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Eco-biology of the blue pansy butterfly, *Junonia Orithya* L. (Lepidoptera: Rhopalocera: Nymphalidae) from the east coast of Southern India

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Abstract
This study presents the first description of the life history of the Blue pansy butterfly *Junonia orithya* L., focusing on larval performance, specifically food consumption and utilization, and the duration of its life cycle on its host plant *Evolvulus alsinoides* (L.) L. The research was conducted in 2023 in Visakhapatnam, South India (17° 42' N and 82° 18' E). The complete life cycle of *Junonia orithya* spans 25 to 32 days (mean 27.40±2.89 days), with the following stages: Egg: 3 days; Larva: 16 to 22 days; Pupa: 6 to 7 days. Nutritional indices were measured across various instars under laboratory conditions, with a temperature of 28±2 °C and a relative humidity of 80±10%. The Approximate Digestibility (AD) ranged from 44.13% to 92.39%, Efficiency of Conversion of Digested food (ECD) from 1.92% to 25.56%, and Efficiency of Conversion of Ingested food (ECI) from 1.77% to 11.28%. These high ECD and ECI values likely contribute to the ecological success of *J. orithya* in the studied environment.

Keywords: Life history, *Junonia orithya*, captive rearing, immature stages, food utilization indices

Introduction
Butterflies are celebrated for their undeniable beauty and the vibrant colors of their wings, making them an integral part of the natural aesthetic. Besides their beauty, they play a crucial role as pollinators, which underscores the importance of their conservation - a global concern (New *et al*., 1995; Withgott, 1999) [13, 26]. Despite this, conservation efforts often overlook butterflies, even though they are deserving of protection (Owen, 1971) [14]. Accurate life history data of butterfly species in a given region is essential for effective conservation management. Unfortunately, such knowledge is lacking for many Indian butterflies (Gay *et al*., 1992; Gunathilagaraj *et al*., 1998) [8, 10]. Efforts to fill this gap have been made, particularly for butterflies in South India (Atluri *et al*., 2004; Samatha *et al*., 2008; Atluri *et al*., 2010) [2, 19, 1]. In this study, we provide detailed information on the immature stages, larval performance on the host plant *Evolvulus alsinoides* (L.) L., and the duration of the life cycle from egg to adult emergence for the Blue pansy butterfly *Junonia orithya* L. 1758.

Materials and Methods
This study was carried out in Visakhapatnam, located on the eastern coast of India in Andhra Pradesh, during the year 2023 (latitude 17° 42' N, longitude 82° 18' E). The methodology for captive rearing involved collecting eggs from wild-mated females, raising the larvae to adulthood in a controlled environment, and subsequently releasing the adult butterflies or pupae back into the wild, following the protocol outlined by Crone *et al*. (2007) [7]. Observations of the reproductive activities of the Blue pansy butterfly *Junonia orithya* were conducted daily between 0800 and 1500 hours at two locations: the Andhra University campus and a nearby Zoo Park, approximately 5 km from the campus. After identifying oviposition sites, the leaves bearing eggs were collected in Petri dishes (15 cm × 2.5 cm) and brought to the laboratory. The leaf pieces with eggs were placed in smaller Petri dishes (10 cm × 1.5 cm) lined with moistened blotter paper to prevent desiccation. These dishes were then stored in a clean, spacious cage fitted with a wire gauge. Since no ant predation was observed, no special protection against predators was necessary. The eggs were monitored every 6 hours to record hatching times.

Newly hatched larvae were transferred to clean Petri dishes lined with moistened blotter paper using a camel hairbrush. The larvae were provided daily with a weighed amount of fresh leaves from the host plant. Fecal matter and leftover food were collected and weighed daily (over a 24-hour period). As the larvae grew, their space requirements increased, and they were transferred to larger Petri dishes (15 cm × 2.5 cm). Larval performance in terms of food utilization indices was calculated using the method described by Waldbauer (1968) [25].

All parameters were studied with five replications, using fresh weight measurements. The development of the pupa from the fully grown larva was recorded, including details on color, shape, size, weight, and the timing of adult emergence. Millimeter graph paper was used for all measurements. The laboratory conditions were maintained at a temperature of 28±2 °C and a relative humidity of 80±10%, with normal indirect sunlight, varying between 12 hours in November/January and 14 hours in June/July.

For describing adult characteristics, butterflies that emerged from the pupae in the laboratory, as well as those captured in the wild, were used.

