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Morphological study and DNA barcoding of *Junonia atlites* collected from Jharkhand state of India

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Abstract

Butterflies provide many ecosystem services including pollination, role in increasing biodiversity, acting as food source for other organisms such as birds, reptiles, amphibians etc. They are also sensitive to environmental change, so their numbers can indicate the health of environment. Keeping these apprehensions in mind, the present work was undertaken to perform morpho-molecular study of *Junonia atlites* belonging to Nymphalidae. The butterflies of the Jharkhand state of India are very poorly studied, some preliminary works from this region are just limited to preparation of checklist and hobby studies lacking any scientific explanation and validation. In this work 10 sites from Jharkhand were surveyed for morpho-molecular studies on *Junonia atlites*, the evolutionary relationships among the studied specimens were determined following the sequencing of CO1 gene, followed by preparation of distance matrix and phylogenetic tree. Significant variation was exhibited by specimen collected from Horhap Forest and St. Xavier's College, Ranchi when compared with the other eight specimens, which is evident by the matrix score and phylogenetic tree analysis.

Keywords: *Junonia atlites*, morphomolecular, morphoanatomical, distance matrix, phylogenetic tree, blast

Introduction

Every species is a bountiful creation of nature, and every species of butterfly is a work of natural beauty. Due to their extreme sensitivity to even the smallest changes in habitat and climatic conditions, butterflies have been shown to be an effective indicator group for biodiversity within tropical land-use systems, (Kremen 1994; Libert 1994; Brown 1997; Larsen, 2008; Özden *et al*. 2008; Pozo *et al*. 2008) [1, 2, 3, 4. 5, 6] because they react quickly to these anthropogenic changes (Akite, 2008) [7]. Because of their brief life span, limited range, and low mobility, butterflies are particularly susceptible to changes in land cover and the patterns of land use (Dover and Settele 2009) [8]. The characteristics of butterflies, such as their relative ease of capture, ease of manipulation, and group identification, make them good bioindicators (Rogo and Odulaja 2001; Fitzherbert *et al*. 2006; Marin *et al*. 2009; Nyamweya and Gichuki 2010) [9, 10, 12]. Because of these characteristics, butterflies are considered significant creatures for biomonitoring. By being present, absent, abundant, or rare in a habitat, they reveal changes in biodiversity, habitat, and environmental conditions. (Kremen 1992; 1994; Howard *et al*., 2000; Cleary 2004; Cleary and Mooers 2004) [1, 13, 14, 15, 16], they also highlight the effects of landscape and habitat management practices and the disruptions caused by human activity to terrestrial ecosystems, especially when viewed from the perspective of economic growth. (Stork *et al*. 2003; Öckinger and Smith 2008, Théodore Munyuli, 2013) [17, 18, 19]. In addition to their vital function in providing ecosystem services, the identification of butterflies becomes a necessary precondition for their employment in this kind of monitoring. Three agroclimatic zones with 29.61% forest cover can be found in Jharkhand, which is located in India's seventh agroclimatic zone. These varied environments are ideal for the presence and abundance of butterflies. Jharkhand's butterflies play a significant function, but their study has not been structured or highlighted. In fact, the review of literature (Hembrom and Sinha, 2012; Singh and Ahmad, 2017; Kumari *et al*., 2021; Kumari and Thakur, 2022; Kumar and Keshari, 2023) [20, 21, 22, 23, 24] reveals complete lack of taxonomic studies for identification and description of butterfly species found in the state. Keeping the above in background and also unavailability of authentic information pertaining to taxonomic study a morphoanatomical as well as morhpomolecular approach of study have been taken up for the first time for identification and description of butterfly species of Jharkhand. In this communication we report the description and identification of *Junonia atlites* belonging to Lepidopteran family Nymphalidae, collected from Horab (Horhap) Forest (23.334038 N 85.464701 E) of Chatra district of The Jharkhand State of India.

