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***Research Article** (Open Access)*

PHYLOGENETIC INSIGHTS AND LIFE HISTORY DYNAMICS OF *DELIAS EUCHARIS* (COMMON JEZEBEL) IN SOUTHERN ANDHRA PRADESH - INDIA

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ABSTRACT
The Common Jezebel*, Delias eucharis* (Drury 1773), placed egg clusters on *Dendrophthoe pentandra* near the Buggavanka Area 14.2559.007"N 7852'51.841"E. Due to the fact that it was multivoltine, it was commonly found in Southern Andhra Pradesh throughout the year and was approaching rarity in the summer. It takes 25–29 days to complete the life cycle from egg to adult. During the previously described period, 80–100% of adults, larvae, and pupae develop effectively. With the discovery of its early stages on *Dendropthoe pentandra*, the lifecycle of *Delias eucharis* monophagus was fully explained along with their phylogenetic tree and the sequence data was done on the barcode region of the mitochondrial gene Cytochrome c oxidase sub unit 1 (COI), which comprises the systematics of the Common Jezebel.

Keywords: *Delias eucharis, Common Jezebel, Phylogenetics, Systematics, Butterflies, Morphological Characteristics*

INTRODUCTION
The order Lepidoptera, which also includes moths, is home to the diverse group of insects known as butterflies (Veronika K.S, *et al.,* 2025) (D. Sowbagya and S. P. Venkata Ramana, 2025).The Common Jezebel or *Delias eucharis* (Drury, 1773) (Westwood, John Obadiah (1773) (Lepidoptera: Pieridae, Pierinae), is a very beautiful butterfly that can be found world wide from sea level to the greatest heights of 6000–7000 feet (Wynter-Blyth, 1957). It is wide spread in India, with the exception of desert areas.This butterfly can be found in both natural and artificial forests, cities, villages, private gardens, nurseries and cultivated areas, where trees harbor *Dendrophthoe sp.,* a semi-parasitic mistletoe (Wynter-Blyth, 1957) a feeding plant for larvae (Varshney and Smetacek, 2015).
An illustration of the evolutionary links between biological entities, typically sequences or species, is a butterfly's phylogenetic tree. The amount of evolutionary change (branch lengths) and topology (branching order) between nodes capture relationships between items. The root serves to clarify ancestry and give these linkages direction (Edwards, 2019). The mitochondrial gene Cytochrome c oxidase sub unit 1 (COI) barcode region has been successfully used to identify butterfly species (Laiho and Stahl's, 2013; Ashfaq *et al.,* 2013), as well as morphologically similar species (Hebert *et al.,* 2004; Gillespie *et al.,* 2013). It has also been used to identify phylogenetic relationships and population genetic structure among species (Dai *et al.,* 2012; Ashfaq *et al.,* 2013; Seraphim *et al.,* 2014; Silva-Brandao *et al.,* 2015; Karthika *et al.,* 2017).

Adult Behaviour
The wingspan of both males and females ranges from 6.5 to 8.5 cm. Its wings are closed when it is at rest, revealing its vividly coloured underside. Its vivid colouring indicates that the toxins the larvae from the host plants have accumulated have made it unappealing. The painted saw tooth, *Prioneris sita*, mimics the common Jezebel, among other distasteful butterflies. The orange-red markings on the hind wing are shaped differently on the common Jezebel. The patches are more arrowhead-shaped in the common Jezebel than they are in the painted saw tooth, where they are much more squarish.

MATERIALS AND METHODS

Methodology

Freshly laid eggs of *Delias eucharis* were collected from *Dendrophthoe pentandra* from the premises of Buggavanka Area 14.2559.007"N 7852'51.841"E., and brought to the Entomology Laboratory of Department of Zoology, Yogi Vemana University and placed in paired petri dishes. The experiment was conducted in semi-natural conditions at 23ºC-27ºC temperature and 50%-60% RH during the months of June (2023)–May (2024). After emergence of the larva, it consumed its egg-shell after that this larva can be transferred into fresh leaves. The remaining of the leaves was replaced by the fresh leaves every 12 hours. During the study development time based on moulting, the different instars were identified. Instars particulars were recorded. Quantitative information on food consumption and usage was gathered for every instar of the species of butterfly and well documented by (Venkata Ramana *et al.,* 2021). After being weighed independently, the host leaves and larvae were put in petri dishes. The weights of the larvae, remaining leaf material, and faeces in the petri dish were measured after the larvae had been permitted to feed on the leaves for a full day. Every 24 hours, weights were recorded and fresh food was provided. Growth and food utilization indices were calculated using fresh weight values. These factors included growth rate (GR), consumption index (CI), and approximate digestibility (AD), also referred to as absorption efficiency. The adult emerged butterfly was preserved in 90% Ethyl alcohol for the molecular work.