Results

Adult Stage (Refer to Figure 1a)

Field Characteristics: The female butterfly is noticeably larger than the male. The upper side of the forewing exhibits a black hue at the base, transitioning to light brown towards the apex. In the hindwing, there is a blue marking, which is more prominent in the male than in the female. Both wings have two orange ocelli. The underside of both sexes is grayish, adorned with white lines and spots, with ocelli visible only on the forewings. The wingspan ranges from 40 to 60 mm.

Behavior

This butterfly predominantly flies close to the ground and frequently pauses to bask on the ground.

Nectar Sources

In the study area, the butterflies feed on nectar from plants like *Antigonon letopus* Hook & Arn., *Lantana camara* L., and *Spermacoce hispida* L.

Oviposition Host Plant

The plant used for egg-laying in the study area was *Evolvulus alsinoides* (L.) L.

Egg Stage (Refer to Figure 1b)

Oviposition occurs between 1000 and 1400 hours. The gravid female deposits eggs singly on either the underside near the edge or the upper side near the petiole of both fresh and mature leaves. Typically, 6 to 10 eggs are laid at a time, but on different leaves. The eggs are green, spherical, with longitudinal ridges, and measure between 0.80 to 0.90 mm in height. They hatch within 3 days. Immediately after hatching, the larva consumes its eggshell and proceeds through 6 instars over a period of 16 to 22 days.

Larval Stage (Refer to Figure 1c-h)

Instar I

This stage lasts 3 to 4 days. The larva, upon hatching, measures approximately 2.00 mm in length, eventually growing to between 2.20 and 2.40 mm in length and 0.80 to 0.85 mm in width. The head is black, measuring between 0.70 and 0.80 mm in diameter. The body is chocolate-colored, covered in fine hairs.

Instar II

Lasting 2 to 3 days, the larva reaches a length of 2.85 to 3.65 mm and a width of 0.85 to 1.20 mm. The head measures between 1.00 and 2.40 mm in diameter. The body becomes thicker and darker, nearly black, and is cylindrical in shape.

Instar III

Lasting 1 to 2 days, the larva grows to a length of 4.00 to 7.00 mm and a width of 1.13 to 1.80 mm. The head measures 2.50 to 3.50 mm in diameter. A yellowish band appears just behind the head, marking this stage.

Instar IV

This stage lasts 2 days. The larva reaches a length of 6.00 to 11.00 mm and a width of 1.65 to 2.80 mm. The head measures 3.50 to 4.00 mm in diameter. The yellowish band that emerged in the previous stage becomes more prominent, and the larva develops six rows of hairs along its body length and twelve rows across its body.

Instar V

This stage lasts 1 to 2 days. The larva reaches a length of 13.50 to 16.50 mm and a width of 2.56 to 3.70 mm. The head measures 5.00 to 6.00 mm in diameter, and the body turns black with shiny hair bases.

Instar VI: Lasting 4 days, the larva grows to a length of 25.75 to 28.80 mm and a width of 3.48 to 4.50 mm. The head measures 6.00 to 7.50 mm in diameter. The yellowish band fades to black as the larva ceases feeding and contracts in preparation for pupation.

Pupal Stage (Refer to Figure 1i)

This stage lasts 6 to 7 days. The pupa measures between 15.00 and 17.00 mm in length and 5.00 to 6.00 mm in width at its broadest point. It is chocolate-colored with two rows of small, grayish-white spiny projections along its dorsal side. The pupa weighs between 284.30 and 351.50 mg.

The specific biological observations are detailed in Table 1.

Duration of Life Cycle

The total duration of the life cycle, from egg to adult emergence, ranges between 25 to 32 days (mean 27.40±2.89 days). The egg stage lasts 3 days, the larval stage 16 to 22 days, and the pupal stage 6 to 7 days.