Materials and methods

Butterfly sampling

Various parts of the Jharkhand state (table 1) of India were surveyed for specimens of *Junonia atlites*. Several butterflies, were captured and released after preliminary identification was done by examining the traditional morphological characteristics of wings, locale and other information as per the butterfly identification keys (Kunte, 2006; Smetacek, 2022) [25, 26]. For GSA (genome sequence analysis) the specimens from each sampling sites were preserved in 70% alcohol for DNA (deoxy ribonucleic acid) extraction, PCR (Polymerase Chain Reaction) amplification and sequencing. One specimen each from every survey sites, after sacrificing under the ethyl acetate fumes was pinned, spread and submitted to the ICRI (Insect Collection, Record and Identification), Entomology section, Department of Zoology, St. Xavier's College, Ranchi.

Table 1: Details of sampling sites, date of survey, GPS coordinates of sampling sites

Sites	Sampling Site (Jharkhand, India)	Date of Survey	GPS Co-ordinates (N/E)	Voucher No (ICRI)
1	Lotwa Dam, Hazaribag	5th April, 2023	24.150798, 85.382147	SXCRAN/ENT/0423/S16A
2	Horhap Forest, Chatra	21st April, 2024	23.334038, 85.464701	SXCRAN/ENT/0424/S16
3	Raisia, Kolebira	28th March, 2023	22.676007, 84.749159	SXCRAN/ENT/0323/S16B
4	Kasdega, Simdega	25th March, 2024	22.559980, 84.507465	SXCRAN/ENT/0324/S16C
5	St. Xavier’s College, Ranchi	15th April, 2024	23.367615, 85.326720	SXCRAN/ENT/0424/S16D
6	Village Kolomdega, Simdega	21st April, 2024	22.568485, 84.776583	SXCRAN/ENT/0424/S16E
7	Chaibasa Forest, Khuntpani	29th March, 2023	22.632304, 85.711545	SXCRAN/ENT/0324/S16F
8	Latehar Forest, Latehar	8th May, 2024	23.740401, 84.552293	SXCRAN/ENT/0524/S16G
9	Dhangi Pahad, Dhanbad	9th March, 2022	23.821008, 86.486962	SXCRAN/ENT/0322/S16H
10	Masanjor Dam, Dumka	8th March, 2024	24.111078, 87.313855	SXCRAN/ENT/0324/S16I

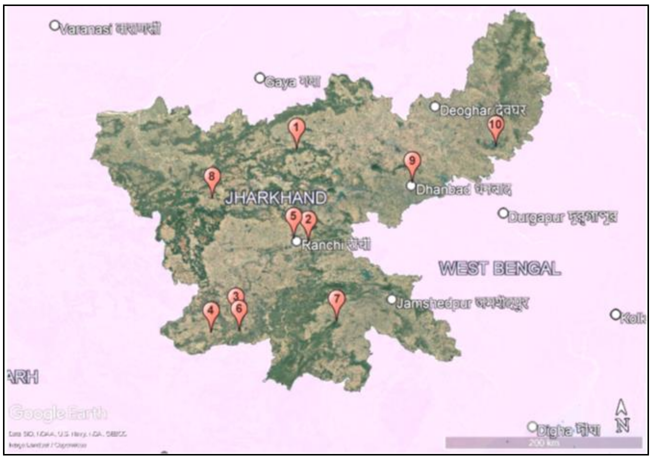

Fig 1: Map of Jharkhand State of India (Google Maps), Showing different Survey locations for *Junonia atlites (1: Lotwa Dam, Hazaribag; 2: Horhap Forest, Chatra; 3: Raisia, Kolebira; 4: Kasdega, Simdega; 5: St. Xavier’s College, Ranchi; 6: Kolomdega village, Simdega; 7: Chaibasa Forest, Latehar; 9: Dhangi Pahad, Dhanbad; 10: Masanjor Dam, Dumka)*

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. In comparison to males, female *Junonia atlites* have thicker and more pronounced markings. On females, there is an ochre tint to the area between the transverse fasciae. The female *Junonia atlite*'s wings are canary-yellow on the ventral side rather than light-grey, and its eye markings are darker and more noticeable (Kunte, 2006; Smetacek, 2022; Mehra *et al*., 2018) [25, 26, 27].