CI (Consumption index) = Weight of food consumed/Weight of instar x Number of feeding days
GR (Growth rate) = Weight gained by the instar/ Mean weight of instar x Number of feeding days
AD (Approximate digestibility) = Weight of food ingested – Weight of faeces/Weight of food ingested x 100
ECI (Gross conversion efficiency = Weight gained by the instar/Weight of food ingested x 100
ECD (Net conversion efficiency = Weight gained by the instar/Weight of food consumed-Weight of faeces x 100
The weights were expressed in units of milligrams (mg). The values were based on five different observations for each parameter; standard deviations were also calculated.

DNA Extraction and PCR Amplification

Prior to DNA extraction, mesothorax and metathorax of the selected specimen was collected and they were stored at -20 ^{0}C until analysis. DNA was extracted using a Veriti 96 well (Applied Biosystems) and the QIAGEN DNeasy (USA) extraction kit in accordance with the manufacturer's instructions. PCR amplifications were performed using a thermal cycler. Composition of Taqmaster mix: high-fidelity DNA polymerase, 0.5mM dNTPs, 3.2mM MgCl2, PCR enzyme buffer.

PCR Amplification of COI Gene: The~0.8kbp, LCO-HCO-rDNA fragment was amplified using high–fidelity PCR polymerase. The PCR product was sequenced Bi-directionally.166 ng of extracted DNA is used for amplification along with 10pM of each primer and the sequence data is processed for further findings.

Table 1. PCR conditions.

PCR amplification conditions	Volume
DNA	1 ul
LCO forward primer	2 ul
HCO reverse primer	2 ul
dNTPs (2.5mM each)	4 ul
10X Taq DNA polymerase assay buffer	10 ul
Taq DNA polymerase enzyme (3U/ ml)	1 ul
Water	30 ul
Total reaction volume	50 ul

The PCR programme used for the COI region was 94°C for 3 minutes followed by 40 cycles of 94°C for 1 minute, 54°C for 1 minute and 72°C for 1.5 minutes, with a final extension of 72°C for 10 minutes. PCR products were visualized on a 2% Agarose gel and the purified PCR products were sequenced in the forward and reverse directions.

Table 2. Primers used for PCR reaction.

No.	Oligo name	Sequence (5`à 3`)	Tm (°C)	GC-content
1	LCO Forward	GGTCAACAAATCATAAAGATATTGG	46	32%
2	HCO Reverse	TAAACTTCAGGGTGACCAAAAAATCA	48	34.62%

RESULTS

Egg Stage: Eggs were round flask shaped being narrow at the top, with longitudinal ribs on the surface. Eggs were about 1.00-1.20(1.10±0.20) mm in length and 1.48-1.69(1.60 ±00.9) mm in diameter. The freshly laid eggs were recorded as pale yellow in colour which turned gradually into yellow and finally into yellowish gray before hatching. Egg stage lasted for 3-4 days.

Larval Stage

Instar-I: This stage lasted 2-3 days. Body color was yellowish brown later it becomes yellowish. The freshly hatched larva first feeds on the empty egg shell before moving on to the leaf lamina.When it consumed a leaf-based diet, body develops a noticeable green undertone. Head is black in colour and measures about 0.30-0.40 mm(0.30±0.01)mm in width. The instar body length reached around 1.40–1.55(1.50±0.10)mm on the first day and it grew to a length of 5.20–6.00(5.60±0.40)mm by the end of the instar period. The spines measures about 2.0mm. The caterpillar moults into the second instar after approximately 3days in the first instar. It weighed about 2.81±0.09mg.

Instar-II: This stage lasted 2-3 days. Body color was yellowish brown later it becomes yellowish. The body length reached around 9.5-11.5(10.0±2.0)mm. The spines measures about 4.8mm. It weighed about10.6±0.10mg. The head measures about 0..4 –0.4 (0.45±0.02) mm in diameter.

Instar-III : This stage lasted 3-4 days. Body color was yellowish brown later it becomes yellowish. The body length reached around 17.00–22.00(20.0±1.30)mm. The spines measures about 5.4mm. It weighed about 61.0±0.14mg. The head measures about 0.61–0.69mm (0.63±0.01) in diameter.

