CO I gene, Dysphania militaris, Tirumala septentrionis, Euploea core.

Introduction

Butterflies play important ecological roles in pollination, food webs, and biodiversity, and they also have educational and cultural significance. Protecting butterfly habitats and conserving butterfly populations is important for maintaining healthy ecosystems and the many benefits they provide. The biodiversity counts to 1.4 million species, of which 0.53 are insects. Among which, about 15000 species houses the most common and broadly recognized insect order Lepidoptera. Order Lepidopterans comprises of moths, butterflies, skippers, under 46 super families and 126 families. Lepidoptera usually have four well developed wings covered with overlapping scales. Butterflies and moth, major group of organisms in Lepidoptera, differ in their biological and ecological features. The former has colourful thin and smooth body while the later are dull coloured and thick bodied. The antennae of butterflies seem to be in rounded clubs on the end whereas the antennae of moths are feathery and thin. Butterflies are active during daylight, but moths are nocturnal. The resting posture of wings are vertical in butterflies. In contrary, the wings are held flat against body while resting in moths. Geometridae, Crambidae. Noctuidae, Sphingidae are few families of moths. Nymphalidae, Hesperiidae, Lycaenidae, Papilionidae are few families of butterflies. Nymphalidae is the largest family of butterflies with more than 7000 species and Noctuidae is the largest family among moths.

Species identification and description are important in biology. Identification using molecular data has revolutionised taxonomy as they bring about precise characterisation of species. Analysing of molecular information aids in identification of cryptic species, which is infeasible with traditional taxonomy. The extension of molecular taxonomy led to DNA barcoding, a cost efficient and reliable identification method. MT-CO1 gene segments are generally used for barcoding as their sequences are highly conserved among related

species. Moreover, CO I gene sequence decipher evolutionary relationships.

Based on the published study of combined analysis of DNA sequences from three genes, morphological data for 57 taxa representing all major lineages from the three super families of butterflies (Hedylidea, Hesperiidea, and Papilionoidea) as well as outgroups representing other lepidopteran families were taken into consideration for sequencing. This was the first phylogenetic hypothesis for butterflies and skippers that had strong evidence from both molecular and morphological characteristics. A nearly similar genus was sequenced for this investigation. Wingless (403 bp), Elongation factor 1 alpha, and Cytochrome oxidase subunit 1 (CO1, 1531 bp). Numerous unusual relationships are found as a consequence of the data analysis. The monophyly of all conventionally recognised higher taxa is strongly supported by the study of the combined molecular and morphological information. Additionally, it supports the theory that the skippers and butterflies are more closely related to the Hedylidae than they are to the geometrids in which they were formerly placed.[1].

Euptychia butterflies are the most species rich subtribe of Neotropical Satyrine.[2] The morphological characters were used to examine the phylogenetic relationship within Euptychia [3]. For this, 103 morphological attributes taken from the adult phase, 45, 48, 10 from wing pattern, genitalia, wing venation respectively. Maximum Parsimony was used to examine the resulting data matrix under both equal and extended implied weights. The data results are contrasted with molecular phylogenies that were previously accessible. All the analysis recovered Euptychia as monophyletic [4]. This study shows that morphological data are largely in agreement with molecular data, therefore provides the mean to identify synapomorphies for major clades and it is an incentive for future research not only for Euptychia but also for the other insect groups [5]."

The most widely utilised molecular diversity marker is mitochondrial DNA. As mitochondrial DNA is relatively easy to amplify, strongly conserved, with very little duplication, no intron and a very short intergenic region, this is been used for genetic identification of species [6]. Lepidopteran recent divergence events

have been resolved using the mitochondrial CO1 gene, especially at the genus and species level. It is useful in fully resolving topologies within species of Nymphalidae [7] and also the precision with which the chosen DNA sequence's intraspecific variation and interspecific divergence can be distinguished. The use of the CO1 as a barcode to identify animals in which only 648bp of the mitochondrial CO1 gene near its 5' end was used [8]. The advantage of CO1 is that it is short enough to identify variation among species [9].

Since ancient times, a wide range of biological research topics, including evolution, genetics, mimicry, population dynamics, and biodiversity conservation, have been studied using butterflies as model animals. They also act as an important connection in a thriving ecosystem web chain. The morphological characters are agreeable with the mitogenomic data in resolving the phylogeny of Nymphalid butterflies and compared them with other nymphalid mitogenes [10]. Therefore, all the genes identified are similar to the mitochondrial genes with normal sizes.