DNA extraction, PCR amplification and sequencing

DNA was extracted from the hind leg of a butterfly specimen. The quality was assessed using a 1.0% agarose gel. A single band of high-molecular-weight DNA was observed. Fragments of the mitochondrial cytochrome c oxidase subunit I (CO1) gene were amplified using particular forward and reverse primers. When resolved on an agarose gel, only one distinct PCR amplicon band were seen. The sequences obtained from each samples varied in their length. The PCR amplicon was further purified to eliminate impurities. The PCR amplicon was sequenced with forward and reverse primers using the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyser (ThermoFisher, 2024) [28].

Molecular sequence analysis tools

The Sangner sequencing approach yielded a distinct PCR amplicon. The resulting PCR amplicon was compared to data in nucleotide databases using the BLAST (Basic local alignment and search tool) software available at NCBI (National Centre for Biotechnology Information). The nucleotide blast (BLASTn) option was employed. BLASTn compares the nucleotide query (our sequence) with the subject sequence (data stored in databases). BLASTn was run with the following parameters: search set: standard database; program selection: highly comparable sequences (megablast). Megablast is used to identify sequences and compare them within species (Srivastava *et al*., 2024) [29].

Submission of nucleotide sequence to the GenBank

The butterfly specimens were determined to be *Junonia atlites* based on the BLASTn search results. Furthermore, the CO1 nucleotide sequences were submitted to the DNA Databank of Japan (DDBJ) and accession IDs for nucleotide sequences were obtained.

Phylogenetic tree and Distance Matrix

The distance matrix and phylogenetic tree were created using all ten *Junonia atlites* species' CO1 nucleotide sequences that were gathered from various survey locations around the state of Jharkhand. The Maximum Composite Likelihood technique was used to construct the evolutionary distances (matrix), which are given in units of base substitutions per site (Tamura *et al*., 2004) [30]. Ten nucleotide sequences were used in this investigation. For every pair of sequences, all ambiguous locations were eliminated (pair-wise deletion). The final dataset contained 1002 locations in total.

The Kimura 2-parameter model and the Maximum Likelihood approach were used to derive the evolutionary history (Kimura, 1980). By applying the Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood technique, the initial tree for the heuristic search was automatically generated. The topology with the highest log likelihood value was then chosen. Ten nuelcotide sequences were used in this investigation. MEGA X (Molecular Evolutionary Genetics Analysis) software, version 10.2.6, build 10210527-x86_64 (Windows 11), was used to conduct these analyses (Kumar *et al*., 2018; Srivastava *et al*., 2024) [29, 31].

Results and discussion

Morphological description

The species belongs to family Nymphalidae of Lepidoptera.

Nymphalidae

This family contains two groups which differ much in general appearance. The fritillaries are orange on the upper sides marked with black, while the under sides have patterns often marked with silver. The other includes aristrocrats some most brilliant coloured butterflies. (Kunte, 2006) [25].

Junonia (Hubner, 1819)

The medium-sized, multicoloured butterflies of this genus have noticeable maculae in the discoidal cell of the forewings. The species has been identified using ocelli, or eye spots, in the hind wings (Brakefield, 1996; Bhojoo *et al*., 2015) [32, 33]. Different eye spot hues, including pink, blue, and orange, as well as variations in size, shape, and arrangement, are characteristics of *Junonia* species (Kodandramaiah and Wahlberg, 2007, Bhojoo *et al*., 2016) [35].

Atlites

Pale-white body with corn-colored eyes followed by large maxillae and gradient orange-tipped antennae along with moderate-sized legs, the maxillae are slightly larger compared to other lepidopterans. Wing span: 54 – 65 mm.

On the dorsal side the butterfly exhibits dark greyish-white wings with a darkened grey boarder line followed by 6 eye spots present on the forewings. Radiating longitudinal black dashes are present on the upper portion of the forewings alongside greying-brown latitudinal bands with transverse, radiating smaller dark bands running longitudinally along the apical half portion. Wing borders are composed of 2-3 dark bands running across the boundaries. The central portion of the hindwings is speckled lightly with glamorous silver scales along with 4 elliptical ocelli aligned together. Pale clouded white wings followed by dark-brown eyes spots with tawny-orange cornea and gunmetal-coloured pupils. The boundaries of the wings are rough and bumby the dark bands present at the borderline run in a meandering pattern throughout.

