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INTEGRATIVE MORPHO-MOLECULAR ANALYSIS OF *Papilio polytes*, *Papilio polymnestor*, AND *Euploea core* FROM JHARKHAND (INDIA) USING ADVANCED BIOTECHNOLOGICAL AND BIOINFORMATIC APPROACHES

Kumar Manoj [1]
Ranjan Rakesh [1]
Pratik Raj [1]
Tirkey Suraj [1]
Kumar Aman [1]
Hembrom Twinkle [1]
Prasad S. Manoranjan [2]
Singh R. Bharti[1]

[1]St. Xavier's College, Faculty of Science, Department of Zoology, Jharkhand, India

[2]Ranchi University, Faculty of Science, Department of Zoology, Jharkhand, India

E-mail: dr17mk@gmail.com

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Plants and butterflies have a coevolutionary relationship, and butterflies are essential to the ecosystem. They serve as ecosystem indicators as well since research on their populations and behaviour can reveal how healthy an environment is. They are efficient pollinators, and particular species have been known to migrate great distances to spread pollen, which causes genetic variety in plant species and increases their chances of surviving.

Aim. This work aimed to study the morpho-molecular features of *Papilio polytes, Papilio polymnestor,* and *Euploea core* from the Jharkhand state of India.

Methods. The study spans different areas from five districts, viz. Chatra, Koderma, Giridih, Godda, Ramgarh of the state. The specimens were collected, examined, and physical specimens of each species were submitted to the Insect Collection, Record and Identification, of the Department of Zoology, St. Xavier's College, Ranchi, and Voucher numbers were obtained. Modern biotechnological and bioinformatic tools were used in this work, five specimens of each species were sequenced for the mitochondrial cytochrome oxidase subunit 1 (CO1), on the basis of which the BLAST (Basic Local Alignment and Search Tool) search was performed for identification of the species on the basis of matching scores with sequences present in the nucleotide sequence databases.

Results. The latest biotechnologies were used. After identification, the sequences were submitted to GenBank, and accession IDs were obtained. The sequences were used to prepare a phylogenetic tree to ascertain the relationships among the collected specimens.

Conclusions. There is a paucity of knowledge related to the morphology and taxonomy of butterflies of the state; thus, this study is the first attempt of its kind. The study revealed significant intraspecific variation among specimens of *Euploea core*. The least variation was exhibited among specimens of *Papilio polymnestor*. The study contributes to the knowledge related to butterfly species of *Papilio polytes*, *Papilio polymnestor*, *and Euploea core*. It will be helpful in further studies related to the conservation and monitoring of the species.

Key words: morpho-molecular, Jharkhand, India, *Papilio polytes, Papilio polymnestor, Euploea core,* BLAST, phylogeny.

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Butterflies are keystone species that act as driver species [1] in the context of ecosystem services as pollinators. Mostly, some anthropogenic activity and, to some extent, also the natural result in habitat loss, the spread of invasive species, pathogens, and climate change. Pollinator populations, including the butterflies, have been declining in many parts of the world [2–4]. Consequently, the decline in pollinators has raised concerns about the consequences of the loss of one of the most critical ecosystem services, i.e., pollination [5]. Pollination, without doubt, is a vital ecosystem service that is accomplished through the involvement of multiple species of pollinators, among which insects like moths and butterflies share a notable contribution [6]. As per an estimate [7], the value of crop pollination on a global level in economic terms happens to be over €153 billion annually. More than 75% of crops, particularly fruits and vegetables, are dependent, to a considerable extent, on pollinators in order to provide optimized yield [5]. Having perceived that the pollinator species with a significant share of butterflies and moths, in the context of our global economy and food production, are essential ecosystem service providers [8], it is not only surprising but also alarming that there is a paucity of knowledge on the rapidly declining pollinator populations and vanishing of different species [8, 9].

The status of pollinators as a whole and butterflies in particular has attracted much attention from their identification and conservation viewpoint. More than 30 countries are members of Promote Pollinators, the Coalition of the Willing on Pollinators (initiated at the CBD CoP 13), committing themselves to take action to identify and conserve pollinators.

O'Connor et al. [10] reported the unavailability of data on declining population and community change for many taxonomic groups, including butterflies. This underlines the gap in knowledge pertaining to proper identification and subsequent efforts of conservation. The leading cause behind the gaps is that monitoring biodiversity is a task [4, 11, 12] that requires information from gene to species and ecosystem level. Biodiversity conservation demands, at its first step, the identification of the species with the genetic variation within the species. The situation is not different in this region. Butterflies of this region have not been adequately identified. Most of the information on butterflies from this region (Jharkhand) is either a survey or preliminary reports, lacking any morphological study and taxonomical observation [13–16]. For instance, in 2023, Kumar and Keshari prepared a checklist of the variety of butterflies encountered at Bhagwan Birsa Biological Park in Ormanjhi, Ranchi, Jharkhand. They enlisted *Papilio polytes, Papilio polymnestor,* and *Euploea core* among 86 butterfly species that belong to six families.

In contrast to their methods, which used a random encounter approach and involved taking pictures of butterflies, the whole paper lacked even a single photograph of the reported butterflies, including *Papilio polytes, Papilio polymnestor,* and *Euploea core*. Mahto et al. [17] studied the status of diversity and conservation of Rhopalocera in urban Ranchi. Osga et al. [18] studied the diversity of butterfly species in a work that was limited to the campus of St. Xavier's College, Ranchi.