Instar-IV: This stage lasted 2–3days. Body color was yellowish brown later it becomes yellowish. The body length reached around 26.00–30.00(29.0±0.12) mm and 4.20–5.30(5.00±0.02) mm in width. The spines measures about 6.4mm. It weighed about 338.0±2.0mg. Head and tail turns to be black in colour. The head measures about 0.80–0.90mm (0.85±0.01) in diameter.

Instar–V: This stage lasted 3–4 days. Body color was golden colour. The body length reached around 48.0±40.00 (46.0±0.16)mm and 5.40-6.40 (6.10±0.02)mm in width. The spines measures about 7.4mm. It weighed about 739.0±2.20mg.

Pupa: This stage lasted 8-10 days. The pupa appears to be lemon yellow in colour. The body length reached around 24.3 mm and width 6.9mm. Its colour turns from yellow into black in meanwhile the body gets black dots both dorsally and ventrally.

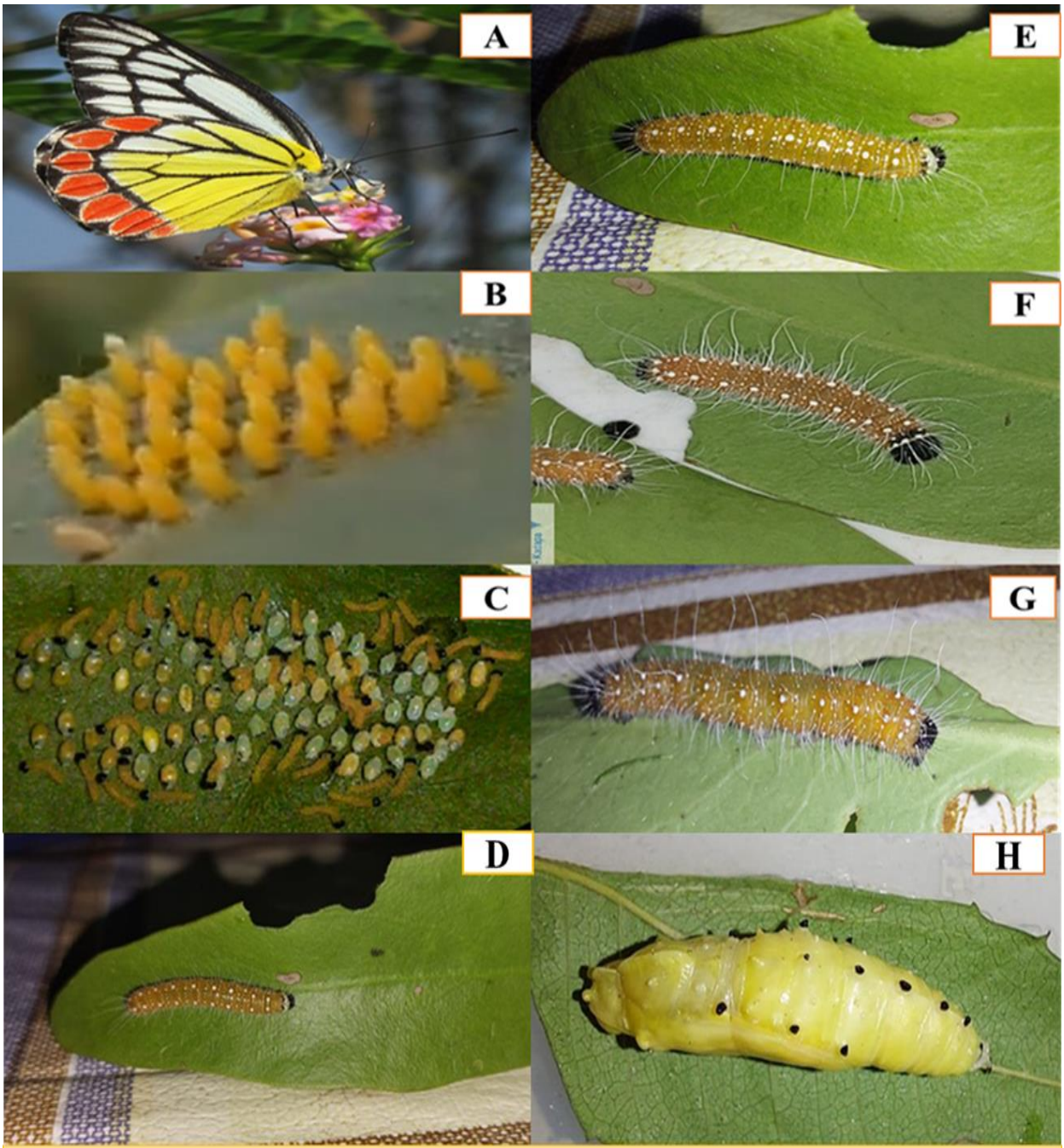

Fig. 1. Delias eucharis life cycle: **A. Adult, B. Egg, C. I-Instar, D. II-Instar, E. III-Instar, F. IV-Instar, G. V-Instar, H. Pupa.**