On the ventral the wings are ghostly-white resembling a dried-out leaf complete with highly detailed complex system of venations., the forewings ventral side has a tint of lavender on the apical halves along with dashes of periwinkle, the females have the light-grey proportion replaced by canary-yellow and darker, more-clearly visible eye-spots. The border lines are extremely darkened on the ventral side.

Fig 2: Photograph of the Underside of the specimen of *Junonia atlites*

Fig 3: Photograph of the Upper side of the specimen of *Junonia atlites*

Forewings

Costa with an open discoidal cell, a broad apex, a broad triangular projection downward at the discal cell, a sinuated termen, angular tornus, concave-shaped termen, rounded tornus, and a slightly arched dorsum; The subcosta (Sc) extends half the length of the costa from the base of the wing; From the base of the wings, Radius (R1) splits into Radius (R2), which is followed by Radius (R3), Radius (R4), and Radius (R5); R4 and R5 reach the termen; Radius (R2) anastomoses with Radius (R3) and reaches the end at the wing's apex; Median (M1) originates from the discoidal cell's angle, Median (M2) originates from the discoidal cell's lower side, and Median (M3) anastomoses with Median. Anal vein (A2) originates from the base of the wing and then separates from the discoidal cell up to the tornus. Cubitus (Cu1) originates from the underside of the discoidal angle, whereas Cubitus (Cu2) is parallel to the cubitus (Cu1) (Bhojoo *et al*., 2016; Mehra *et al*., 2018) [27, 35].

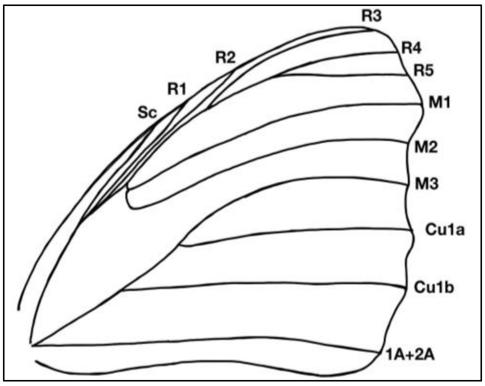

Fig 4: Venation in the forewings of *Junonia atlites*

The hind wings have a leaf-like form, with a rounded apex and costa that arches from the base. The discal cell is open, and the veins Subcosta and Radius (Sc+R) emerge from the base of the wings and are followed and separated from Radius (R1). Humeral (Hu) travel close to the costal margin and emerge from Sc+R and Rs, reaching the termen. The median (M1) emerges from the upper angle of the discoidal cell.

The discoidal cell's bottom side is where the median (M2) originates, while the lower side of the discoidal cell is where the median (m3) anastomoses with the cubital (Cu1). After emerging from a discoidal cell for an uneven distance, cubital Cu1a and Cu1b reach the term, 3A emerges from the base of the wing and reaches half of the dorsum, while anal veins 1A and 2A originate from the base of the cell and reach the dorsum (Bhojoo *et al*., 2016; Mehra *et al*., 2018) [27, 35].

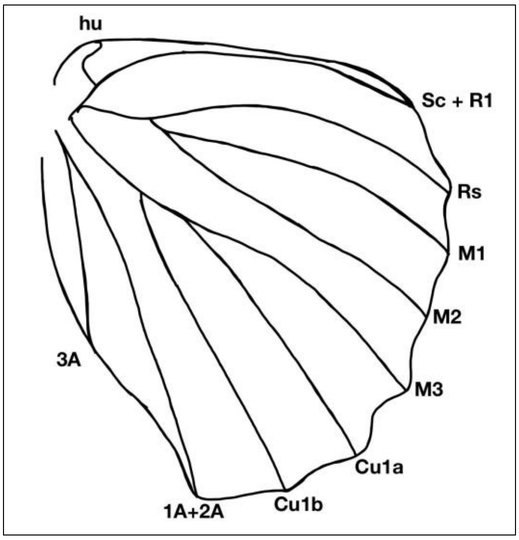

Fig 5: Venation in the hindwings of *Junonia atlites*

Distribution: India, Sri Lanka, Myanmar, Bangladesh, Southern China, Cambodia, Indochina, Malay Peninsula, Western and Central Indonesia, Philippines (Smetacek, 2022) [26].

