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Cover: Golden-headed Lion Tamarin *Leontopithecus chrysomelas*. Watercolor and acrylics by P. Kritika.

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ARTICLE

Tricho-taxonomic prey identifications from faeces of Indian Rock Python *Python molurus* (Linnaeus, 1758) (Reptilia: Squamata: Pythonidae) in Moyar River Valley, Tamil Nadu, India

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Abstract: Identifying the prey species is crucial for successful conservation and landscape-level management of large predators whose feeding ecology is incompletely known. Assessment of faecal samples is a viable method for achieving this goal. The Indian Rock Python *Python molurus*, one of India's largest snakes, is an ambush predator trophically-equated to apex predator in its ecosystem. Yet, compared to trophically-similar big cats and canids there is a notable lack of research on its prey items in southern Indian regions. In this study, 31 faecal samples from radio-tagged pythons and other pythons inhabiting the Moyar River Valley were studied. These samples were dried and washed to obtain the hairs to identify the species. Hairs were obtained from 11 faecal samples, and prey species were identified using the tricho-taxonomy. Our findings revealed that mammals constitute the majority of consumed prey. Pythons preferred the palm squirrel *Funambulus palmarum* as the most desirable prey. Other large prey species include the Sloth Bear *Melursus ursinus*, the Sambar Deer *Rusa unicolor*, and the Spotted Deer *Axis axis*. Seasonal variation in prey species intake peaked during September–November, while no prey species were recorded during April–August.

Keywords: Behavior, diet, ecology, feeding, giant snake, hair, mammals, predation, prey, reptile.

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Author contributions: All authors contributed to the study. JN: manuscript writing (original draft), visualization, formal analysis, methodology, writing, review, & editing. CR: writing, review, editing, project administration, supervision, methodology, investigation, funding acquisition, & conceptualization. CSV: sample collection, writing, review, & editing. AB: writing, review, editing, validation, methodology, formal analysis, investigation, & supervision.

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INTRODUCTION

Food is central to all animals' biology and life history, and recognising, locating, capturing, ingesting, and digesting prey are critical activities for their survival and reproductive success (Slip & Shine 1988). Diet studies provide knowledge about species trophic ecology and population dynamics necessary for species management and conservation policies (Morrison et al. 1990; Sutherland et al. 2004; Marti et al. 2007). The feeding habits of giant snakes are of particular interest due to their remarkable adaptations for locating, capturing, subduing, and ingesting large prey (Slip & Shine 1988). They rely on several sources of sensory input to locate food, including vision, vibration, heat, and colour (Slip & Shine 1988). Since large-bodied snakes such as pythons are mostly restricted to tropical areas, presumably due to thermal constraints in temperate climates, detailed information on thermal biology, diet, and other ecological factors for giant snakes inhabiting tropical regions is scarce (Shine & Madsen 1996; Shine et al. 1998). The Indian rock python *Python molurus* is a giant-bodied non-venomous and habitat generalist snake species with a mean adult snout-vent length of 3–4.5 m, with some individuals exceeding 6 m (Bhupathy 1990). It is widely distributed in India, Sri Lanka, Nepal, and parts of Pakistan (Smith 1943; Whitaker 1993; Daniel 2002; Whittaker et al. 2004) inhabiting a wide range of habitats, including wetlands, open forests, scrublands, harsh deserts, rainforests, woodlands, grassy marshes, river valleys, rocky slopes, and savannas (Murphy & Henderson 1997).

Its home range size in the Moyar River Valley Landscape, southern India, is 4.2 km^2 (Vishnu et al. 2023a). Python often functions as a top-level predator that may influence local prey populations, including reptiles, birds, and small to medium-sized mammals, particularly in ecosystems where they are abundant (Bhupathy et al. 2014; Gangaiamaran et al. 2023). Avian prey has been routinely recorded in the diets of giant snakes, such as pythons and anacondas, many times, indicating that almost all of the giants do eat birds (Murphy & Henderson 1997). Six species of birds were found in the diet of *P. molurus* in Keoladeo National Park, Rajasthan (Bhupathy & Vijayan 1989), often including domestic birds (Murphy & Henderson 1997). Studies in Pakistan and Sri Lanka have also reported reptiles such as monitor lizards *Varanus* sp. as prey species of *P. molurus* (Deraniyagala 1955; Minton 1966). Mammals most likely comprise the bulk of the diet of giant snakes (Ernst & Zug 1996; Bhupathy et al. 2014). Mammals of suitable size that overlap spatially and temporally with *P. molurus* may be vulnerable to predation (Murphy & Henderson 1997). Pythons' diet also frequently include domesticated cats and dogs, rodents, ungulates, and monkeys (Murphy & Henderson 1997). Unusual prey like horned mammals with the potential to cause serious injuries when swallowed have also been recorded in the diet of *P. molurus, P. sebae,* and *Malayopython reticulatus* (Duarte 2003). These prey include small to medium-sized bovids such as *Kobus kob* (Hay & Martin 1966), *Aepyceros melampus* (Gasc 1994), *Axis porcinus, Gazella thomsoni, Munticaus muntjac* (Greene 1997), and antelopes (Spawls & Branch 1995).

