

Mating Behaviour and Egg Laying Pattern of the Areca Nut White Grub, *Leucopholis lepidophora* (Coleoptera: Scarabaeidae)

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ABSTRACT

The mating behaviour and egg laying pattern of the areca nut white grub, *Leucopholis lepidophora*, were studied in the Shivamogga district's Hosanagara taluk. Females were active between 18:40 and 19:20 hrs, when light intensity dropped to 83.62 lux. The most males approached the virgin female at 18:50 hrs (15.94 lux) and the session ended at 19:20 hrs (0.26 lux). The typical mating behaviour of *L. lepidophora* was investigated. Female beetles began abdominal expansion and contraction, which could indicate that semiochemical secretion has begun. Males then approached, calling out to females. Male raised all of its legs, turned its body ventrally, and became suspended by the genitalia after inserting genitalia into the female abdomen. Copulation lasted an average of 74.60±6.61 minutes. The adult female's pre-oviposition period, oviposition period, and postoviposition period were 9.56± 2.04 days, 3.72± 2.35 days, and 5.24± 4.67 days, respectively. Female longevity ranged from 11 to 28 days, with an average of 18.52± 4.91 days. The incubation period was 9.56± 2.04 days, with varying fecundity and clutch intervals ranging from 24-48 hours. Experiment on egg laying pattern revealed that females waited an average of 9.56± 2.04 days before oviposition began, after which they began to lay varying numbers of eggs at an average clutch interval of approximately 24-48 hours.

Keywords: Lux, semiochemical, Copulation, fecundity, virgin females.

Adarsha, S. K., Kalleshwaraswamy, C. M., & Shivanna, B. K. (2023). Mating behaviour and egg laying pattern of the areca nut white grub, *Leucopholis lepidophora* (Coleoptera: Scarabaeidae), *Journal of the Entomological Research Society*, 25(2), 241-251.

Received: September 28, 2021

Accepted: March 19, 2023

INTRODUCTION

White grubs are the severe arecanut pest in India. Many species of white grubs are notorious pests of many cultivated crops in India. The arecanut root grubs belong to the Melolonthinae subfamily, and the genus *Leucopholis* Dejean, (Coleoptera: Scarabaeidae: Melolonthinae) is an important pest species feeding on roots of areca nut in malnad and coastal belts of Karnataka (Adarsha, 2014; Kalleshwaraswamy, Adarsha, Naveena, & Sharanabasappa, 2015). The genus *Leucopholis* were successfully identified by male genital structures such as aedeagus and endophallus differed significantly between three species namely *L. lepidophora* Blanchard, *L. burmeisteri* Brenske and *L. coneophora* Burmeister. They were successfully identified by employing COXI gene (Mahadeva Swamy, Asokan, Kalleshwaraswamy, & Adarsha, 2019). Grubs of these *Leucopholis* beetles cause damage to the roots by feeding resulting in symptoms like yellowing of leaves, stem tapering, and nut fall ultimately leading reduced vigour, yield loss and death of palm (Kumar, 1999). Affected palms loose anchorage due to loss of roots and topple down when disturbed (Nair & Daniel, 1982). Six to eight grubs are enough to kill palms (Prakash, Kumar, & Ravikumar, 2011). Severe damage and yield loss noticed in paddy field converted areca nut gardens because of sandy loam soil (Adarsha, 2014). Kalleshwaraswamy, et al, 2015 reported that there was 27.86-36.97 per cent damage by *L. lepidophora* with a yield reduction of 39.79-41.60 per cent in different districts of hilly regions of Karnataka. *L. burmeisteri*, found to be restricted to the coastal area and reported to cause 28.80 per cent damage with a yield reduction of 39.79-41.16 per cent.