Considering the paucity of authentic information on taxonomy and phylogeny of the region, we have initiated a project exploiting the latest biotechnological and bioinformatic tools for identification of the butterflies of this region based on morphological, anatomical, as well as DNA bar coding based morpho-molecular approach; so based on the latest biotechnologies.

The present communication is a part of the project stated above, and deals with morphological and molecular identification of *Papilio polytes* (*Papilio*nidae), *Papilio polymnestor* (*Papilio*nidae), and *Euploea core* (Nymphalidae) collected from different sites spread along the Jharkhand State of India.

Materials and Methods

Butterfly Sampling

Various parts of the Jharkhand state (Table 1) of India were surveyed for the collection of butterfly samples. Several butterflies were captured and released after preliminary identification based on study of traditional morphological characteristics of wings, locale, and other information as per the butterfly identification keys [19, 20]. In this work, the latest biotechnologies were used to rule out any error in the identification of morphologically similar (cryptic) species. For genetic level identification, the COI gene is preferred due to its high interspecific variation leading to species-level resolution, maternal inheritance, and universal primer compatibility. It also exhibits negligible intraspecific variation. This barcode gap makes it ideal for distinguishing species, thus for genome

Table 1

Details of sampling sites, date of survey, GPS coordinates of Sampling sites

Sp.	District	Site Code	Locality	GPS Co-ordinates (N/E)	Date	Voucher No. (SXCRAN-ENT-)
Papilio polytes	Chatra	A	Kathautia Talab	24.20585802, 84.85986758	04 Apr, 2024	0424-S17A
	Koderma	B	Raja Talab	24.46980312, 85.59268970	10 Apr, 2023	0423-S17B
	Giridih	C	S.S. U. Children Park	24.180694277, 86.30340222	26 Mar, 2023	0323-S17C
	Godda	D	Biodiversity Park	24.79002746, 87.220366	1 May, 2024	0524-S17D
	Ramgarh	E	Gadh Baba Mandir	23.63961972, 85.52425085	20 Apr, 2024	0424-S17E
Papilio polymnestor	Chatra	F	Nawi Talab	24.202032144, 84.87149307	4 May, 2023	0523-S18A
	Koderma	B	Raja Talab	24.469585704, 85.59092695	6 May, 2024	0524-S18B
	Giridih	G	Pampoo Talab	24.183487830, 86.31396327	9 June, 2023	0623-S18C
	Godda	H	Godda Park	24.839396892, 87.21427081	27 Apr, 2023	0423-S18D
	Ramgarh	I	Radha Rani Van	23.60687392, 85.5150253	4 June, 2024	0624-S18E
Euploea core	Chatra	J	Puraniya Talab	24.21051887, 84.87502824	10 Apr, 2024	0424-S19A
	Koderma	K	Koderma Town Station	24.465371166, 85.61008921	19 Apr, 2023	0423-S19B
	Giridih	L	Shashtri Nagar	24.1963680, 86.30239845	28 Mar, 2023	0323-S19C
	Godda	M	Biodiversity Park	24.7904938, 87.22146098	3 May, 2024	0524-S19D
	Ramgarh	N	Bijuliya Talab	23.620302331, 85.51636776	20 Apr, 2024	0424-S19E

(COI) sequence analysis. At least one specimen from each sampling site was preserved in 70% alcohol for DNA (deoxyribonucleic acid) extraction, PCR (Polymerase Chain Reaction) amplification, and sequencing [21]. Two to three specimens from every survey site, after being sacrificed under the ethyl acetate fumes, were pinned and spread for further reference. One representative of each specimen was submitted to the ICRI (Insect Collection, Record and Identification), Entomology Section, Department of Zoology, St. Xavier's College, Ranchi, and Voucher numbers were obtained (Table 1).

Morphological Investigations

Morphological cues were used to describe and record each sample's sex and colour pattern. Sex was investigated based on the wing markings [19, 20, 22].

DNA extraction, PCR amplification, and sequencing

A butterfly specimen's hind limb was used for extracting DNA. A 1.0% agarose gel was used to evaluate the quality. There was only one band of high-molecular-weight DNA visible. Using specific forward and reverse primers, fragments of the mitochondrial cytochrome c oxidase subunit I (COI) gene were amplified. There was only one identifiable PCR amplicon band visible when the sample was resolved on an agarose gel. The length of the sequences that were extracted from each sample varied. To get rid of contaminants, the PCR amplicon was subjected to further purification. The BDT v3.1 Cycle sequencing kit was used on an ABI 3730xl Genetic Analyser (ThermoFisher, 2024) to sequence the PCR amplicon using forward and reverse primers [23].

Molecular sequence analysis tools

The latest biotechnological tools were used, unique PCR amplicon was obtained by the Sanger sequencing method. The NCBI's (National Centre for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) software was used to compare the resultant PCR amplicon with information found in nucleotide databases. The option of nucleotide blast (BLASTn) was used. The nucleotide query (our sequence) and the subject sequence (data from databases) are compared by BLASTn. The following parameters were used when running BLASTn: Program selection: highly similar sequences (megablast); search set: standard database. Sequences can be identified and compared within species using Megablast [24].

Submission of nucleotide sequence to GenBank

Based on the biotechnology of the BLASTn search results, the butterfly specimens were identified as *Papilio polytes, Papilio polymnestor, and Euploea core*. Additionally, accession IDs for nucleotide sequences were acquired after the COI nucleotide sequences were submitted to GenBank and the DNA Data Bank of Japan (DDBJ).