Molecular identification and phylogenetic relationships of seven satyrinae butterflies in Bangladesh using cytochrome C Oxidase subunit 1 gene was studied [11]. When they compared the sequences to related satyrinae species sequences that had previously been deposited in the NCBI gene library, they found 97 to 100 similarities, indicating that the identification of these Bangladeshi satyrinae species was accurate. The analysis of Euptychia came out as monophyletic [12]. This work provides the mean to determine synapomorphies for major clades by demonstrating that morphological data are mainly consistent with genetic data. Studies observed Cytochrome C Oxidase subunit 1 gene based phylogenetic description of common Mormon butterfly, as that the best phylogenetic inferences to be created through moderately divergent nucleotide data from mitogenomes [13]. The analysis of the species belonging to the subfamily Nymphalidae was performed using partial sequences of the COI gene region compared with existing data and constructed a phylogenetic tree including several clades [14]. The goal of the current work is to accurately identify species using DNA barcoding using the mitochondrial gene Cytochrome C Oxidase (CO1), and to validate butterfly species through BLAST DNA sequence analysis.

Materials and Methods

Sample Collection

Dysphania militaris, Tirumala septentrionis and *Euploea core* were collected, preserved in 70% ethyl alcohol were used for the isolation of DNA. Specimens of approximately same size were freshly collected from St. Joseph's College, Devagiri, premises, Calicut.

DNA Isolation

Preparation of Sample

A 1.5ml microcentrifuge was filled with 25mg of tissue that had been chopped into small pieces. The tissue was centrifuged for one minute at 12,000 rpm. After disposing of the flow-through, resuspend the cell pellet in 200µL of buffer GA. After adding 20µL of Proteinase K, everything was well combined by vortexing. until the tissue is fully lysed, the same was incubated at 56°C. 200µl of Buffer GB was added to the sample, and it was thoroughly mixed by vortexing. Afterwards, it was incubated for 10 minutes at 70°C to produce a uniform solution. The 1.5 ml microcentrifuge tube was briefly centrifuged to extract the droplets from the interior of the lid. 200µl of ethanol (96-100%) was added to the sample, and it was well mixed for 15 seconds by vortexing. When ethanol is added, a white precipitate could occur. The 1.5 ml microcentrifuge tube was briefly centrifuged to extract droplets from the interior of the lid. The mixture was pipetted into a 2 ml collecting tube and centrifuged for 30 seconds at 12,000 rpm in the Spin Column CB3. After disposing of the flow-through, insert the spin column into the collection tube. 500µl of Buffer GD was added to spin column CB3, and after 30 seconds of centrifuging at 12,000 rpm, the flow-through was disposed of and the spin column was placed into the collecting tube. After adding 700µl of Buffer PW to spin column CB3, the centrifuge was run for 30 seconds at 12,000 rpm. Placed the spin column within the collection tube and disposed of the flow-through. 500µl of Buffer PW was added to spin column CB3, and it was centrifuged for 30 seconds at 12,000 rpm. inserted the spin column into the collecting tube and disposed of the flow-through. Centrifuged for two minutes at 12,000 rpm to thoroughly dry the membrane. After putting the

spin column CB3 in a fresh, clean 1.5 ml microcentrifuge tube, pipette 50-200µl of distilled water or Buffer TE straight to the membrane's centre. After letting it sit at ambient temperature (15-25°C) for two to five minutes, centrifuged it for two minutes at 12,000 rpm.

Amplification

Using the forward primer 5'3' and the reverse primer 5'3', the COI gene-containing region of the mitochondrial DNA genome was amplified. In a PCR tube, 25µL of Emerald Amp GT Master mix (Takara), 2µL of COI forward and reverse primer, 20µL sterile water, and 1µL DNA sample (50ng/ml) were combined to create a reaction mixture under cold conditions. The PCR reaction was applied to the DNA samples. There was a five-minute initial denaturation at 95°C, and then 35 cycles of denaturation at 95°C for ten seconds each. The cycle was finished with primer annealing at 54°C for 45 seconds, primer extension at 72°C for 45 seconds, and final elongation at 72°C for three minutes. The temperature was maintained at 4°C.

Electrophoresis

The PCR product was confirmed by Agarose gel electrophoresis using a 1.5% gel prepared in 1X TAE buffer (EtBr) and visualization of the bands were observed under UV trans-illuminator. The size of the product can be estimated with the help of a 1 Kb base pair ladder.