The early instar larvae generally feed on the humus for survival (Veeresh, 1977) which may not call for closer association with the host plants. Later instars are near to the root zone and feed on the roots of the areca nut (Veeresh, 1977; Kumar, 1999; Adarsha, 2014). Several integrated management strategies have been adopted to tackle the menace of different species of areca white grubs (Veeresh, Vijayendra, Reddy, & Rajanna, 1982; Kumar, 1999). This includes larval collection by digging soil, soil application of biocontrol agents and insecticides, flooding with insecticidal use and adult collection during their emergence (Veeresh et al, 1982; Kumar, Prakash, Belavadi, & Chandrashekara, 2011; Prabhu, Rakesha, & Balikai, 2011; Adarsha, Kalleshwaraswamy, Pavithra, & Sharanabasappa, 2015b; Adarsha, Shivanna, & Kalleshwaraswamy, 2017; Kalleshwaraswamy, Adarsha, Naveena, & Kumar, 2017; Adarsha, Pavithra, Kalleshwaraswamy, & Kavita Hegde, 2018). Generally, control of soil-dwelling pests of areca nut through insecticide application not exposed to direct contact of insects with the chemicals and other soil properties may hinder the effectiveness of the chemicals (Arakaki et al., 2004b). Chemical applications to soil may create an adverse effect on soil arthropods, earthworms and other natural enemies as well (Adarsha, Kalleshwaraswamy, & Pavithra, 2015a) and also contaminate the groundwater (Fukaya et al, 2009). Hence, there is a need to develop eco-friendly and environmentally safe management practices.

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Adults of *L. lepidophora* emerge from June to October from the ground to mate in the evening (Kumar, 1999; Chanakeshava, 2006; Adarsha, 2014; Kalleshwaraswamy, Adarsha, Naveena, & Sharanabasappa, 2016). The previous studies have indicated that a female beetle secretes sex pheromone to which the male beetles are attracted. Once emerged, the males are attracted to the pheromone and hover around the female. By this, an innovative idea of a female baited trap was designed to attract and collection of male beetles. Synthetic sex pheromones have the potential for monitoring and control but also the advances knowledge of *L. lepidophora* mating behaviour, and egg laying pattern is required. Hence, this study is planned to find out information on the mating behaviour of areca nut white grub beetles.

MATERIALS AND METHODS

Experimental site

The study conducted in white grub infested areca nut garden at Gulukoppa (Hosanagara taluk, Shivamogga district; 13°52' N; 75°12' E, 692 msl). Twenty-year-old gardens with a white grub incidence of about 50 per cent selected for the experiment.

Calling time and male attraction by virgin females

Virgin females placed in a bucket trap with a small plastic box. These traps were individually tied on the stems of areca nut at 5 feet height and 10 m distance. The virgin females were set at 18:30 hours. This experiment repeated for five times in different days to get more accuracy. Calling posture and attraction of male beetles were recorded at every 10 minutes interval. All males that approached the females were caught in the bucket trap, every 10 min count of the males were taken. Light intensity was measured at 5 feet above the ground for every 10 min from 18:30 to 19:30 hrs. Light intensity measured using a digital lux meter (B. S. K. Technologies, Equinox™ Model number: EQ-1301).

Mating behaviour and copulation duration

Field observations made during the emergence period immediately after dusk, i.e., between 18:30 to 21:00 hrs by visually searching throughout the arecanut garden. Mating behaviour and copulation duration of 15 newly emerged *L. lepidophora* were observed in areca nut garden at different time intervals. From 18:30 to 21:00, every 15 min intervals pairs in mating were visually surveyed with a head torch. The time of start and ending of copulation were recorded.

Egg laying pattern of the field-collected mated female of *L. lepidophora*

A laboratory experiment was conducted by using field mated beetles. Freshly mated females were collected from the field and then transferred to the laboratory for further studies. Field collected mating pairs were placed individually in plastic containers (17cm ht. X 13 cm dia.) for egg laying. These plastic containers were filled with moist sandy loam soil which was previously taken from the white grub infested

field. The adults collected were maintained until the females die. Pre-oviposition, Oviposition, post-oviposition fecundity and longevity of female beetle were recorded. Observations were taken daily and clutch sizes, inter-clutch interval and eggs per clutch were recorded.

RESULT

Calling and male attraction by virgin females

Virgin females activity started at around 18:40 hrs when light intensity fell to 83.62 lux (Table 1). This considered as a calling period of adult female beetles. Calling behaviour of virgin female beetles increased at 18:50 hrs and light intensity was 15.94 lux, but it was ceased at 19:20 hrs (0.26 lux). Males are started to approach calling virgin females at 18:40 hrs (83.62 lux). The highest number of males was approached to virgin female at 18:50 hrs (15.94 lux) and terminated at 19:20 hrs (0.26 lux).