Phylogenetic Tree and Distance Matrix

Phylogenetic tree and Distance Matrix were obtained using other novel biotechnological and bioinformatic tools. The 15 sequences (5 from each species) derived from the COI nucleotide sequences of the three species collected from various survey sites restricted to the five districts (Chatra, Koderma, Giridih, Godda, and Ramgarh) of the state of Jharkhand were used to create the distance matrix and phylogenetic tree. The evolutionary distances (matrix), which are expressed in units of base substitutions per site (Tamura et al., 2004), were constructed using the Maximum Composite Likelihood approach [25]. In this study, ten nucleotide sequences were employed. All unclear sites were removed (pairwise deletion) for each pair of sequences.

Other biotechnological and bioinformatic tools were used for determining the evolutionary history. The evolutionary history was derived using the Maximum Likelihood method and the Kimura 2-parameter model [26]. The initial tree for the heuristic search was automatically created by applying the Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances determined using the Maximum Composite Likelihood approach. Next, we selected the topology with the highest log likelihood value. In this study, fifteen nucleotide sequences were employed. These analyses were performed using MEGA X (Molecular Evolutionary Genetics Analysis) software, version 10.2.6, build 10210527-x86_64 (Windows 11) [24, 27, 28].

Results and Discussion

Morphological Investigations

Papilionidae

The *Papilionidae* family of butterflies includes the swallowtail and Parnassian species. This is a family of big, vibrant butterflies. They vary in size from 60 to 180 mm. The genus Ornithoptera includes the largest butterflies in the world, the birdwing butterfly. From behind the hind wing, many swallowtail butterflies have long tails or extensions [29-31].

Papilio polytes

Commonly referred to as "Common Mormon," butterflies belonging to the *Papilio polytes* family come in three female forms: cyrus, stichius, and romulus, with only one male. Males are black with a white stripe across each hindwing and white specks along the outer margin of the forewing. The majority of the adult female *Papilio polytes*' polymorphism mimics that of other distasteful butterfly species. The cyrus form, which is present throughout the typical Mormon's entire range, resembles males but has paler colouring and more pronounced red crescents. Common Mormon populations from the Himalayas to Japan, Sulawesi, and Sri Lanka are home to the most prevalent stichius form, which resembles the typical rose (*Pachliopta aristolochiae*). The dull-coloured romulus form is similar to the Crimson rose (*Pachliopta hector*). This predator escape technique is an example of Batesian mimicry since *Papilio polytes* mimics unpleasant butterfly species and is palatable [21, 32, 33].

Wing venation of *Papilio polytes*

Forewing: *Papilio polytes* has a big, triangular forewing. Costa has a somewhat arched posture. Dc (discal cell), which is more than half the length of the wing, is closed. The Sc (subcostal) vein is located at the costal edge. R2 (radius 2) originates behind the Dc. It ends at the apex, while R1 (radius 1) emerges at one half of the costal edge near Sc. R3 (radius 3) forms an arch at the apex, emerging from the Dc's anterior corner.

Papilio polytes (female) *Papilio polytes (male)*

Fig. 1. **Photograph of Dorsal Side of specimen of *Papilio polytes***

Papilio polytes (female) *Papilio polytes (male)*

Fig. 2. **Photograph of Ventral Side of specimen of *Papilio polytes***

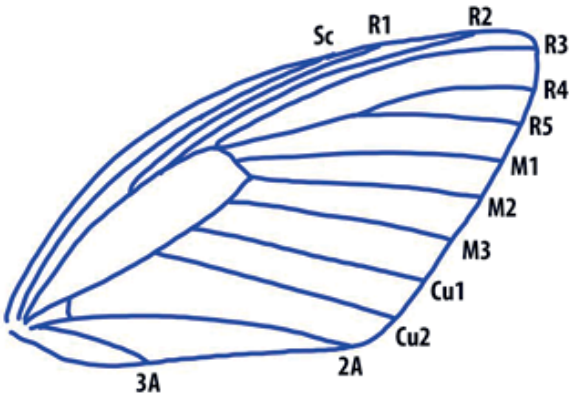

Fig. 3. **Venation in the forewing of *Papilio polytes***

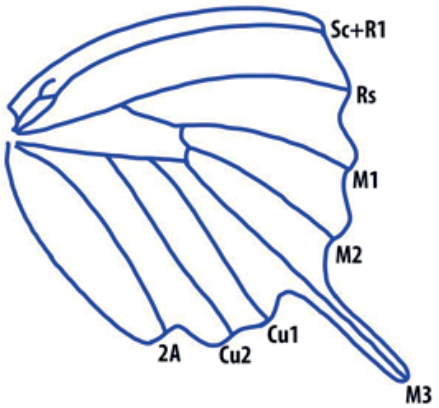

Fig. 4. **Venation in the hindwings of *Papilio polytes***

From a similar vein that emerges from the tip of Dc at the source of R3, R4 (radius 4), and R5 (radius 5) contact the wing's margin. The lower apex of Dc is where M1 (median 1), M2 (median 2), and M3 (median 3) originate, and each of the three median veins has a roughly equal length. Starting independently from the lower portion of Dc, the Cu1 (cubitus 1) and Cu2 (cubitus 2) reach the wing edge close to the tornus. Anal vein 2A (anal 2) emerges from the back and ends at the tornus' edge. 3A (anal 3) is extremely short, starting at the base and ending at the dorsum near the wing border.