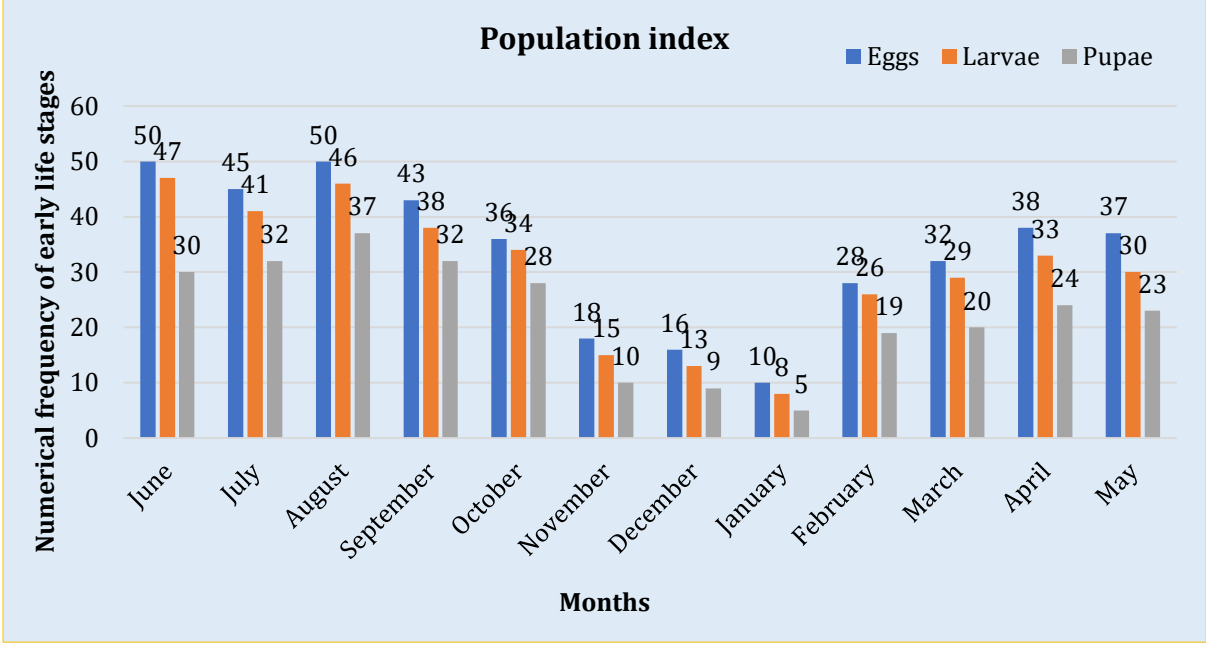

Figure 2. Month wise distribution and numerical frequency of three different early stages of *Delias eucharis*.

Population Index

The numerical frequency of the natural occurrence *of Delias eucharis* life stages–eggs, larvae and pupae, on host plant *Dendrophthoe pentandra* was given.

Table 3. Population index of different life stages of *Delias eucharis* on *Dendrophthoe pentandra* leaves in the field.

Calendar month	Adults' abundance	Number of eggs	Number of larvae	Number of pupae
June	Very common	50	47	30
July	Very common	45	41	32
August	Very common	50	46	37
September	Common	43	38	32
October	Common	36	34	28
November	Rare	18	15	10
December	Rare	16	13	9
January	Rare	10	8	5
February	Common	28	26	19
March	Common	32	29	20
April	Common	38	33	24
May	Common	37	30	23

Duration of Life Cycle

As, the egg stage lasted 3–4 days, larval stage 11–16 days, and the pre-pupal and pupa period 7–8 days, the total developmental period from egg to adult stage spanned over 21–28 days.

Development Success of Eggs, Larvae and Pupae

The laboratory study results hatching success rate of 50–90% during June–January with higher rate recorded during July–October. The success rate of larval development varied between 50-89%, with

higher rate occurring during July to October. Pupal development varied between from 50–88%, with the higher rate being evident during July–October.