Table 1. Calling females and male attraction by virgin females of *L. lepidophora* in the arecanut ecosystem in Gulukoppa, Hosanagara.

Time of day (hrs)	Number of males attracted by virgin female*	Calling female (%)*	Light intensity (Lux)
	Mean±SD	Mean±SD	Mean±SD
18:30-18:40	0.00	0.00	222.40±90.25
18:40-18:50	10.40±4.77	41.33±19.67	83.62±41.63
18:50-19:00	45.80±23.89	52.00±7.30	15.94±5.18
19:00-19:10	12.40±3.65	53.33±12.47	2.96±0.85
19:10-19:20	3.20±2.59	17.33±13.82	0.54±0.18
19:20-19:30	0.00	0.00	0.26±0.21

*N=75

Mating behaviour of adult *L. lepidophora*

The sequential mating behaviour of adult *L. lepidophora* represented in figure 2. Before mating, males were emerged from the soil after dusk at 18:30 hrs onwards. Males made a repeated zigzag flight search for mates, by making buzzing sound throughout the garden at the height of approximately 15 to 20 feet. Copulating pairs (N=15) were observed with the aid of a head torch, in the areca nut field. At the same time, females emerged from the soil and flew for a short distance and settled on the host plants and took calling posture. Female beetles commenced abdominal expansion and contraction which may be an indication of starting secretion of semiochemicals. Subsequently, males were observed to fly toward calling females. The males were found to alight near the females with buzzing sounds. All females were observed to be receptive, and no case of mate refusal was observed (N = 15). The precopulatory phase began when the male approached the female. The male then moved closer to the female and immediately mounted. Female retracted her elongated abdomen to stop calling when male beetle mounted her. The male moves around the female and mounts her by climbing on to her abdomen. Male completely moved on her and held the thorax region with forelegs. Next, the male moved in a posterior direction as the abdomen bent ventrally and the aedeagus was extruded and lifted the female

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abdomen by its hind legs. In the copulatory phase, the male inserted its genitalia into the female abdomen. When the genitalia had successfully joined, the male raised all of its legs, turned its body ventrally and became suspended by the genitalia. The copulatory phase ended with the withdrawal of the aedeagus from the genital chamber of the female. In the postcopulatory phase, the male dismounted immediately after genital retraction. Soon after copulation, males returned to the soil by dropping, while females stayed for a few minutes on the surface and flew to the soil for oviposition. Most unmated males also returned to the soil shortly after the active flight period at dusk. The copulation of fifteen beetles was observed between 18:47 hours to 19:05 hrs. The first termination of copulation occurred at 19:55 hrs and the last at 20:29 hrs. Copulation lasted for 74.60 ± 6.61 min on average (mean \pm SD; range 64-85, N = 15). Copulatory phase was larger than the precopulatory 2.73 ± 1.58 min on average (mean \pm SD; range 1-6, N = 15) and postcopulatory phase 1.67 ± 0.90 min on average (mean \pm SD; range 1-3, N = 15) (Table 2).

Table 2. Mating duration of female beetles in the field during-2016.

Parameters	Minutes	
	Mean \pm SD#	Range
Pre copulation	2.73 \pm 1.58	1-6
Copulation	74.60 \pm 6.61	64-85
Post copulation	1.67 \pm 0.90	1-3
#N=15		

Egg laying pattern of field-collected mated females of *L. lepidophora*

In a pooled analysis of 2015 and 2016, the longevity of female ranged from 11-28 days with an average of 18.52 ± 4.91 days. The pre-oviposition period, Oviposition period and postoviposition period of an adult female with an average of 9.56 ± 2.04 days (Mean \pm SD, range 4-13 days), 3.72 ± 2.35 days (Mean \pm SD, range 1-11 days) and 5.24 ± 4.67 days (Mean \pm SD, range 1-13 days), respectively (Table 3).

Table 3. Fecundity and longevity of *L. lepidophora* females during 2015-16 and 2016-17.