Hindwing: The hindwing resembles something of a fan. DC is closed, and the coastal margin is somewhat arched. The humoral vein is located at the base's anterior-most point. Sc and R1 combine to form Sc+R1, which emerges from the base and travels parallel to the costa to the apex, or wing margin. The anterior sector of Dc is the origin of the radial sector vein (Rs), which ends at the anterior part of the termen at the wing's border. Both M1 and M2 emerge from Dc's terminal end and arrive at the margin at the term. The M3 vein lengthens along the wing margin to create a protrusion that resembles a tail. From the bottom portion of Dc, Cu1 and Cu2 emerge, reaching the wing

Fig. 5. **Photograph of Dorsal Side of specimen of *Papilio polymnestor***

Fig. 6. **Photograph of Ventral Side of specimen of *Papilio polymnestor***

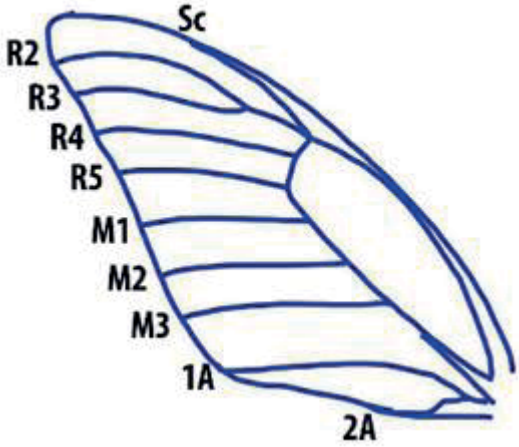

Fig. 7. **Venation in the forewings of *Papilio polymnestor***

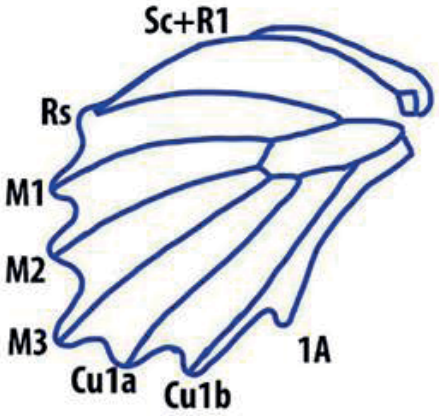

Fig. 8. **Venation in the hindwings of *Papilio polymnestor***

margin near the tornus. 2A rises from the wing's base to the tornus, which is the wing's edge.

Papilio polymnestor, often known as Blue Mormons, are giant swallowtail butterflies that are found in Sri Lanka, Southern India, and Northeastern India [34]. It is the state butterfly of Maharashtra, an Indian state. Between 120 and 150 mm is its wing span. It is the fourth-largest butterfly in India. The wings at the back have a glistening blue tint. In addition to the buff-coloured female of Sri Lanka's *Papilio polymnestor parinda* Moore, it has several characteristics with the latter. A few preliminary reports of it have been made from the state of Jharkhand [13-16], but none of them have included morphological, molecular, or documentation (photographs, sketches, sample preservation, etc.) research.

Wing venation of *Papilio polymnestor*

Forewing: The forewing contains twelve veins, as well as a vast, closed discal cell from which numerous veins radiate. The base is where the first and last of the 12 veins emerge, while the discal cell is where the others appear. Subcostal (Sc) joins the R5 vein on the forewing. There are five branches of the radial veins (R1–R5). The lower discal is the origin of the median veins (M1–M3). The last vein is the anal vein, which contains two branches, 1A and 2A, followed by the cubital veins, Cu1a and Cu1b.

Hindwing: Sc+R1 (fused) is the result of the first radial vein in the hind wing fusing with the subcostal. The term "radial sector" refers to the unbranched radius (Rs). There are three branches of the median vein (M) (M1-M3). There are cubital veins Cu1a and Cu1b. There is only one anal vein (1A). The Humeral vein is a little spur that extends towards the costa close to the base of the eighth vein.

Fig. 9. **Photograph of Dorsal Side of specimen of *Euploea core***

Fig. 10. **Photograph of Ventral Side of specimen of *Euploea core***

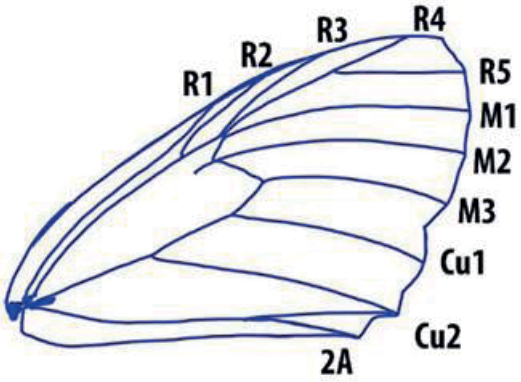

Fig. 11. **Venation in the forewings of *Euploea core***

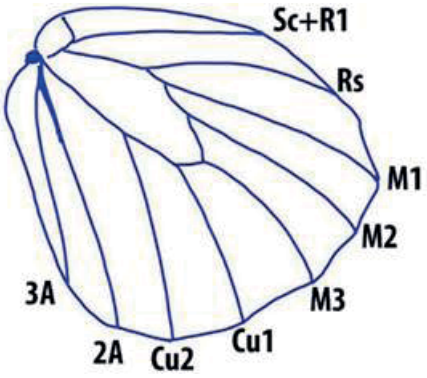

Fig. 12. **Venation in the hindwings of *Euploea core***

Nymphalidae

Two groups in this family are very different from one another in appearance. While the undersides of the fritillaries frequently have silver markings, the upper surfaces are orange and feature black markings. The other contains some of the brightest-coloured butterflies [19, 20, 28].