Morphomolecular description

DNA Extraction, PCR amplification and Nucleotide Sequencing

A single discrete PCR amplicon of CO1 region were obtained using specific primer. The details of the sequences obtained from the 10 specimens are listed in table 2.

Table 2: Nucleotide frequencies and NT length of CO1 nucleotide region of the butterfly samples collected from Different parts of Jharkhand

Site Code	Survey Sites	Accession Ids	Nucleotide Frequencies (%)				
			T(U)	C	A	G	Total
1	Lotwa Dam, Hazaribag	LC847173.1	37.4	17.5	30.4	14.7	326
2	Horhap Forest, Chatra	LC819329.1	38.5	15	30.9	15.6	1002
3	Raisia, Kolebira	LC847172.1	36.5	17.7	30.1	15.7	356
4	Kasdega, Simdega	LC847171.1	38.4	17.3	30.7	13.7	365
5	St. Xavier's College, Ranchi	LC847170.1	38.5	15	30.9	15.6	630
6	Kolomdega Village, Simdega	LC847169.1	37.4	17.5	30.4	14.7	326
7	Chaibasa Forest, Khuntpani	LC847168.1	37.4	17.5	30.4	14.7	326
8	Latehar Forest, Latehar	LC847167.1	37.8	17.3	31.8	13.1	381
9	Dhangi Pahad, Dhanbad	LC847166.1	37.8	17.3	31.8	13.1	381
10	Masanjor Dam, Dumka	LC847165.1	37.2	17.5	30.5	14.8	325

All the resultant nucleotide sequences (query) were compared with the nucleotide databases using BLASTn program based at NCBI. The BLASTn search setting were – Search Set: Standard database; program selection: Highly Similar sequences (megablast). The megablast is used for sequence identification and intraspecies comparision (Zheng *et al*., 2000; Aleksandr *et al*., 2008) [36, 37]. Based on the BLASTn similarity search the specimens were confirmed to belong to the species *atlites* of genus *Junonia*. All the sequences were then submitted to the DDBJ (DNA Databank of Japan) to obtain accession IDS (Table 2). The full definition of CO1 sequences submitted to DDBJ can be viewed by scanning and visiting the links provided in as QR (quick response) codes (Figure 6)

LC847173.1 LC819329.1 LC847172.1 LC847171.1 LC847170.1

LC847169.1 LC847168.1 LC847167.1 LC847166.1 LC847165.1

Fig 6: Accession IDs and QR codes for accessing the full definition (online) of CO1 sequences submitted to DDBJ

Distance matrix and phylogenetic tree

The MEGA X program was utilised to create a distance matrix based on the CO1 nucleotide sequences from ten *Junonia atlites* specimens. Phylogeny uses distance matrices as a non-parametric distance approach. A phylogenetic tree is then created by reconciling these distances. A measure of "genetic distances" between the sequences under study is explicitly used in distance matrix phylogenetic analysis techniques (Mount, 2004) [38].

FASTA-aligned sequences of all the 10 sequences stated in above paragraph were aligned using CLUSTAL-W alignment in MEGA X, and distance matrix phylogenetic tree was constructed using neighbour-joining statistical method. Bootstrap method was employed for test of phylogeny (Saitou and Nei, 1987) [39].

The distance matrix is presented as table 3. The genetic distance among the 10 CO1 sequences ranged from a minimum of 0.000 to a maximum of 0.005. Most distant relationship was observed between the Dhangi Pahad (Dhanbad) strain and Xavier's College (Ranchi) with a genetic distance of 0.005. Similar distance was observed between Dhangi Pahad (Dhanbad) strain and Horhap Forest (Chatra) strain. The Latehar Forest (Latehar) forest strain showed genetic distance (maximum) of 0.005 with Xavier's College (Ranchi) and Horhap Forest (Chatra) strain. On the basis of the distance matrix value obtained, it can be concluded that the specimens of Masanjor Dam (Dumka), Dhangi Pahad (Dhanbad), Latehar Forest (Latehar), Kolomdega Village (Simdega), Kasdega (Simdega), Raisia (Simdega) and Lotwa Dam (Hazaribag) are very closely related to each other with a distance matrix value of 0.000.