Parameter	2015*		2016**		Pooled#	
	Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD
Pre-oviposition period (Days)	4-11	9.31 \pm 2.02	8-13	9.83 \pm 2.12	4-13	9.56 \pm 2.04
Oviposition period (Days)	1-11	3.92 \pm 2.72	1-7	3.50 \pm 1.98	1-11	3.72 \pm 2.35
Post-oviposition period (Days)	1-13	7.31 \pm 4.80	1-13	3.00 \pm 3.44	1-13	5.24 \pm 4.67
Fecundity (Eggs/female)	1-32	15.69 \pm 10.12	4-31	19 \pm 8.23	1-32	17.04 \pm 9.37
Adult longevity (Days)	11-28	20.54 \pm 5.13	11-24	16.33 \pm 3.73	11-28	18.52 \pm 4.91
*N=13; **N=12; #N=25						

Clutch size in relation to the clutch number

Data from egg laying pattern on a number of eggs in first, second, third, fourth and fifth clutches produced by females are summarized in Table 4. Mean number of eggs per clutches decreased from first through second, third fourth to the fifth (Mean \pm SD: first clutch, 8.36 ± 6.18 ; range 1-20, Second clutch, 5.52 ± 3.20 , range 1-13, Third clutch 5.47 ± 3.27 , range 1-14, fourth clutch 2.29 ± 1.80 , range 1 to 6 and fifth clutch 3.20 ± 1.60 , range 1-6).

Table 4. Clutch size and mean number of eggs laid by female beetle *L. lepidophora* in laboratory condition.

Clutches	Mean number of eggs/ clutches	
	Mean±SD	Range
1	8.36±6.18	1 -20
2	5.52±3.20	1 - 13
3	5.47±3.27	1-14
4	2.29±1.80	1 - 6
5	3.20±2.17	1-6
*N=25		

Inter- clutch interval in relation to the clutch number

The number of days between successive clutches changed over time (Mean±SD). Time to between first and second clutch with an average of 41.05±27.66 hrs (N=21), between second and third clutch with an average of 37.09±43.41 hrs (N=11), between third to fourth clutch with an average of 37.71±18.88 hrs (N=7) and between fourth to fifth clutch with an average of 32±18.88 hrs (N=3) (Table 5).

Table 5. Inter clutch interval in relation to a clutch number of adult female beetle *L. lepidophora* in laboratory condition.

Clutches	Inter clutch interval (Hours)
	Mean±SD
1-2*	41.05±27.66
2-3**	37.09±43.41
3-4#	37.71±18.88
4-5###	32±18.88
*N=21, **N=11, #N=7, ###N=3	

DISCUSSION

Females are more active at around 18:40-19:20 hrs, where light intensity fell to 83.62 lux (Fig. 1). Present findings are in confirmation with Arakaki et al, (2004b) where *Dasylepida ishigakiensis* females were observed to emerging from the soil at 18:30-19:00 hrs and light intensity was less than 300 lux. Peak emergence of adult beetles occurred between 19:00 and 20:00 hrs. The emergence of *L. lepidophora* began at ~16:30 when illuminance fell below 65.5±12.5 lx and extended till 2100 (2.5±0.5 lx) (Kalleshwaraswamy et al, 2016). Further studies needed to confirm the relationship between the emergence of adult beetles and light intensity.

In this study, emerged females of *L. lepidophora* showed an abdominal expansion and contraction, indicating a typical sequence of calling behaviour to males and secrete sex pheromone. Similar behaviour was observed in *D. ishigakiensis* (Arakaki et al, 2004b) and *Lepidiota mansueta* (Bhattacharyya et al, 2015). Female *Exomala orientalis* (Waterhouse), also exhibited leg raising and stroking, which may aid in the dissemination of the pheromone (Facundo, Linn, Villani, & Roelofs, 1999). Precopulatory, copulatory and postcopulatory mating behaviour of *L. lepidophora* was closely matched with the *Dasylepida ishigakiensis* (Arakaki et al, 2004b; Fukaya et al, 2009; Harano et al, 2010; Tokuda et al, 2010) and *Lepidiota mansueta* (Bhattacharyya et al, 2015). Male and female of *Liogenys fusca* emerged between 19:00 and 23:30 h