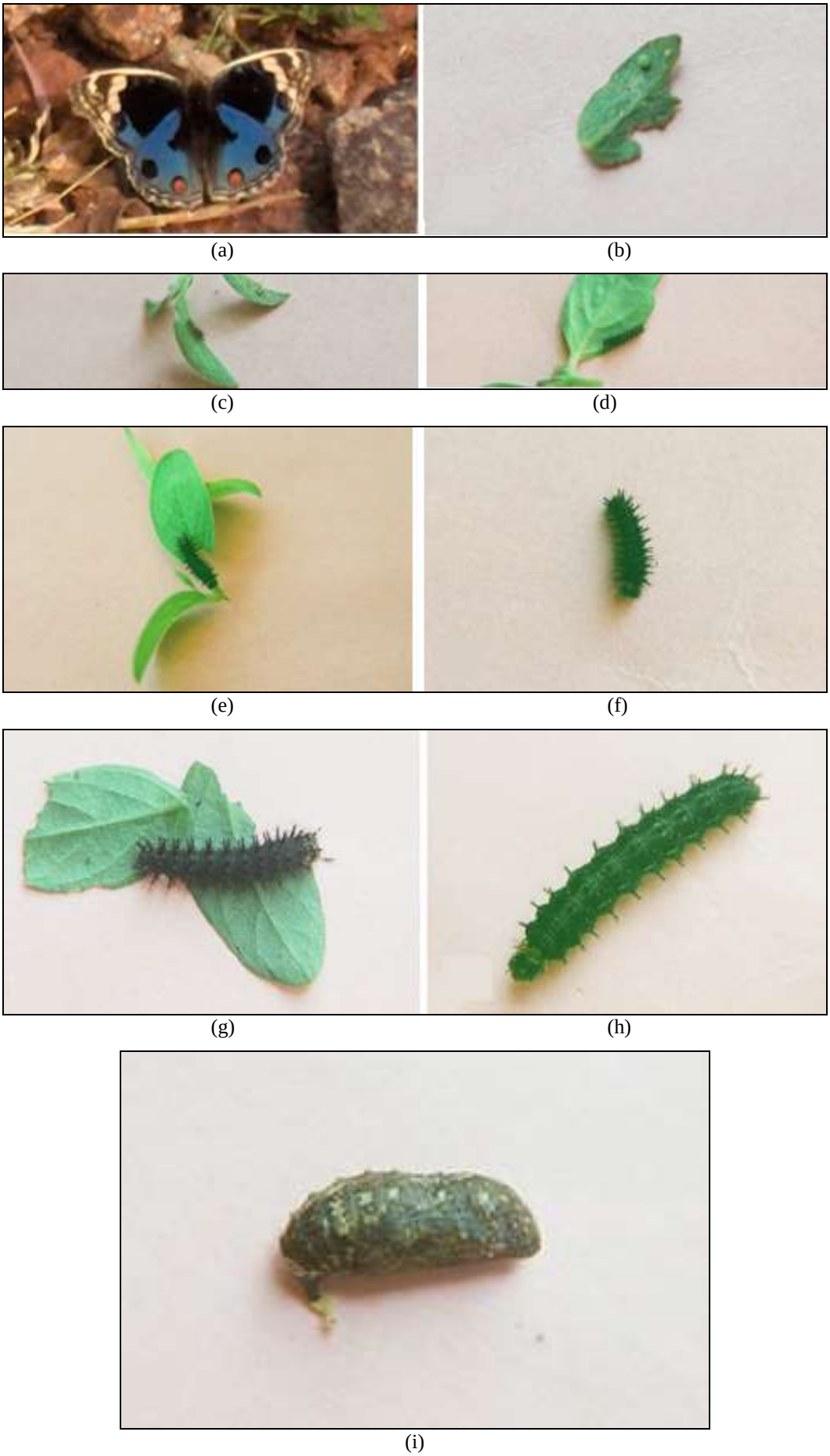

Fig 1: Life stage of *Junonia orithya* L. (a) Adult, (b) Egg, (c) Instar I, (d) Instar II, (e) Instar III, (f) Instar IV, (g) Instar V, (h) Instar VI, (i) Pupa.

Table 1: Biological observations of early life stages of *Junonia orithya* on *Evolvulus alsinoides*

Stage	Length(mm)			Width (mm)			Duration (Days)	
	Min.	Max.	AV.±S.D.	Min.	Max	AV. ±S.D.	Range	AV.±S.D.
Egg	0.80	0.90	0.88±0.04	0.80	0.90	0.88±0.04	3-3	3.00±0.00
Larva								
Instar I	2.20	2.40	2.31±0.07	0.80	0.85	0.81±0.02	3-4	3.20±0.45
Instar II	2.85	3.65	3.33±0.29	0.85	1.20	1.02±0.12	2-4	3.00±1.00
Instar III	4.00	7.00	5.50±1.41	1.13	1.80	1.33±0.27	2-4	2.80±0.84
Instar IV	6.00	11.00	8.85±2.19	1.65	2.80	1.97±0.48	2-4	2.40±0.89
Instar V	13.50	16.50	14.59±1.40	2.56	3.70	2.97±0.48	2-5	3.20±1.10
Instar VI	25.75	28.80	26.94±1.22	3.48	4.50	4.03±0.38	4-5	4.60±0.55
Total larval period days							16 - 22	18.20±2.49
Pupa	15.00	17.00	16.00±1.00	5.00	6.00	5.40±0.55	6-7	6.20±0.45