Euploea core

The medium-sized butterfly *Euploea core*, also called the "Common Crow," is a member of the Nymphalidae family. It has a glossy black appearance with white dots on the wing edges. The range of the wingspan is 85 to 95 mm. The female's forewing has a straight hind border, but the males have a bow-shaped one. Australia and South Asia are home to it. It is also occasionally called the "Common Indian Crow" in India and the "Australian Crow" in Australia.

Wing venation of *Euploea core*

Forewing: The *Euploea* core's forewing has a roughly triangular form. The Dc is closed. From the base of the wing, R1 arises to the edge in the centre of the costa. R2 emerges from Dc's anterior end and travels to the costa. The anterior terminal end of Dc is where R3 and R4 originate. R3 contacts the wing margin shortly before the apex, while R4 reaches the wing margin at the apex. R5 emerges from R4 as a branch and reaches the edge immediately below the wing's apex. The terminal end of Dc is where M1, M2, and M3 originate.

M2 enters the Dc for a very brief distance. The posterior portion of Dc gives rise to anterior Cu1 and posterior Cu2, to reach the wing's edge close to the tornus. As a shared vein, 2A and 1A emerge from the wing base and split apart near the wing's tornus, just short of the margin. At the margin, the 2A and Cu2 meet.

Hindwing: The rear wing of the *Euploea* core resembles a fan and achieves an oval-triangular form. The anterior-most vein, the humeral vein, begins at the base of the wing and ends close to the base at the wing's border. Sc and R1 combine to create Sc+R1, which emerges from the base of Dc and meets the wing edge close to the apex. The near-anterior tip of Dc is where Rs, M1, and M2 originate from. At the top, Rs satisfies the margin.

Table 2

Nucleotide frequencies and NT length of CO1 nucleotide region of the butterfly samples collected from Different parts of Jharkhand

Site Code	District	Butterfly species	Accession Ids	Nucleotide Frequencies, %				
				T(U)	**C**	**A**	**G**	**Total**
A	Chatra	*Papilio polytes*	PQ565876	40.1	15.6	29.8	14.5	704
B	Koderma		PQ565875	40.3	15.4	30.1	14.2	837
C	Giridih		PQ565873	40	16	29.8	14.3	658
D	Godda		PQ565874	40	16	29.8	14.3	658
E	Ramgarh		PQ565877	39.6	16.7	29.8	14	642
F	Chatra	*Papilio polymnestor*	PQ569085	39.7	16.2	30.3	13.8	630
B	Koderma		PQ569086	39.6	16.5	29.9	14	642
G	Giridih		PQ569087	39.7	16.3	30.1	13.9	638
H	Godda		PQ569088	38.9	16.3	30.7	14.1	596
I	Ramgarh		PQ569089	38.8	16.4	30.7	14.1	596
J	Chatra	*Euploea core*	LC849501	42.7	14	28.8	14.5	708
K	Koderma		LC849500	41.8	15.1	28.9	14.2	650
L	Giridih		LC849499	43.1	13	29.3	14.6	583
M	Godda		LC849498	42.9	14.4	28.9	13.9	613
N	Ramgarh		LC849497	42.9	14.3	28.9	14	630

The margin is met at the term by M1 and M2. M3 emerges from the Dc tip to reach the wing margin in the middle of the period. Cu1 emerges from the Dc's near-posterior end to meet the wing margin at the termen.

The 2A extends from two-thirds of the lower DC's length to the wing border at the termen above the tornus. The base of the wing is where anterior 2A and posterior 3A originate. Tornus is where 2A touches the wing's margin, whereas dorsum is where 3A touches the wing's margin.

Molecular analysis

DNA extraction, PCR amplification, and Nucleotide Sequencing

The process of verifying an organism's identity at the molecular level is known as molecular identification **[35].** One way for identifying an organism is DNA barcoding, which uses a brief DNA fragment from a particular gene to identify an organism. To determine the organism, the DNA sequence is compared to a reference library [36]. For example, the cytochrome c oxidase I (COI) gene is utilised for animals, the internal transcribed spacer (ITS) rRNA gene is utilised for fungi, and ribulose bisphosphate carboxylase/ oxygenase (RuBisCO) is utilised for plants **[37].** In this study, the COI gene sequence is used for DNA barcoding (identification of organisms at the molecular level).

Using a particular primer, a single distinct PCR amplicon of the COI region was obtained. Table 2 lists the specifics of the sequences that were extracted from the 15 specimens from the three species — *Papilio polytes, Papilio polymnestor, and Euploea core.* Using the NCBI (National Centre for Biotechnology Information)-based BLASTn program, all of the resulting nucleotide sequences (query) were compared with the nucleotide databases. The BLASTn search parameters were: program selection: highly comparable sequences (megablast); search set: standard database. The megablast is employed for intraspecies comparison and sequence identification **[38, 39].** The findings of the BLASTn similarity search were used for the identification of the specimens at the molecular level.