Gel elution

The DNA fragment-containing gel slice was excised using a clean scalpel. To reduce the gel volume, the cut was made as close to the DNA as possible. After preweighing the 2 ml tube, insert the gel slice into it and weigh it. The weight of the gel slice was noted, and binding buffer (volume: weight=1:1) was added. Until the gel slice was entirely dissolved, the gel mixture was incubated at 55°C. To speed up the melting process, flip the tube every few minutes. After quickly vortexing the gel combination, the solubilized gel solution was moved to the Gene JET purification column. centrifuged at 10,000 rpm for one minute. Re-inserted the column into the same collection tube and disposed of the flow-through. To the GeneJET purification column, 700µl of wash buffer was added. After

centrifuging for one minute, the column was put back into the original collection tube and the flow-through was disposed of. The empty GeneJET purification column was centrifuged and placed into a sterile 1.5 ml micro centrifuge tube. centrifuged for one minute after adding 20 20µL of elution buffer to the purification column membrane's centre. The pure DNA was kept at -20°C and the GeneJET purification column was disposed of.

Sequencing of the Gene

Sequencing of the sample was done at Sci-Genom, Kakkanad, using COI gene specific forward and reverse primers of insect.

Sequence Analysis

The sequences for the CO I gene of insect species were obtained. The ends of the sequence were trimmed and aligned using MEGA 10 (Molecular Evolutionary Genetic Analysis) software.

Analysis through BLAST

The sequence was analysed using the BLAST Bioinformatics tool for comparing the species

Results and Discussion

Dysphania militaris, Tirumala septentrionis, Euploea core were sampled for the study. *Dysphania militaris* is a moth, belonging to family Geometridae, were as *Tirumala septentrionis, Euploea core* are butterflies belonging to family Nymphalidae.

Dysphania Militaris

Phylum: Arthropoda

Class: Insecta

Order: Lepidoptera

Family: Geometridae

Carl Linnaeus originally described *Dysphania militaris* in the 10th edition of *Systema Naturae* in 1758. Its wingspan ranges from 80 to 96 mm. Male forewings are slender and lengthy. The fovea developed robustly. With purplish stripes, the head, thorax, and abdomen have a golden yellowish colour. The inner margin of the forewings is irregularly sinuous, with a deep purplish outer half and a golden-yellow basal half. There are two oblique basal purple fascia, with a patch occasionally detaching from the lower fascia. Two locations near the base of the costa. Three dots form an oblique antemedial series that are frequently combined. The outside region, where vein 3 is where the outer termination is located, has two pale blue maculate bands. Golden yellow hindwings featuring a sizable purple disco-cellular patch and a region beneath the cell. A band protruded between veins 3 and 5. It was postmedial. a sequence of submarginal spots that grow into massive, connected patches at the apex. The yellow area's dots are incredibly erratic.