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and copulation occurred between 19:30 and 21:00 hr, and characterized by a typical behavioural sequence (Rodrigues, Moron, Gomes, & Bento, 2016). Whereas, soon after the emergence, both sexes leading to rapid mounting and successful mating on host plants. Copulation lasted for about 75 min. This duration was longer than those for other scarab species: 20 min in the green chafer, *Anomala albopilosa sakishimana* (Arakaki et al, 2004a) and the oriental beetle, *Exomala orientalis* (Waterhouse) (Facundo et al, 1999). It was less than those of scarab beetles like *Dasylepida ishigakiensis* (2 hours) (Arakaki et al, 2004b) and 85 min in the *Phyllophaga cuyabana* (Moser) (Oliveira & Garcia, 2003), *Lepidiota mansueta* (Bhattacharyya et al, 2015) and 15 min and 8 min in case of Chinese rose beetle *Adoretus sinicus* Burmeister (Hession, Arita, Furutani, & Fukada, 1994) and *Liogenys fusca* (Rodrigues et al, 2016), respectively. The male and female beetles of *L. lepidophora* exhibited protandrous type (males emerging before females) of emergence pattern during the emergence period (Kalleshwaraswamy et al, 2016). Soon after the emergence of female, the large number of males were approached and leads to rapid mounting and mating on nearby plants or soil surface. This study indicated males and females of *L. lepidophora* emerged from soil specifically for mating. Hence, pheromone may be useful for management of areca nut white grub. This will be an aid to develop synthetic sex pheromone for mating disruption and mass trapping of male beetles.

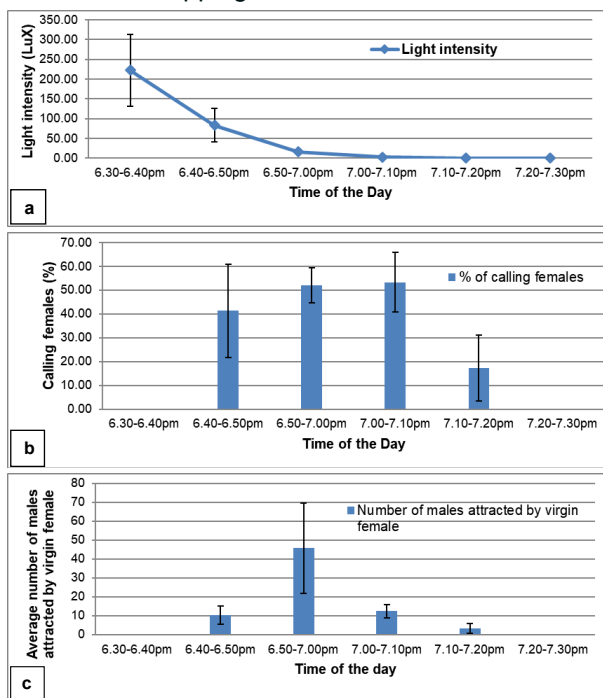


Figure 1. Calling female and male attraction by virgin females of *L. lepidophora* in the areca nut ecosystem in Gulukoppa, Hosanagara, a) light intensity, b) percentage of calling female (N=75), c) number of males attracted by virgin females.