Several methods have been used to study the food habits of reptiles, especially the gut content analysis (Delany & Abercrombie 1986; Lobo et al. 2005; Dove et al. 2011), including stomach flushing (Fitzgerald 1989; Rivas et al. 1996), and faecal sample analysis (Sylber 1988; Bhupathy et al. 2014). Passive and non-abrasive approaches, such as faecal analysis, can be used to determine the feeding behaviour of the study of animals without disrupting their habitat ecology. In snakes, bone and tooth fragments are often poorly preserved in faecal matter due to digestive efficiency, whereas, hair remains relatively undigested and can serve as a reliable tool for prey identification (Quadros & Monteiro-Filho 1998). Data on the diet of *P. molurus* in northern India were reported by Bhupathy et al. (2014) from Keoladeo National Park. Therefore, to provide more information on the dietary patterns of *P. molurus* from southern region of India, we studied the prey items of *P. molurus,* using tricho-taxonomic analysis on faecal samples collected from the Moyar River Valley, Tamil Nadu.

MATERIALS AND METHODS

Study area and field method

The study was conducted in Moyar River Valley, between two protected areas, Sathyamangalam and Mudumalai Tiger Reserves in Tamil Nadu (Figure 1). The region is known for its rich biodiversity assemblage (Thirumurugan et al. 2021). During radio-tracking field surveys, faecal samples (n = 31) from pythons were opportunistically collected between 2018 and 2020. The samples (n = 20) were collected from 11 tagged python individuals, while the remaining samples were collected from other individuals in the wild. The samples were collected in zip-lock bags and labelled with markers. Subsequently, the samples were dried in the shade and then stored in a refrigerator at temperatures below

30°C for almost a year with proper packaging to prevent contamination.

Laboratory method

Hair analyses were done as per Souza & Azevedo (2021). Evidence of mammalian prey species found in the faecal samples was identified following tricho-taxonomy techniques (Bahuguna et al. 2010). The dried faecal samples (n = 31) were at least one year old and were broken down with the help of a mortar and pestle and then cleaned with the help of the sieving method. The hairs were extracted using the forceps. The hair samples obtained from the faecal samples were washed in acetone to remove any dirt present in them. Microslides were prepared to identify the hair of the potential prey species of the Indian Rock Python. The medulla was identified, and a cross-section was done to determine the shape of the medulla, which is visible through cross-section (Bahuguna et al. 2010). All photographs used in the identification were captured using a digital micro camera. The nomenclature of the hair medulla type was adopted from Wildman (1954), and the cuticular and cross-section types were followed as given by Brunner & Coman (1974); Medulla: to identify the medulla type, the whole amount of cleaned hair samples was mounted in DPX (Dibutylphthalate Polystyrene Xylene) without staining, allowing visualisation of internal structures. It was ensured that the hairs were well separated, and individual hairs could be observed. The medulla was observed using a light microscope at 40x magnification. Cross Section: for the present study, hair cross-sections were obtained by simple hand sectioning after mounting the hair in paraffin wax and sectioning the wax block by using surgical blades. The cross-sections were selected based on the clarity of the structure. This method was successfully followed by Bahuguna et al. (2010). No microtome was used for cross-sections. These sections were placed on slides coated with egg albumen and observed at 100x. Scale Casts: to study the scale pattern, 3% gelatin as a special medium was used to obtain