After identification on the basis of BLASTn search results, the 15 sequences obtained in this study were submitted to DDBJ (DNA Databank of Japan) and bankit to obtain accession IDs (Table 2). By looking up the accession IDs using the ENTREZ search engine, one can view the whole definition of CO1 sequences for each of the 15 sequences online. Additionally, each sequence's link has been provided with its QR (quick response) code, which is represented

as Fig. 13 (*Papilio polytes*), Fig. 14 *(Papilio polymnestor*), and Fig. 15 (*Euploea core*). The QR codes can be scanned to view the complete definition of the sequence online.

Distance Matrix and Phylogenetic Tree

Phylogenetic tree and Distance Matrix *were* obtained using other novel biotechnological and bioinformatic tools described below. The MEGAX software's ClustalW alignment function was used to align the 15 sequences, using a gap opening penalty of 15.00 and a gap extension penalty of 6.66. Following that, the alignment was exported in MEGA format. The distance matrix was subsequently created using this alignment (Table 3). There are many applications for distance matrices in bioinformatics, including phylogenetic tree construction, protein structure representation, protein structure comparison and alignment, inferring protein-protein interactions, and protein structure determination. A distance matrix is a two-dimensional array that contains the pairwise distances between a set of elements (in this case, nucleotide sequences) [40, 41]. The distance between two people, or the number of loci that separate them, is measured by pairwise distance. The more species separate from one another, the more unrelated they are, or the greater the distance matrix value, the more variety there is between any two species **[28].** In the distance matrix presented as Table 3, the value of pairwise distance ranged from 0.00 to 0.175.

Among the samples of *Papilio polytes*, the matrix score ranged from a minimum of 0.000 (close relationship) to a maximum of 0.017 (distant relationship). These ranging values indicated the intraspecific variation among the populations of *Papilio polytes* of the sampling regions. The specimen collected from Giridih was very closely related to the sample from Godda (0.00). The most distant relation was between *Papilio polytes* collected from Chatra and Godda; Chatra and Giridih, with a distance matrix score of 0.009 in each case.

Fig. 13. **Accession IDs and QR codes for accessing the full definition (online) of CO1 sequences of *Papilio polytes* submitted to GenBank**

Fig. 14. **Accession IDs and QR codes for accessing the full definition (online) of CO1 sequences of *Papilio polymnestor* submitted to GenBank**

Fig. 15. **Accession IDs and QR codes for accessing the full definition (online) of CO1 sequences of *Euploea core* submitted to DDBJ**

Table 3

Distance Matrix prepared using the 15 different sequences of *Papilio polytes, Euploea core* and *Papilio polymnestor* to estimate the evolutionary divergence between sequences

		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Giridih_*P.polytes*_PQ565873	I		0.000	0.003	0.017	0.009	0.160	0.159	0.148	0.145	0.168	0.081	0.081	0.081	0.079	0.078
Godda_*P.polytes*_PQ565874	II	0.000		0.003	0.017	0.009	0.160	0.159	0.148	0.145	0.168	0.081	0.081	0.081	0.079	0.078
Koderma_*P.polytes*_PQ565875	III	0.003	0.003		0.016	0.007	0.164	0.166	0.162	0.144	0.175	0.074	0.076	0.074	0.073	0.070
Chatra_*P.polytes*_PQ565876	IV	0.017	0.017	0.016		0.001	0.155	0.155	0.162	0.137	0.161	0.080	0.082	0.080	0.079	0.077
Ramgarh_*P.polytes*_PQ565877	V	0.009	0.009	0.007	0.001		0.155	0.155	0.150	0.138	0.156	0.080	0.079	0.079	0.079	0.077
Ramgarh_*E.core*_LC849497	VI	0.160	0.160	0.164	0.155	0.155		0.000	0.000	0.144	0.000	0.158	0.158	0.158	0.163	0.165
Godda_*E.core*_LC849498	VII	0.159	0.159	0.166	0.155	0.155	0.000		0.000	0.140	0.000	0.159	0.159	0.159	0.162	0.164
Giridih_*E.core*_LC849499	VIII	0.148	0.148	0.162	0.162	0.150	0.000	0.000		0.141	0.010	0.160	0.160	0.160	0.160	0.163
Koderma_*E.core*_LC849500	IX	0.145	0.145	0.144	0.137	0.138	0.144	0.140	0.141		0.141	0.132	0.134	0.134	0.127	0.125
Chatra_*E.core*_LC849501	X	0.168	0.168	0.175	0.161	0.156	0.000	0.000	0.010	0.141		0.158	0.159	0.160	0.162	0.164
Chatra_*P.polymnestor*_PQ569085	XI	0.081	0.081	0.074	0.080	0.080	0.158	0.159	0.160	0.132	0.158		0.000	0.000	0.000	0.002
Koderma_*P.polymnestor*_PQ56908(	XII	0.081	0.081	0.076	0.082	0.079	0.158	0.159	0.160	0.134	0.159	0.000		0.000	0.000	0.002
Giridih_*P.polymnestor*_PQ569087	XIII	0.081	0.081	0.074	0.080	0.079	0.158	0.159	0.160	0.134	0.160	0.000	0.000		0.000	0.002
Godda_*P.polymnestor*_PQ569088	XIV	0.079	0.079	0.073	0.079	0.079	0.163	0.162	0.160	0.127	0.162	0.000	0.000	0.000		0.002
Ramgarh_*P.polymnestor*_PQ56908(	XV	0.078	0.078	0.070	0.077	0.077	0.165	0.164	0.163	0.125	0.164	0.002	0.002	0.002	0.002	