Figure 7 displays the phylogenetic tree that was derived using the Maximum Likelihood approach and the Kimura 2-parameter model. Presently displayed is the tree with the highest log probability (-1508.93). Next to the branches, the percentage of trees where the related taxa clustered together is displayed. The Neighbor-Join and BioNJ algorithms were used to automatically generate the initial tree(s) for the heuristic searcher.

Table 3: Distance matrix depicting genetic distances between the 10 specimens of *Junonia atlites* collected from different sampling sites of Jharkhand, India (the colour code depicts the degree of relatedness between the sequences)

Accession IDs	Collection Sites		A	B	C	D	E	F	G	H	I	J
LC847165.1	Masanjor_dam	A		0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.003	0.000
LC847166.1	Dhangi_pahad	B	0.000		0.000	0.000	0.000	0.005	0.000	0.000	0.005	0.000
LC847167.1	latehar_forest	C	0.000	0.000		0.000	0.000	0.005	0.000	0.000	0.005	0.000
LC847168.1	Chaibasa_forest	D	0.000	0.000	0.000		0.000	0.003	0.000	0.000	0.003	0.000
LC847169.1	kolomdega	E	0.000	0.000	0.000	0.000		0.003	0.000	0.000	0.003	0.000
LC847170.1	Xavier_College	F	0.003	0.005	0.005	0.003	0.003		0.003	0.003	0.000	0.003
LC847171.1	Kasdega	G	0.000	0.000	0.000	0.000	0.000	0.003		0.000	0.003	0.000
LC847172.1	Raisia	H	0.000	0.000	0.000	0.000	0.000	0.003	0.000		0.003	0.000
LC819329.1	Horhap_forest	I	0.003	0.005	0.005	0.003	0.003	0.000	0.003	0.003		0.003
LC847173.1	Lotwa_dam	J	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.003	

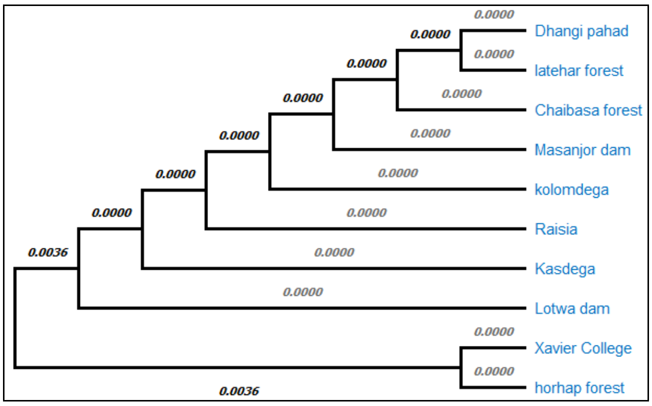

Fig 7: Phylogenetic tree prepared using CO1 sequences of the 10 specimens of *Junonia atlites* collected from different sites of Jharkhand

The phylogenetic tree shows a close relationship between the *Junonia atlites* samples collected from Horhap forest and St. Xavier's College, Ranchi; they both belong to a separate clade as sister taxon. Also their branch length is longest, thus suggesting greater variation. Which is also evident by the distance matrix table, which shows that the samples of *Junonia atlites* collected from Horhap Forest and St. Xavier's College, Ranchi are more distantly related to other samples studied in this work. Rest 8 samples fall into a major clade. The *Junonia atlites* samples collected from Dhangi pahad and Latehar forest also shows close relationships and are present a separate clade as sister taxon. Many preliminary works have been attempted to study the butterflies of the region (Hembrom and Sinha, 2012; Singh and Ahmad, 2017; Kumari *et al*., 2021; Kumari and Thakur, 2022; Kumar and Keshari, 2023) [20, 21, 22, 23, 24], but all of them are limited to just preparation of checklists, which lack any concrete identification criteria for the butterflies, in contrast this work is the first attempt of its kind and it reports the results of morpho-molecular analysis of the *Junonia atlites* specimens collected from various (ten) collection sites spread throughout the Jharkhand state.