Food Consumption, Growth, and Utilization

Table 2 presents data on the food consumed by each of the six larval instars and the corresponding weight gain. The percentage of total food consumed by each instar was as follows: 0.53% (Instar I), 0.69% (Instar II), 2.30% (Instar III), 4.36% (Instar IV), 11.74% (Instar V), and 80.37% (Instar VI). Correspondingly, the weight gain percentage for each instar was 0.08%, 0.25%, 1.44%, 5.12%, 13.91%, and 79.20%. Thus, over 92% of the total food consumption and 93% of the total weight gain occurred in the fifth and sixth instars combined. A direct relationship was observed between food consumption and growth across the six instars (Refer to Figure 2).

The growth rate (GR) values increased until instar IV and then decreased by instar VI, while the consumption index (CI) decreased progressively from the first to the final instar. GR values ranged between 0.16 to 0.47 mg/day/mg, and CI values ranged between 1.84 to 9.02 mg/day/mg.

Table 2 also includes indices of food utilization efficiency: Approximate Digestibility (AD), Efficiency of Conversion of Ingested food (ECI), and Efficiency of Conversion of Digested food (ECD). The AD values decreased from 92.39% in the first instar to 44.13% in the final instar. ECD values increased progressively, ranging from 1.92% to 25.56%. ECI values increased from the first to the fifth instar but decreased in the final instar, ranging between 1.77% and 13.56%. There was an inverse relationship between AD and both ECD and ECI.

Table 2: Food consumption, growth and food utilization efficiencies of *Junonia orithya* larva fed with *Evolvulus alsinoides* leaves.

Instar	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gained by larva (mg)	GR (mg/day/mg)	CI (mg/day/mg)	AD (%)	ECD (%)	ECI (%)
I	23.66±02.85	1.80±00.50	0.42±00.05	0.16	09.02	92.39	01.92	01.77
II	30.54±04.25	2.94±00.48	1.26±00.58	0.25	06.05	90.97	04.56	04.12
III	101.60±10.19	10.42±01.48	7.28±00.98	0.38	05.38	89.74	07.98	07.16
IV	192.54±14.78	36.41±05.09	25.89±04.26	0.47	03.50	81.09	16.58	13.45
V	518.84±45.52	214.58±11.56	70.38±09.87	0.26	01.94	58.64	23.13	13.56
VI	3551.70±96.74	1984.22±48.27	400.59±15.53	0.21	01.84	44.13	25.56	11.28