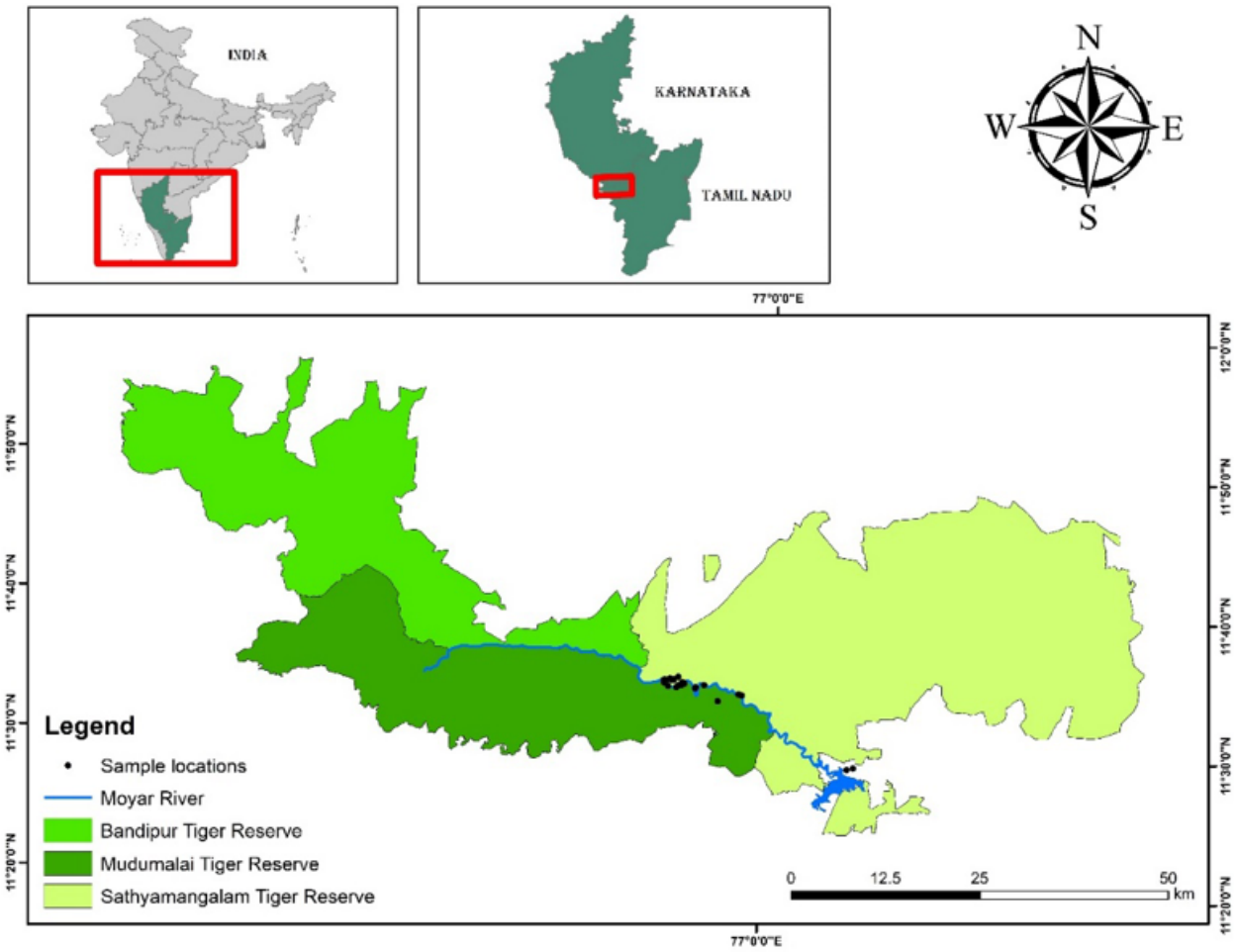

Figure 1. Sampling sites along the Moyar River Valley, India, from where python faecal samples were studied.

the hair impressions. The gelatin is a special medium prepared by mixing the gelatin powder in lukewarm water at a temperature of 50°C. Eosin or hematoxylin was mixed directly into the gelatin mixture to observe the impressions properly. For getting the hair impression or cast, the method by Brunner & Coman (1974) was followed. A thin film of gelatin medium was made. The cleaned hair samples were then directly placed into the glass slide using fine forceps. After drying of gelatin, the hair was removed gently using forceps. This left the hair impression on the glass slide. Different regions (distal, mid and proximal) of hair were examined through 40x microscope to study cuticle patterns.

RESULTS

Hairs were examined from 11 faecal samples out of 31 collected samples because the remaining samples (n = 20) either lacked identifiable remains or were too degraded to analyse. Only the mid-portion of the hair was considered, along with other major factors for scale pattern and cuticular surface. To differentiate the hairs obtained from faecal samples, the hair microstructure (cuticle and medulla) and hair macrostructure (thickness and colour), were taken into consideration (Table 1). Eight mammals were found in the faecal samples of *P. molurus* (Table 2). Of the prey species identified from the samples, the Three-striped Palm-squirrel *Funambulus palmarum* was identified in eight out of the 11 samples contributing to at least 22% of prey individuals, followed by the Grey Mongoose *Herpestes edwardsii* as 20% (n = 7), the Sambar Deer *Rusa unicolor* as 17.14% (n = 6), the Spotted Deer *Axis axis* 14.28% (n = 5), the Black-footed Gray Langur *Semnopithecus hypoleucos* 5.71% (n = 2), the Jungle Cat *Felis chaus* 8.57% (n = 3), the Sloth Bear *Melursus ursinus* 8.57% (n = 3), and the Wild Boar *Sus scrofa* 2.85% (n = 1) in terms of percentage composition of individuals ingested by *P. molurus*. Feathers were present in one sample, which could not be identified to the genus or species level because of severe digestion. Feather types can be identified through DNA extraction; since this study is limited to morphology, the feather types were not identified up to the species level.