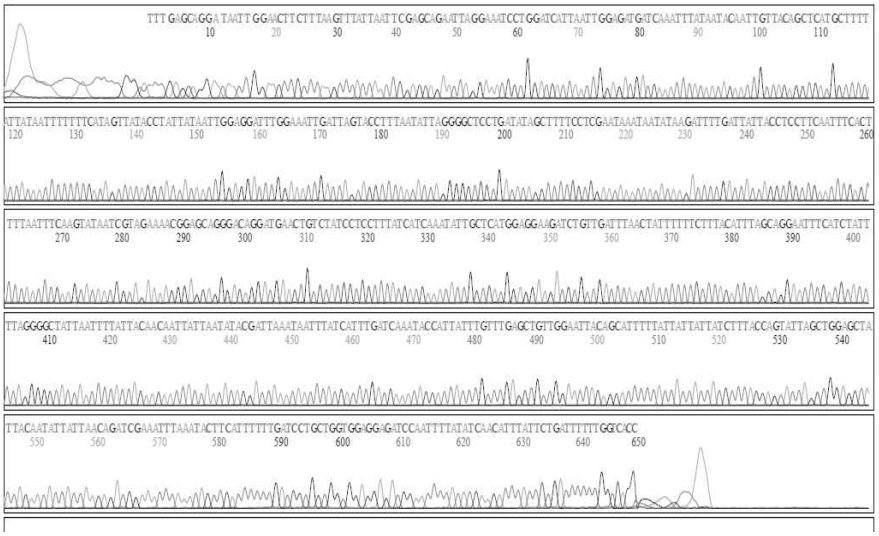

Fig 1: The chromatogram of COI forward sequence of *Dysphania militaris.*

>2_FOM.Primer.Forward_25836-4_P3596,Trimmed Sequence(647 bp)
TGAGCAGGATAATTGGAACTTCTTTAAGTTTATTAATTCGAGCAGAATTAGGAAATCCTG
GATCATTAATTGGAGATGATCAAATTTATAATACAATTGTTACAGCTCATGCTTTTATTA
TAATTTTTTTCATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTT
TAATATTAGGGGCTCCTGATATAGCTTTTCCTCGAATAAATAATATAAGATTTTGATTAT
TACCTCCTTCAATTTCACTTTTAATTTCAAGTATAATCGTAGAAAACGGAGCAGGGACAG
GATGAACTGTCTATCCTCCTTTATCATCAAATATTGCTCATGGAGGAAGATCTGTTGATT
TAACTATTTTTTCTTTACATTTAGCAGGAATTTCATCTATTTTAGGGGCTATTAATTTTA
TTACAACAATTATTAATATACGATTAAATAATTTATCATTTGATCAAATACCATTATTTG
TTTGAGCTGTTGGAATTACAGCATTTTTATTATTATTATCTTTACCAGTATTAGCTGGAG
CTATTACAATATTATTAACAGATCGAAATTTAAATACTTCATTTTTTGATCCTGCTGGTG
GAGGAGATCCAATTTTATATCAACATTTATTCTGATTTTTTGGTCAC

Fig. 2- Consensus sequence of COI sequence from *Dysphania militaris.*

Dysphania militaris voucher 11834 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: MG014753.1 **Length:** 1371 **Number of Matches:** 1