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References

1. Kremen C. Biological inventory using target taxa: A case-study of the butterflies of Madagascar. Ecol Appl,1994:4(3):73-80.
2. Libert M. Monitoring of numbers and species composition of the butterfly community of two forested hills near Yaounde, Cameroun. Rev Ecol-Terre Vie,1994:25(2):151–175.
3. Brown JKS. Diversity, disturbance, and sustainable use of Neotropical forests: insects as indicators for conservation monitoring. J Insect Conserv,1997:1(1):25–42.
4. Larsen TB. Forest butterflies in West Africa have resisted extinction ...so far (*Lepidoptera*: *Papilionoidea* and Hesperioidea). Biodivers Conserv,2008:17(12):2833–2847.
5. Özden Ö, Ciesla WM, Fuller WJ, Hodgson DJ. Butterfly diversity in Mediterranean islands and in Pentadaktylos Pinus brutia forests of Cyprus. Biodivers Conserv,2008:17(12):2821–2832.
6. Pozo C, Luis-Martínez A, Lorente-Bousquets J, Salas-Suárez N, Vargas-Fernández I, Warren AD. Seasonality and phenology of the butterflies (*Lepidoptera*: *Papilionoidea* and Hesperioidea) of Mexico's Calakmul region. Fla Entomol,2008:91(3):407–422.
7. Akite P. Effects of anthropogenic disturbances on the diversity and composition of the butterfly fauna of sites in the Sango Bay and Iriiri areas, Uganda: implications for conservation. Afr J Ecol,2008:46(1):3–13.
8. Dover J, Settele J. The influences of landscape structure on butterfly distribution and movement: a review. J Insect Conserv,2009:13(1):3–27.
9. Rogo L, Odulaja A. Butterflies in two forest fragments at the Kenya coast. Afr J Ecol,2001:39(3):266–275.
10. Fitzherbert E, Gardner T, Davenport TRB, Caro T. Butterfly species richness and abundance in the Katavi ecosystem of western Tanzania. Afric J Ecol,2006:44(3):353–362.
11. Marín L, León-Cortés JL, Stefanescu C. The effect of an agro-pasture landscape on diversity and migration patterns of frugivorous butterflies in Chiapas, Mexico. Biodivers Conserv,2009:18(4):4919–4934.
12. Nyamweya NH, Gichuki NN. Effects of plant structure on butterfly diversity in Mt. Marsabit Forest–northern Kenya. Afric J Ecol,2010:48(2):304–312.
13. Kremen C. Assessing the indicator properties of species assemblages of natural areas monitoring. Ecol Appl,1992:2(2):203–217.
14. Howard PC, Davenport TRB, Kigenyi FW, Viskanic P, Baltzer MC, Dickinson CJ, Lwanga J, Matthews RA, Mupada E. Protected area planning in the tropics: Uganda's national system of forest nature reserves. Conserv Biol,2000:14(3):858–875.
15. Cleary DFR. Assessing the use of butterflies as indicators of logging in Borneo at three taxonomic levels. J Econ Entomol,2004:97(2):429–364.
16. Cleary DFR, Mooers A. Butterfly species richness and community composition in forests affected by ENSO induced burning and habitat isolation in Borneo. J Trop Ecol,2004:20(4):359–367.
17. Stork NE, Srivastava DS, Watt AD, Sen TB. Butterfly diversity and silvicultural practice in lowland rainforests of Cameroon. Biodivers Conserv,2003:12(3):387–410.
18. Öckinger E, Smith HG. Do corridors promote dispersal in grassland butterflies and other insects? Landsc Ecol,2008:23(1):27–40.
19. Théodore Munyuli MB. Drivers of species richness and abundance of butterflies in coffee–banana agroforests in Uganda. Int J Biodivers Sci Ecosyst Serv Manag,2013:9(4):298–310. http://dx.doi.org/10.1080/21513732.2012.709539
20. Hembrom A, Sinha BK. A report on Butterfly of reclaimed OBDs of Kathara Coalmine Area, Jharkhand. Asian J Exp Sci,2012:26(2):53-60.
21. Singh N, Ahmad J. A preliminary list of *lepidopteran* insects from Palkot Wildlife Sanctuary, Jharkhand. J Entomol Zool Stud,2017:5(3):654-661.
22. Kumari K, Oraon PK, Kumari K, Kumari A, Banra S, Thakur AK. Inventory report of butterflies (*Lepidoptera*: Rhopalocera) of family *Nymphalidae*, Pieridae and Papilionidae at Kanke, Ranchi. Biospectra,2021:16(2):169–174.
23. Kumari K, Thakur AK. Studies on the biodiversity of some families of butterflies (*Lepidoptera*: Rhopalocera) at Kanke, Ranchi, Jharkhand, India. Biospectra,2022:17(1):93-98.
24. Kumar V, Keshari S. A checklist of butterfly diversity in Bhagwan Birsa Biological part, Ormanjhi, Ranchi, Jharkhand. Biospectra,2023:18(2):163-166.
25. Kunte K. India-A lifescape: Butterflies of Peninsular India, 2006. ISBN: 8173713545. Universities Press: Hyderabad, India.