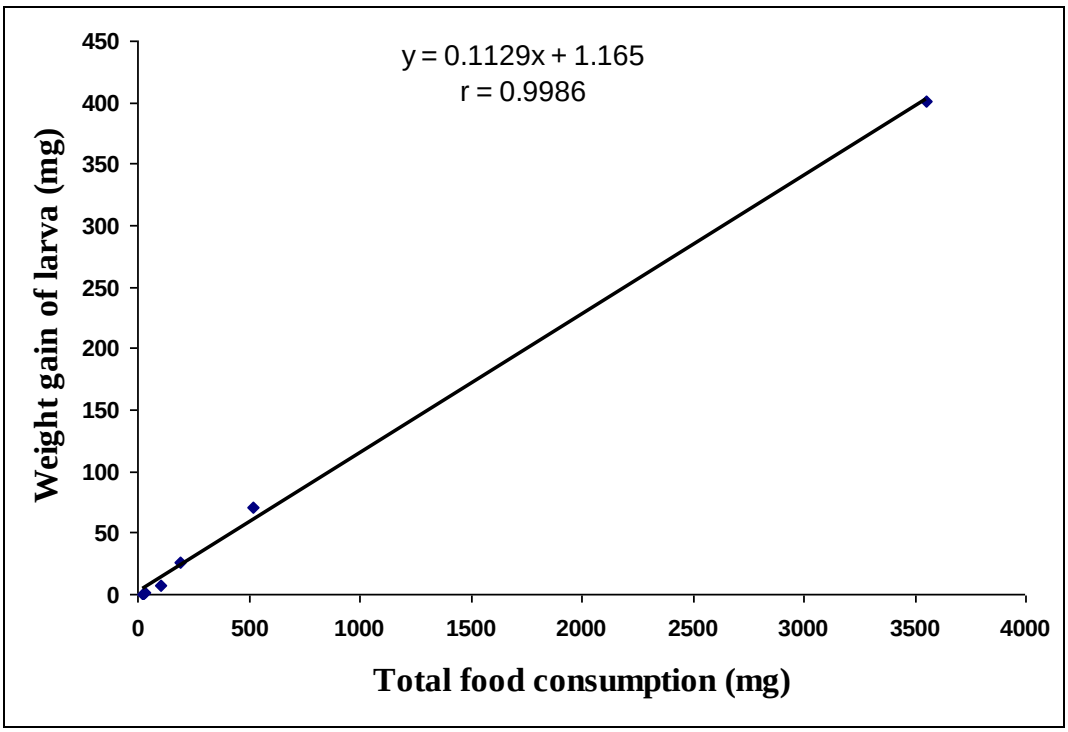

Fig 2: Relationship between food consumption and growth in *Junonia orithya* on *Evolvulus alsinoides.*

Discussion

The total development time from egg-laying to adult emergence was approximately 27.40±2.89 days at around 28±2 °C, consistent with expectations of short life cycles in tropical butterflies (Owen 1971) [14]. As temperature influences the duration of instar stages and overall development time (Mathavan & Pandian 1975; Palanichamy *et al*. 1982; Pathak & Pizvi 2003; Braby 2003) [11, 15, 18, 6], the life cycle duration may vary depending on prevailing temperatures. However, since Visakhapatnam experiences minimal temperature extremes, the life cycle duration remained relatively consistent across overlapping seasons.

Throughout its growth, larvae consumed an average of 4.42 g of leaf material, with increased consumption observed in the last two instars. This trend is consistent with general observations in lepidopterous larvae, where greater consumption in the final instars compensates for the energy demands of the non-feeding pupal stage (Pandian 1973; Waldbauer 1968; Mathavan & Pandian 1975; Scriber & Slansky 1981; Palanichamy *et al*. 1982; Selvasundaram 1992; Gosh & Gonchaudhuri 1996) [16, 25, 11, 21, 15, 22, 9]. The CI values are within the range predicted for forb foliage chewers (Slansky & Scriber 1985) [23]. The rate of food consumption depends on the efficiency of converting ingested food to biomass (ECI); higher CI values are associated with lower conversion efficiency and vice versa. This relationship is evident in the high CI value (9.02) observed in the first instar, reflecting the low ECI values at this stage compared to later instars. Growth rates tend to be higher in penultimate instars than in final instars, a pattern observed in *Junonia orithya* (Scriber & Feeny 1979) [20].

The AD values obtained in this study are comparable with the range of values (19% to 81%) reported for lepidopterous larvae (Pandian and Marian 1986) [17]. The average AD value of over 76.16% supports the assertion by Slansky and Scriber (1985) [23] that foliage chewers often achieve high AD values, particularly when the food source is rich in nitrogen and water (Pandian and Marian 1986) [17]. Similar results have been observed in *Pieris brassicae* (L.) (Yadava *et al*. 1979) [27], *Euploea core* (Cramer) (Venkata Ramana *et al*. 2001) [24], *Ariadne merione merione* (Cramer) (Atluri *et al*. 2010) [1], and *Byblia ilithyia* Drury (Bhupathi Rayalu *et al*. 2011) [5], and *Zizula hylax hylax* Fabricius (Bhupathi Rayalu & Suneetha 2022) [4].

ECD values tend to increase from early to late instars, a trend observed in the ECD values of *Junonia orithya*, with the lowest value in the first instar and the highest in the sixth. Although the ECD values were lower than the AD values, such results are not uncommon (Waldbauer 1968) [25], indicating low efficiency in converting digested food into body tissues. This inefficiency may be due to deficiencies in essential nutrients in the food or factors that increase metabolic energy expenditure (Bailey and Mukerji 1976; Muthukrishnan 1990) [3, 12]. The ECI values followed a pattern similar to the ECD values. The ECI values obtained (1.77% to 13.56%) align with the expected range for forb foliage chewers (1% to 78%) (Slansky and Scriber 1985) [23]. The pattern of ECI values paralleled that of AD, as suggested by Waldbauer (1968) [25]. The relatively high ECD and ECI values in the final two instars (23.13% to 25.56% and 13.56% to 11.28%, respectively) indicate efficient tissue and ecological growth, which likely contributes to the successful survival of *Junonia orithya* in the study environment.

Conclusion

The information gathered in this study, including oviposition behavior, larval host plants, larval performance in terms of food consumption, growth, utilization, and the complete life cycle from egg to adult emergence, can be effectively utilized in the conservation and management of *Junonia orithya*. These insights may be particularly beneficial in environments such as parks, zoos, and butterfly houses, which are well-planted with flowering plants and shaded areas conducive to butterfly survival.

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