Range 1: 1 to 633 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1086 bits(588)	0.0	618/633(98%)	0/633(0%)	Plus/Plus

```
Query  15   GGAACTTCTTTAAGTTTATTAATTCGAGCAGAATTAGGAAATCCTGGATCATTAATTGGA  74
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Sbjct  1    GGAACTTCTTTAAGTTTATTAATTCGAGCAGAATTACGAAATCCTGGATCATTAATTGGA  60

Query  75   GATGATCAAATTTATAATACAATTGTTACAGCTCATGCTTTTATTATAAtttttttCATA  134
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GATGATCAAATTTATAATACAATTGTTACAGCTCATGCTTTTATTATAATTTTTTTCATA  120

Query  135  GTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATATTAGGGGCT  194
            |||||||||| ||||||||||||||||||||||||||||||||||||||| ||| |||
Sbjct  121  GTTATACCTATCATAATTGGAGGATTTGGAAATTGATTAGTCCCTTTAATATTAGGAGCT  180

Query  195  CCTGATATAGCTTTTCCTCGAATAAATAATATAAGATTTTGATTATTACCTCCTTCAATT  254
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCTGATATAGCTTTTCCTCGAATAAATAATATAAGATTTTGACTATTACCTCCTTCAATT  240

Query  255  TCACTTTTAATTTCAAGTATAATCGTAGAAAACGGAGCAGGGACAGGATGAACTGTCTAT  314
            |||||||||||||||| || || ||||||||||||||||||||||||||||||||||||
Sbjct  241  TCACTTTTAATTTCAAGAATGATTGTAGAAAACGGAGCAGGGACAGGATGAACTGTCTAT  300

Query  315  CCTCCTTTATCATCAAATATTGCTCATGGAGGAAGATCTGTTGATTTAACTATTTTTTCT  374
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Sbjct  301  CCCCCTTTATCATCAAATATTGCTCATGGAGGAAGATCTGTTGATTTAACTATTTTTTCT  360

Query  375  TTACATTTAGCAGGAATTTCATCTATTTTAGGGGCTATTAATTTTATTACAACAATTATT  434
            ||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  361  TTACATTTAGCAGGAATTTCATCTATTTTAGGAGCTATTAATTTTATTACAACAATTATT  420

Query  435  AATATACGATTAAATAATTTATCATTTGATCAAATACCATTATTTGTTTGAGCTGTTGGA  494
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AATATACGATTAAATAATTTATCATTTGATCAAATACCATTATTTGTTTGAGCTGTTGGA  480
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Fig 3: BLAST alignment of *Dysphania militaris* with database sequence MG014753.1

Sl. No.	Species Name	% of Identity	Accession No.
1	*Dysphania militaris*	97.63	MG014753.1
2	*Dysphania militaris*	99.842	MG251974.1

Table 1: Showing blast analysis of, *Dysphania militaris* and percentage of identity with sequences in the database

Tirumala septentrionis

Phylum: Arthropoda

Class: Insecta

Order: Lepidoptera

Family: Nymphalidae

The Indian subcontinent and Southeast Asia are home to the dark blue tiger, or *Tirumala septentrionis,* a type of danaid butterfly. Its wingspan is between 80 and 105 mm. In the forewing, the short streaks above vein 5 are outwardly never truncate, always sharp; the two streaks in interspace 1 are smaller, never coalescing, the upper one producing an oval detached patch. The lower streak of the discoidal cell never developed into a hook, and the two streaks in the hindwing that are wide apart at their apices are connected at the base. This species underside is often darker than that of *T.*

limniace, with the forewing apices and the entire hindwing ground colour without the pronounced golden-brown hue.

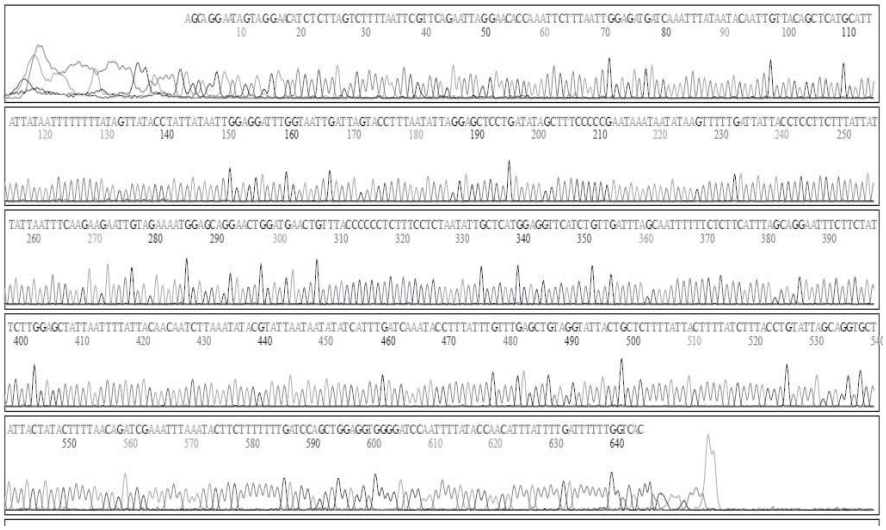

Fig 4: The chromatogram of COI forward sequence of *Tirumala septentrionis*

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>1_FOM.Primer.Forward_25836-1_P3596,Trimmed Sequence(640 bp)
GAATAGTAGGAACATCTCTTAGTCTTTTAATTCGTTCAGAATTAGGAACACCAAATTCTT
TAATTGGAGATGATCAAATTTATAATACAATTGTTACAGCTCATGCATTTATTATAATTT
TTTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTACCTTTAATAT
TAGGAGCTCCTGATATAGCTTTCCCCCGAATAAATAATATAAGTTTTTGATTATTACCTC
CTTCTTTATTATTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATGAA
CTGTTTACCCCCCTCTTTCCTCTAATATTGCTCATGGAGGTTCATCTGTTGATTTAGCAA
TTTTTTCTCTTCATTTAGCAGGAATTTCTTCTATTCTTGGAGCTATTAATTTTATTACAA
CAATCTTAAATATACGTATTAATAATATATCATTTGATCAAATACCTTTATTTGTTTGAG
CTGTAGGTATTACTGCTCTTTTATTACTTTTATCTTTACCTGTATTAGCAGGTGCTATTA
CTATACTTTTAACAGATCGAAATTTAAATACTTCTTTTTTTGATCCAGCTGGAGGTGGGG
ATCCAATTTTATACCAACATTTATTTTGATTTTTTGGTCA
```