Food Consumption and Growth

Table 4. Food consumption and utilization, growth and food utilization efficiencies of *Delias eucharis* on *Dendrophthoe pentandra* leaves.

Instar number	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gain by larvae (mg)	GR (mg/ day)	CI (mg/day)	AD (%)	ECD (%)	ECI (%)
I	30.0 ± 0.22	0.64 ± 0.03	2.81 ± 0.09	0.78	8.1	96	9.57	9.37
II	192.0 ± 1.20	4.90 ± 0.15	10.6 ± 0.10	0.5	4.2	95	12.2	5.52
III	555.0 ± 3.20	50.0 ± 0.62	61.0 ± 0.14	0.44	2.9	89	15.7	11.1
IV	1315.0 ± 3.90	219.0 ± 2.41	338.0 ± 2.00	0.39	2.06	80	23.35	25.7
V	3850.0 ± 12.2	684.0 ± 5.01	739.0 ± 2.20	0.3	1.3	78	30.84	19.2

Growth Rate (GR), Consumption Index (CI), Approximate Digestibility (AD), Efficiency of Conversion of Digested Food (ECD), Efficiency of Conversion of Ingested Food (ECI)

For each of the five instars, the amount of food they eat and the weight they gain are recorded. The larvae's weight and food intake both rose as they progressed through the instars **(Waldbauer, G.P. 1968).** Plotting the instars weight gain against their food intake revealed a clear correlation between the two variables. The percentages of the total meal ingested by the succeeding instars were 0.4, 2.81, 9.00, 21.50, and 62.51%. For weight gain, the corresponding percentages were 0.20, 0.98, 5.19, 28.00, and 63.02%. The weight growth (91.65%) and food consumption (over 82%) profiles thus showed that the last two instars contained a significant portion of both. As the instars grew older, both GR and CI gradually declined. The range of GR values was 0.78 mg/day to 0.30 mg/day, while the range of CI values was 8.10 mg/day/mg to 1.30 mg/day. In both instances, the first instar had the greatest values, while the fifth instar had the lowest.

Phylogenetic Tree

Phylogenetic Tree Builder uses sequences aligned with System Software aligner. A distance matrix is generated using the Jukes-Cantor corrected distance model. When generating the distance matrix, only alignment model positions are used, alignment inserts are ignored and the minimum comparable position is 200. The tree is created using Weighbor with alphabet size 4 and length size 1000.

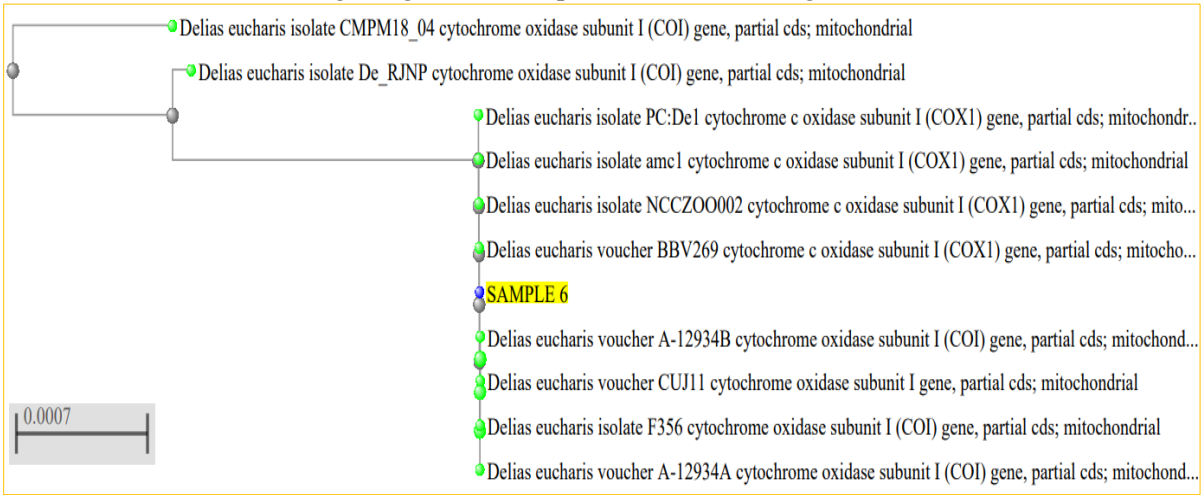

Figure 3. Phylogenetic tree of studied species.