Among the samples of *Euploea core,* the distance matrix score ranged from a minimum of 0.000 to a maximum of 0.144. A very close relationship was exhibited between samples collected from Ramgarh & Godda; Ramgarh & Giridih; Ramgarh & Koderma; Godda & Giridih, Godda & Chatra, exhibiting a matrix score of 0.000. The distant relationship was shown among the specimens collected from Koderma and Ramgarh, with the highest matrix score of 0.144 among *Euploea core.*

Least variation was observed among samples of *Papilio polymnestor*, with a minimum of 0.000 to a maximum score of 0.002; this exhibits lower intraspecific variation among populations of *Papilio polymnestor* collected from the different sampling sites. The six pairs — Koderma & Chatra, Giridih & Chatra, Godda & Chatra, Giridih & Koderma, Godda & Koderma, Godda & Giridih exhibited very close relationships with a matrix score of 0.00.

Regarding interspecific variance, the matrix score varied between 0.070 and 0.175. The number of base substitutions between sequences is displayed for each location. The Maximum Composite Likelihood model was used for the analyses **[25].** For every pair of sequences, all ambiguous locations were eliminated (pairwise deletion). The final dataset contained 986 locations in total **[27].**

A figure that shows the evolutionary descent lines of various species, creatures, or genes from a common ancestor is called a phylogenetic tree, or phylogeny. Phylogenies are helpful for structuring classifications, organising knowledge of biological diversity, and shedding light on evolutionary events. Since Charles Darwin's time, tree diagrams have been employed in evolutionary biology**. [**42].

The Neighbor-Joining approach was used to estimate the evolutionary relationships of taxa [43]. The branches are accompanied by the proportion of duplicate trees where the related species clustered together in the bootstrap test (100 repetitions) [44]. The evolutionary distances are shown in terms of the number of base substitutions per site and were calculated using the Maximum Composite Likelihood approach [25]. Fifteen nucleotide sequences were analysed. In MEGA X, evolutionary studies were carried out [27]. Figure 16 displays the resultant phylogenetic tree.

As exhibited by the phylogenetic tree, the *Papilio polytes* from Giridih, Godda, and Koderma fell in a separate clade, and Chatra and Ramgarh fell in another clade. In the case of *Papilio polymnestor*, the sample from Koderma and Giridih fell in a separate clade as sister taxa. Interestingly, the samples of *Papilio polytes* and *Papilio polymnestor*, both species have a common genus under which fall the two species *polytes* and *polymnestor,* this is visible from the phylogenetic tree, which shows that both have originated from a common ancestor. The sample of *Euploea core* collected from Chatra showed most intraspecific variation, which is also exhibited by a high matrix score of 0.175.

Conclusions

Morpho-molecular studies on *Papilio polytes*, *Papilio polymnestor*, and *Euploea core* collected from various sampling sites from