Fig. 5- Consensus sequence of COI sequence from *Tirumala septentrionis*

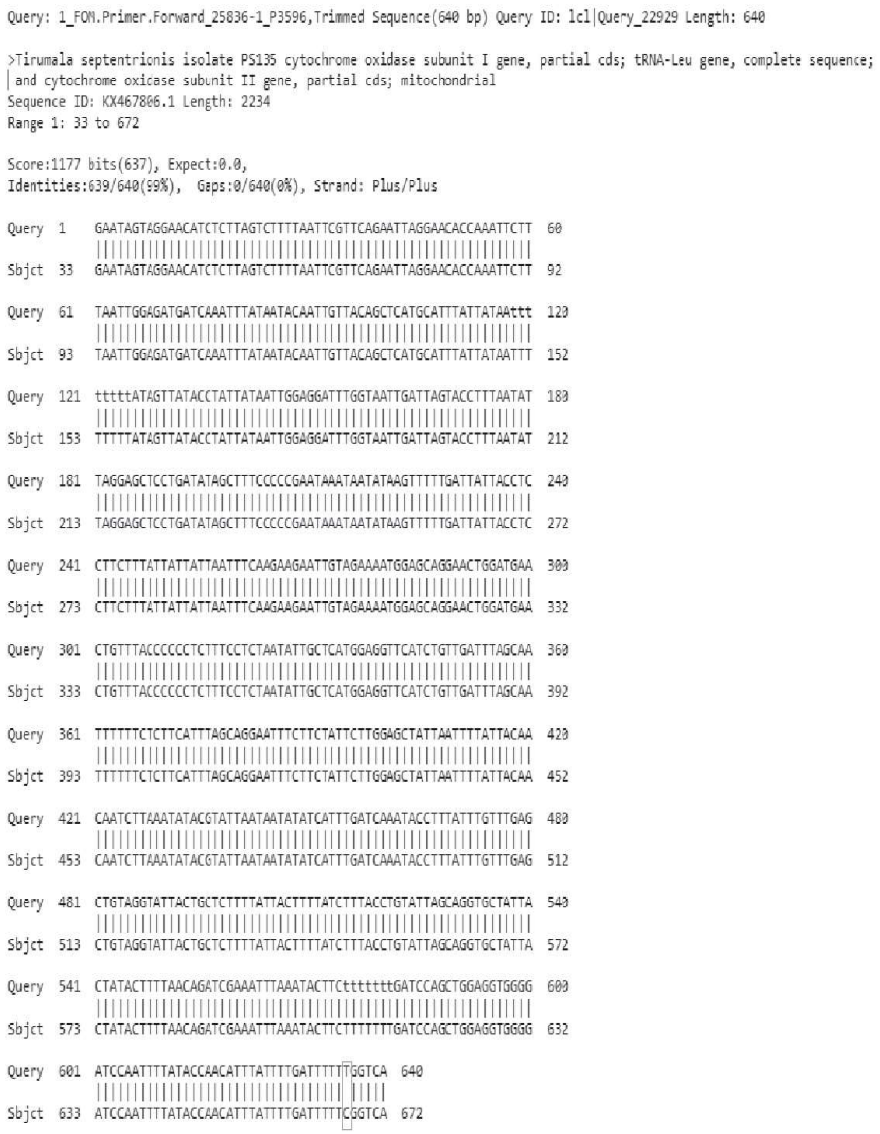


Fig 6: BLAST alignment of *Tirumala septentrionis* with database sequence KX467806.1

Sl. No	Species Name	% of Identity	Accession No.
1	Tirumala septentrionis	99.842	MG251979.1

Sl. No	Species Name	% of Identity	Accession No.
2	Tirumala septentrionis	99.842	MG251974.1
3	Tirumala septentrionis	99.842	MG251970.1
4	Tirumala septentrionis	99.842	MG251969.1
5	Tirumala septentrionis	99.842	MG251968.1

Table 2: Showing blast analysis of *Tirumala septentrionis,* and percentage of identity with sequences in the database.