26. Smetacek P. A naturalist's guide to the Butterflies of India, Bangladesh, Nepal, Pakistan and Sri Lanka, 2022. ISBN978-81-7599-406-5. John Beaufoy Publishing Ltd.
27. Mehra D, Kirti JS, Sidhu AK. A taxonomic study of six species of the genus *Junonia* Hubner, [1819] (Insecta: *Lepidoptera*: *Nymphalidae*) from the northwestern Himalayan region in India. J Threat Taxa,2018:10(7):11934–11947.
28. ThermoFisher. Applied Biosystems 3500 Dx Series Genetic Analyzer (CE-IVD, IVDR). Retrieved on 25th May 2024: 08:02 (IST). Available from: https://www.thermofisher.com/ie/en/home/life-science/sequencing/sanger-sequencing/sanger-sequencing-technology-accessories/applied-biosystems-sanger-sequencing-3500-series-genetic-analyzers/3500-series-genetic-analyzer/3500-dx-cs2-series-genetic-analyzer.html
29. Srivastava R, Kumar M, Ranjan R, Dandapat S, Sinha MP. Morpho-molecular taxonomy and bioinformatics based phylogeny of *Metaphire posthuma* (Sims and Easton, 1972). The Bioscan,2024:19(1):45-52. DOI: https://dx.doi.org/10.2139/ssrn.4847732
30. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA,2004:101:11030-11035. DOI: https://doi.org/10.1073/pnas.0404206101
31. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol,2018:35:1547-1549. DOI: https://doi.org/10.1093/molbev/msy096
32. Brakefield PM. Development, plasticity and evolution of butterfly eyespot patterns. Nature,1996:384:236–242. DOI: https://doi.org/10.1038/384236a0
33. Bhojoo M, Nasreen M, Shakeel M, Mansoor S, Nadir S. First record of two species of genus *Junonia* (*Nymphalidae*: *Lepidoptera*) from Sanghar, Sindh. Pure Appl Biol,2015:4(4):522-527. DOI: https://doi.org/10.19045/bspab.2015.44010
34. Kodandaramaiah U, Wahlberg N. Out-of-Africa origin and dispersal-mediated diversification of the butterfly genus *Junonia* (*Nymphalidae*: Nymphalinae). J Evol Biol,2007:20(6):2181–2191. DOI: https://doi.org/10.1111/j.1420-9101.2007.01425.x
35. Bhojoo M, Memon N, Memon SA, Shah M, Shah NA, Turk JK. Taxonomic note on two species of genus *Junonia* (*Nymphalidae*: *Lepidoptera*). Int J Fauna Biol Stud,2016:3(3):181–184.
36. Zheng Z, Scott S, Lukas W, Webb M. A greedy algorithm for aligning DNA sequences. J Comput Biol,2000:7(1-2):203-14.
37. Aleksandr M, George C, Yan R, Thomas LM, Richa A, Alejandro A, Schäffer. Database Indexing for Production MegaBLAST Searches. Bioinformatics,2008:24:1757-1764.
38. Mount DM. Bioinformatics: sequence and genome analysis, 2nd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 2004.
39. Saitou N, Nei M. The Neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol,1987:4(4):406-425. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a040454