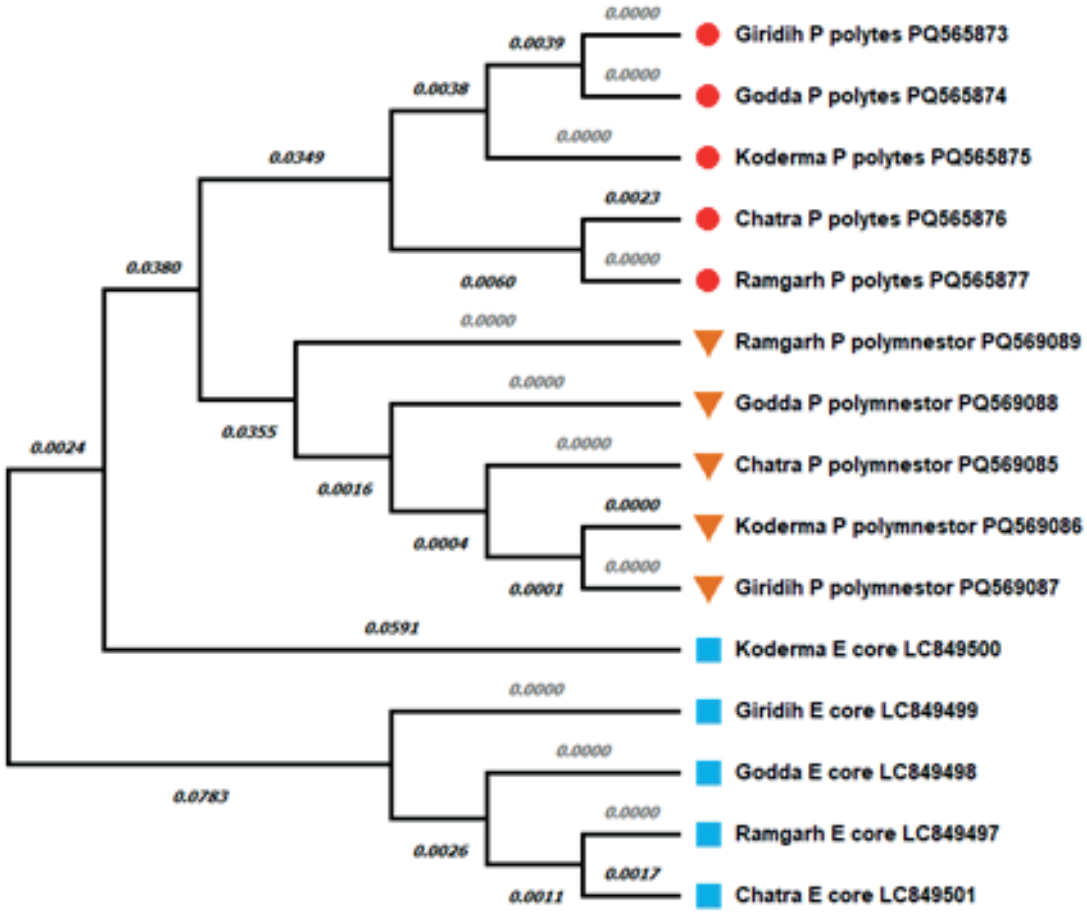

Fig. 16. **Phylogenetic tree prepared using 15 different CO1 sequences of the *Papilio polytes, Euploea core* and *Papilio polymnestor***

Koderma, Giridih, Godda, Ramgarh, and Chatra districts of the Jharkhand state of India were done. The study involved the use of the latest biotechnological and bioinformatic tools. There is a paucity of knowledge related to the morphology and taxonomy of butterflies of the state; thus, this study is the first attempt of its kind. The study revealed significant intraspecific variation among specimens of *Euploea core*. The least variation was exhibited among specimens of *Papilio polymnestor*. The study contributes to the knowledge related to butterfly species of *Papilio polytes*, *Papilio polymnestor*, *and Euploea core*. It will be helpful in further studies related to the conservation and monitoring of the species.

Authors' Contributions

Conceptualization: Kumar M, and Sinha MP; Data curation: Kumar M, and Ranjan R,; Formal analysis: Pratik R, Tirkey S, and Kumar A; Methodology: Kumar M, Hembrom T, Raipat BS; Project administration and Supervision: Sinha MP, and Raipat BS; Software: Kumar M; Writing original draft: Kumar M, Ranjan R, Sinha MP; Writing — review and editing: Raipat BS, Pratik R, Tirkey S, Kumar A, and Hembrom T. All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

Ethical approval

Not Applicable.

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МОРФО-МОЛЕКУЛЯРНІ ДОСЛІДЖЕННЯ ЗРАЗКІВ ОСЕРЕДКУ (?) *Papilio polytes, Papilio polymnestor* ТА *Euploea*, ЗІБРАНИХ У ШТАТІ ДЖАРКХАНД (ІНДІЯ) З ВИКОРИСТАННЯМ БІОТЕХНОЛОГІЧНИХ ТА БІОІНФОРМАТИЧНИХ ІНСТРУМЕНТІВ

Kumar Manoj[1], *Ranjan Rakesh*[1], *Pratik Raj*[1], *Tirkey Suraj*[1], *Kumar Aman*[1], *Hembrom Twinkle*[1], *Prasad S. Manoranjan*[2], *Singh R. Bhart*[1]

[1]St. Xavier's College, Faculty of Science, Department of Zoology, Jharkhand, India;
[2]Ranchi University, Faculty of Science, Department of Zoology, Jharkhand, India.

Email: dr17mk@gmail.com

Рослини та метелики мають коеволюційний зв'язок, і метелики є важливими для екосистеми. Вони також служать екосистемними індикаторами, оскільки дослідження їхніх популяцій та поведінки можуть показати, наскільки здоровим є навколишнє середовище. Вони є ефективними запилювачами, і відомо, що деякі види мігрують на великі відстані для поширення пилку, що спричиняє генетичну різноманітність у видах рослин та збільшує їхні шанси на виживання.

Мета. Вивчити морфомолекулярні особливості осередку *Papilio polytes, Papilio polymnestor* та *Euploea* зі штату Джаркханд в Індії.

Методи. Дослідження охоплює різні ділянки п'яти районів, а саме штати Чатра, Кодерма, Гірідіх, Годда, Рамгарх. Зразки були зібрані, досліджені, а фізичні зразки кожного виду були передані до Відділу колекції, обліку та ідентифікації комах кафедри зоології коледжу Святого Ксаверія в Ранчі, та отримані номери ваучерів. У цій роботі були використані сучасні біотехнологічні та біоінформаційні інструменти, п'ять зразків кожного виду були секвеновані на предмет субодиниці 1 (CO1) мітохондріальної цитохромоксидази, на основі чого було виконано пошук за допомогою BLAST (Basic Local Alignment and Search Tool) для ідентифікації виду на основі збігів із послідовностями, присутніми в базах даних нуклеотидних послідовностей.

Результати. Були використані найновіші біотехнології. Після ідентифікації послідовності були передані до GenBank, і були отримані ідентифікатори доступу. Послідовності були використані для побудови філогенетичного дерева для встановлення взаємозв'язків між зібраними зразками.

Висновки. Існує недостатньо знань про морфологію та таксономію метеликів цього штата; тому це дослідження є першою спробою такого роду. Дослідження виявило значну внутрішньовидову варіацію серед екземплярів *Euploea core*. Найменша варіація спостерігалася серед екземплярів *Papilio polymnestor*. Дослідження сприяє розширенню знань про види метеликів *Papilio polytes, Papilio polymnestor* та *Euploea* і буде корисним у подальших дослідженнях, пов'язаних зі збереженням та моніторингом виду.

Ключові слова: морфомолекулярний, Джаркханд, Індія, *Papilio polytes, Papilio polymnestor, Euploea*, BLAST, філогенія.