Euploea core

Phylum: Arthropoda

Class: Insecta

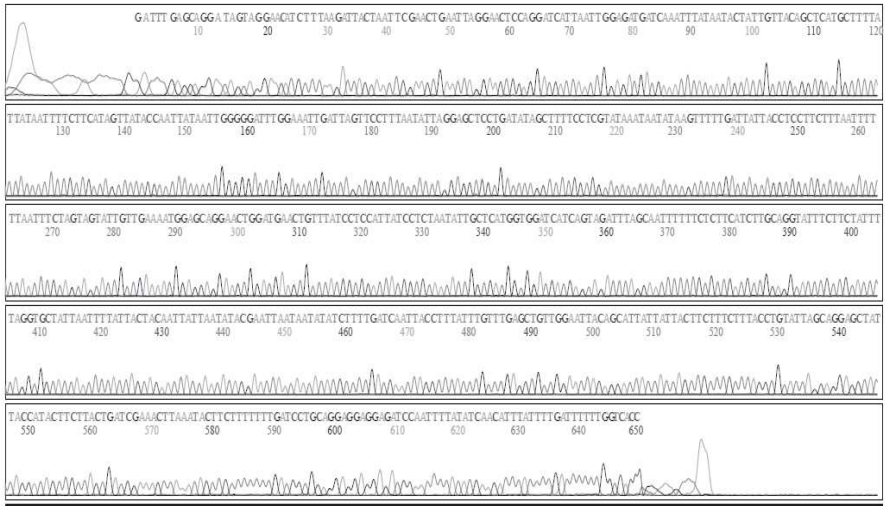
Order: Lepidoptera

Family: Nymphalidae

The common indian crow, *Euploea core* ,The upperside of the butterfly is extremely dark brown, almost black, with very little paler near the terminal margins. The forewing and hindwing have white spots in both the subterminal and terminal series; on the forewing, the subterminal series spots are much larger than the terminal series spots and bent inwards opposite the apex, while the discal series of four or five spots, some of which may be very small or obsolescent, are on the forewing. Chocolate-brown on the underside, with five or six discal tiny dots beyond and a mark near the cell's apex. The white spots are similar to those on the upperside but more pronounced on the hindwing. Head, thorax, abdomen, and antennae are all very dark brown, with the exception of the antennae, which have white spots

underneath. This glossy black, medium-sized butterfly has white markings in rows along the edges of its wings, measuring 85-95 mm in length. This species males visit plants like as *Heliotropium* and *Crotalaria* to restock on pheromones, which are used to entice females during courtship.

Fig 7: The chromatogram of COI forward sequence of *Euploea core*



Sl. No	Species Name	% of Identity	Accession No.
1	*Euploea core*	99.69	GU012626.1
2	*Euploea core*	99.69	KT879874.1
3	*Euploea core*	99.84	MG892098.1

Table 3: Showing blast analysis of *Euploea core* and percentage of identity with sequences in the database

>3_FOM.Primer.Forward_25836-5_P3596,Trimmed Sequence(650 bp)
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CAGGATCATTAATTGGAGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTA
TTATAATTTTCTTCATAGTTATACCAATTATAATTGGGGGATTTGGAAATTGATTAGTTC
CTTTAATATTAGGAGCTCCTGATATAGCTTTTCCTCGTATAAATAATATAAGTTTTTGAT
TATTACCTCCTTCTTTAATTTTATTAATTTCTAGTAGTATTGTTGAAAATGGAGCAGGAA
CTGGATGAACTGTTTATCCTCCATTATCCTCTAATATTGCTCATGGTGGATCATCAGTAG
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TTATTACTACAATTATTAATATACGAATTAATAATATATCTTTTGATCAATTACCTTTAT
TTGTTTGAGCTGTTGGAATTACAGCATTATTATTACTTCTTTCTTTACCTGTATTAGCAG
GAGCTATTACCATACTTCTTACTGATCGAAACTTAAATACTTCTTTTTTTGATCCTGCAG
GAGGAGGAGATCCAATTTTATATCAACATTTATTTTGATTTTTTGGTCAC

Fig 8- consensus sequence of COI sequence from *Euploea core*

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Query  360  GATTTAGCAATTTTTTCTCTTCATCTTGCAGGTATTTCTTCTATTTTAGGTGCTATTAAT  419
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Query  480  TTTGTTTGAGCTGTTGGAATTACAGCATTATTATTACTTCTTTCTTTACCTGTATTAGCA  539
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Sbjct  492  TTTGTTTGAGCTGTTGGAATTACTGCATTATTATTACTTCTTTCTTTACCTGTATTAGCA  551

Query  540  GGAGCTATTACCATACTTCTTACTGATCGAAACTTAAATACTTCtttttttGATCCTGCA  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  552  GGAGCTATTACCATACTTCTTACTGATCGAAACTTAAATACTTCTTTTTTTGATCCTGCA  611