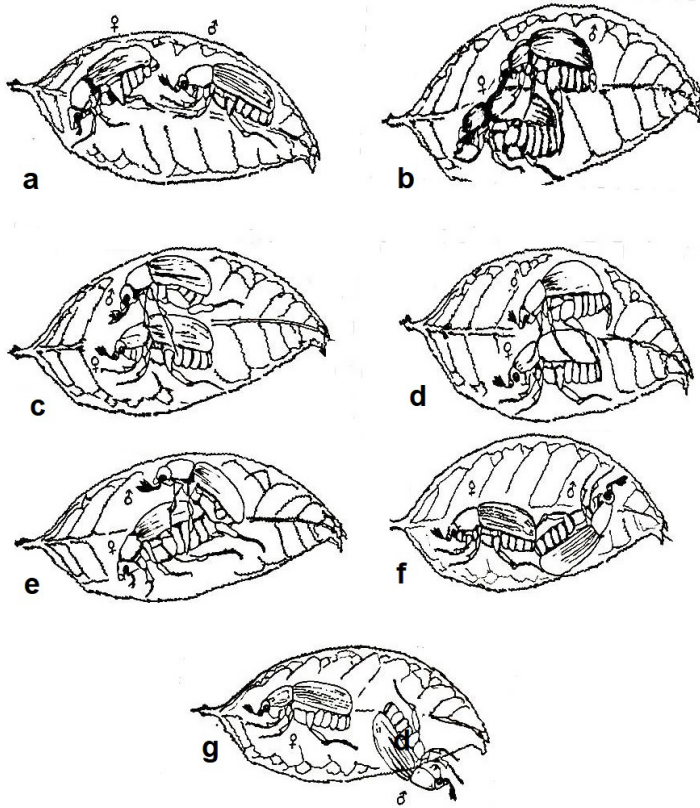


Figure 2. Mating behaviour represented in sequence of *L. lepidophora*. a) male approaches female, b) male mounts the female, c) male moves anteriorly on her and holds the thorax region with forelegs, d) male moved in a posterior direction as the abdomen bent ventrally and the aedeagus was extruded, e) male probes the end of the female's abdomen with his everted aedeagus, f) copulation-male turned its body ventrally and became suspended by the genitalia, g) dismounting and separation.

Experiment on egg laying pattern indicated that female waited on an average 9.56 ± 2.04 days before starting of oviposition, after which time they began to lay the varying number of eggs at an average clutch interval of approximately 24-48 hours. Females of Japanese beetle *Popillia japonica* did not initiate oviposition until several days after emergence, and the number of eggs laid remained relatively constant over time (Timmerman, Switzer, & Kruse, 2000). Female areca nut white grub *L. lepidophora* lay on an average 17.04 ± 9.37 eggs per female (range 1-32) over the one-month adult lifespan. Female laid eggs in multiple clutches after mating with one male. In our study, single mating is enough to fertilize all the eggs. The eggs laid by the female decreased in successive clutches (Fig. 3) may reflect either a cost of present reproduction on future fecundity (Bell & Kofoupanou, 1985), a reduction in overall performance and fecundity with increasing age of female (Park, Gustafsson, & Moreno, 1992). Fecundity of longhorn beetle *Cerambyx welensii* was decreasing

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over time and showed a fluctuating synovigenic pattern (Torres-Vila, Mendiola-Diaz, Conejo-Rodriguez, & Sanchez-Gonzalez, 2016). Many protandrous species lay only one clutch of eggs (Wiklund & Forsberg, 1991) and those species that lay more clutches usually only have two to three clutches with the majority of eggs being laid in the first clutch (Milne, 1960). Decisions on clutch size are amenable to the programming approach because after each oviposition the reproductive state of the female has changed and hence if eggs or reserves are limiting, the optimal size of the next clutch may also have changed (Mangel, 1987). Several internal factors are responsible for clutch size in female beetles like rates of egg maturation, number of mature eggs, number of immature eggs, a capacity of oviducts, energy reserve and also the fitness of females (Wilson, 1989; Griffiths, 1990). Clutch size increases the reproductive success of the female. The present studies were helpful in the planning of integrated pest management strategies.

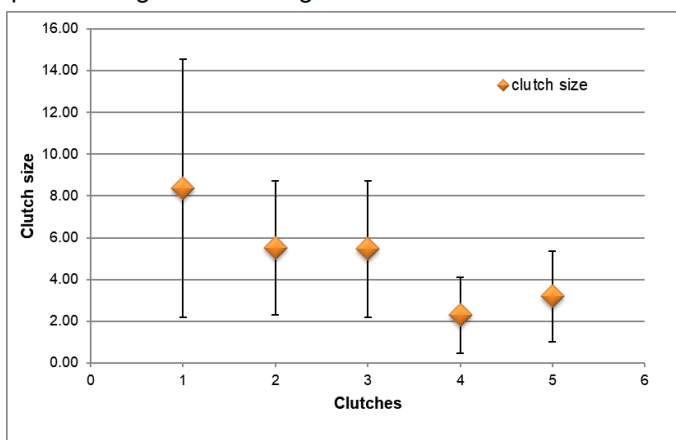


Figure 3. Number of eggs in the different clutches as an egg laying pattern.

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