Table 5. BLAST data

S/N	Organism name	Accession No.	% Match
1.	*Delias eucharis isolate* PC: De1 Cytochrome c oxidase subunit I (COX1) gene	OR483967.1	100.00%
2.	*Delias eucharis isolate* amc1 Cytochrome c oxidase subunit I (COX1) gene	ON358410.1	100.00%
3.	*Delias eucharis isolate* NCCZOO002 Cytochrome c oxidase subunit I (COX1) gene	MW846618.1	100.00%
4.	*Delias eucharis voucher* BBV269 Cytochrome c oxidase subunit I (COX1) gene	OQ254745.1	100.00%
5.	*Delias eucharis voucher* A-12934A Cytochrome oxidase subunit I (COI) gene	KX008047.1	100.00%
6.	*Delias eucharis isolate* F356 Cytochrome oxidase subunit I (COI) gene	KJ422911.1	100.00%
7.	*Delias eucharis voucher* CUJ11 Cytochrome oxidase subunit I gene	KT880647.1	100.00%
8.	*Delias eucharis voucher* A-12934B Cytochrome oxidase subunit I (COI) gene	KX008048.1	100.00%
9.	*Delias eucharis isolate* De_RJNP Cytochrome oxidase subunit I (COI) gene	MH675598.1	99.83%
10.	*Delias eucharis isolate* CMPM18_04 Cytochrome oxidase subunit I (COI) gene	JX978938.1	99.67%

Indices of Food Utilization

The values of AD, ECD and ECI are included. The values of AD decreased from a high of 98%in the first instar to a low of 82% in the fifth instar. Both ECD and ECI showed an increasing trend in their values from the first instar to the fourth instar but slightly decreased from the fourth instar to the fifth instar. Values of ECD ranged from 9.57–30.84% and of ECI from5.52–25.70%. While AD decreased as the larvae aged, ECD and ECI showed an opposite trend.

Phylogeny

To determine the evolutionary relationships between biological species, phylogenetic trees are a crucial tool in bioinformatics (Rafiuddin, 2019). Based on the *Delias eucharis* phylogenetic tree and BLAST data, the barcode region of the mitochondrial gene Cytochrome c oxidase sub unit 1 (COI) had a 98% sequencing success rate. The -696 bp area was amplified and sequenced. The sequence was added to BOLD and is currently in the process under the KSVGP project folder. Additionally, the data showed how well the COI barcode area delineates closely related species. To sum up, this work offers a more thorough phylogeny of *Delias eucharis*. According to our research, some families show a great deal of morphological convergence, which makes conventional taxonomic classifications more difficult. Additionally, this analysis finds a number of potentially new species and taxonomic changes that are supported by molecular data. These discoveries enhance our knowledge of butterfly systematics and offer crucial information for conservation plans meant to maintain butterfly variety in the face of environmental change on a worldwide scale. This finding opens the door for future investigations into ecological interactions, evolutionary biology, and species conservation in the context of Lepidopteran diversity by establishing a thorough phylogenetic framework. However, we have helped to improve knowledge of the *Delias eucharis* taxonomic status, identification, phylogenetic relationships, and bio geographic history.

DISCUSSION

The life history stages of *D. eucharis* were thoroughly examined, and the results showed a strong relationship between environmental conditions, reproductive techniques, and lifespan (Roy choudhury *et al.,* 2005). Important information on *Delias eucharis* evolutionary relationships within the Pieridae family was uncovered by the phylogenetic study. A better understanding of *D. eucharis* evolutionary history is made possible by placing it among closely related species (Akito Y. Kawahara *et al.,* 2023).Its current genetic diversity is influenced by gene flow and possible hybridization occurrences, which are shown by comparison with closely related species(K. S. Veronika, K. Haripriya, and S. P. Venkata Ramana. (2025). The significance of conservation initiatives aimed at particular *D. eucharis* populations is highlighted by the evolutionary findings. For upcoming comparative studies of butterflies, the molecular data presented here provide a baseline (Wiley, *et al.,*1991; William *et al.,*2000). It suggests that the barcode region of -696 bp area of the mitochondrial gene Cytochrome c oxidase sub unit 1 (COI) was amplified and sequenced and its taxonomic status, identification, phylogenetic relationships were known that it has slight difference with 2 species when compared with the 10 neighbour species from BLAST data(Kshanika Goonesekera *et al.,* 2019), (Edwards, 2019) the difference may be due to geographic region, climatic conditions.

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