Query  600  GGAGGAGGAGATCCAATTTTATATCAACATTTATTTTGA  638
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Sbjct  612  GGAGGAGGAGATCCAATTTTATATCAACATTTATTTTGA  650
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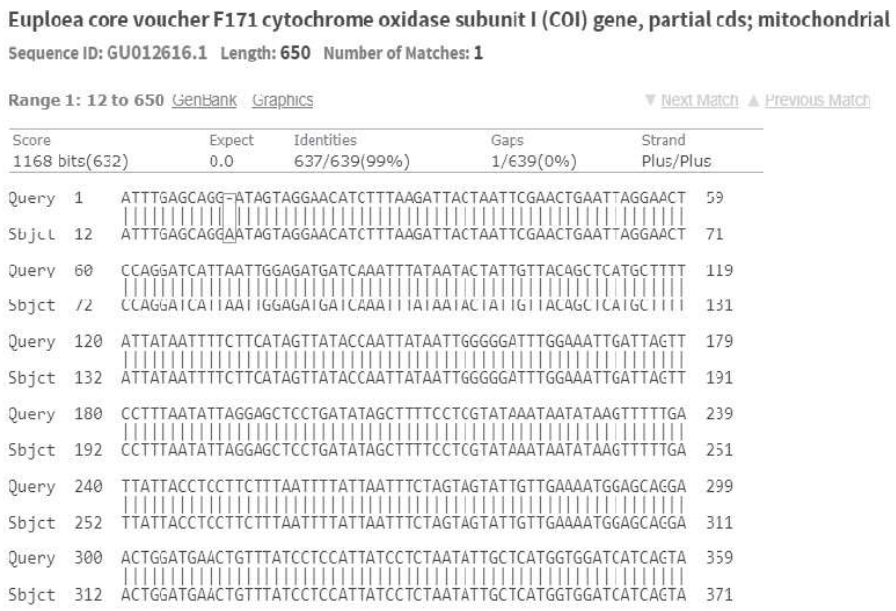

Fig 9: BLAST alignment of *Euploea core voucher* with database sequence GU012626.1

Taxonomy is an amazing world of naming, describing and classifying the entire group of biological organisms using morphological, behavioural, genetic and biochemical observations. It leads to the inclusion of those that are new to science and enumerate the components of biodiversity. The current taxonomy, based on the hierarchy of Linnaeus ranks, said to be unsatisfied by many and they pointed to a rankless system of classification focused on the phylogenetic taxonomy. While considering the morphology, phenotypic plasticity becomes a limitation to species recognition in taxonomy.Morphologically cryptic species are often overlooked; is a lack of taxonomic keys to identify immature specimens of many species.

The invention of traditional taxonomy with specialists can create tremendous progress by vanishing the taxonomic crisis in several groups, geographical area and funding for taxonomic works. Molecular taxonomy, information technology, the development of investment funds [15] and increased utilization of cyber tools [16] like alternate and complementary approaches have been promoted. Among these, DNA barcoding has been particularly successful in the

identification and delimitation of new species from various groups [17].

The present study is based on three species; *Dysphania militaris*, *Tirumala septentriosis, Euploea core* belongs to the order Lepidoptera and these are collected from St. Joseph's college Devagiri campus, Kozhikode. Three of them were initially identified by their morphological characters. For the further confirmation of the species identity genome-based analysis were used. CO 1 gene is taken for the identification of species. DNA was isolated and then amplified. About 710 base pair sent for sequencing. Sequence analyses were done by comparing the sequences with the available sequences in the data base. It was found that from the selected species, first one shows 97.63% similarity to *Dysphania militaris* of the order Lepidoptera. And second species shows 99.84% similarity with *Tirumala septentrionis* coming under order Lepidoptera. The third species shows 99.69% similarities to *Euploea core,* which is also coming under lepidoptera. Here along with the morphological characters, DNA barcoding also helped in the identification of different wispy and delicate species of the order lepidoptera.

Conclusion

Taxonomy, branch of science that deals with species identification, demands extensive knowledge about organisms and their morphological features to facilitate faithful identification. The number of taxonomists with above mentioned level of expertise is limited. In many cases, differences caused by geographical and seasonal variations, leads to misinterpretation of species. Misidentification of sexually dimorphic and mimetic insects is another example were traditional taxonomy gets challenged. To overcome these challenges, taxonomists go for more approaches, like molecular analysis, during species identification.

Three species under the order Lepidoptera were identified using molecular data during the study. It shows many variations of the basic body structure and the most apparent feature is the presence of scales that cover the body, wings and a proboscis. The samples to be identified were successfully collected and were identified based on their morphological features. DNA isolation was done and amplified using PCR and confirmed by gel electrophoresis

Sequencing was done and analysed using BLAST for confirming the species. The species identified and confirmed were *Dysphania militaris*, *Tirumala septentrionis* and *Euploea core*.

Declarations

Conflicts of Interest

The authors hereby confirm that there is no conflict of interest in the present article**.**

Authors' Contributions

JJ and **MM** contributed to the concept design, methodology, analysis of result, preparation and final editing of the manuscript. **AMK** conducted the experiments, data collection and analysis as well as preparation of the initial draft of manuscript. **NJ** contributed to the analysis of data and manuscript editing. All authors have approved the submission to the journal.

Ethics Approval

The Committee of Research Ethics of College provided full approval for this research.

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Consent for Publication

The research does not incorporate any external materials or third-party content.

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