The variation in feeding of *P. molurus* shows higher prey species in September–November (n = 25) as more (Table 2) faecal samples with identifiable prey remains were also found in these months (Figure 2), suggesting higher feeding activity during the post-monsoon period. A few instances of feeding were also observed from January–March. No feeding records were found for April–August and December.

DISCUSSION

The predominance of mammals in the diet of *P. molurus* in the Moyar River Valley of southern India aligns with findings from other ecosystems, such as Keoladeo National Park in northern India (Bhupathy et al. 2014), for *P. bivitattus* in Everglades National Park in Florida (Snow et al. 2007), and the carpet python *Morelia spilota* in Australia (Slip & Shine 1988). Although prey composition varies based on regional faunal availability and habitat structure, mammals consistently form an important component of the diets of other pythons, with large individuals capable of taking large prey (Pope 1961; Branch & Hacke 1980). Avian prey has always been a part of the diet of giant snakes, including pythons (Snow et al. 2007; Bhupathy et al. 2014). In the present study, the lower occurrence of avian prey may reflect either feeding preference, habitat use or the greater digestibility of feathers relative to hairs. One python was observed feeding on poultry (quail &

Table 1. Prey species and their detailed hair characteristics, analysed from faecal samples of *Python molurus* in Moyar River Valley, India.

Prey species (Scientific names)	Prey species (Common names)	Medulla pattern	Scale pattern (mid)	Colour of hair	Cuticular surface (medial margins)
Axis axis	Spotted Deer	wide simple	regular wave	light brown	smooth
Rusa unicolor	Sambar Deer	wide simple	irregular wave	brown	crenate
Semnopithecus hypoleucos	Black-footed Gray Langur	simple fragmented	irregular wave	white	crenate
Felis chaus	Jungle Cat	Simple	irregular wave	grey brown	crenate
Herpestes edwardsii	Grey Mongoose	simple	irregular wave	banded (black and white bands)	crenate
Melursus ursinus	Sloth Bear	narrow simple	irregular wave	black	crenate
Sus scrofa	Wild Boar	narrow simple	irregular wave	brown	rippled
Funambulus palmarum	Three-striped Palm Squirrel	multiseriate	regular wave	brown	crenate

chicken) before faecal collection, suggesting potential anthropogenic feeding opportunities. The low incidence of birds in the diet may also reflect the frequency of arboreal activity as reported by Slip & Shine (1988) in their study on the Australian carpet pythons *Morelia spilota* complex. Use of microhabitats such as burrows, dry bushes, rock crevices, and water bodies by pythons inhabiting the Moyar River valley region (Vishnu et al. 2023b) may facilitate opportunistic predation and access to a broader prey base.

Typical food items consumed by the *P. molurus* include fishes, amphibians, reptiles like lizards and snakes, birds, and mammals (Ernst & Zug 1996). In the Moyar River valley region, *Funambulus palmarum* was found to be the principal prey species of *P. molurus*, having a maximum share. The frequent occurrence of *F. palmarum* in the faecal samples may reflect significant spatial overlap in microhabitats shared by the python and this species. The abundance of small to medium-sized rodents and lagomorphs makes them prime candidates for prey (Murphy & Henderson 1997). *Herpestes edwardsii* was the second most consumed species, which could be attributed to the burrows commonly used by Pythons as well. Pythons have been observed sharing the same burrows with porcupines *Hystrix* sp. and other small mammals (Bhupathy et al. 2014). The presence of hairs of large mammalian species such as Sloth Bear Sambar Deer, and Spotted Deer in the samples suggests possible predation or scavenging, though confirmation of active predation requires further evidence such as kill observations or telemetry data. Similar predation on large mammals by pythons has been reported from studies in India, USA (non-native), and Indonesia (Fredriksson 2005; Snow et al. 2007; Bhupathy et al. 2014). Occurrence of species such as *M. ursinus*, *F. chaus*, and *S. scrofa* is almost similar to that reported by Bhupathy et al. (2014) from northern India.

Prey intake peaked between September–November, consistent with post-monsoon prey availability (Figure 2). These results are consistent with the seasonal variation reported in northern India at Keoladeo National Park, Rajasthan, by Bhupathy et al. (2014). No feeding incidents occurred in the colder month of December, corresponding to the start of mating season when snakes are found near ground burrows, basking in the sun (Ramesh & Bhupathy 2010). Reproductively active snakes typically stop feeding during the breeding season, as feeding is inconsistent with reproductive activities, a pattern seen in colubrids (Shine et al. 2003), viperids, and pythonids (Alridge & Brown 1995; Madsen & Shine

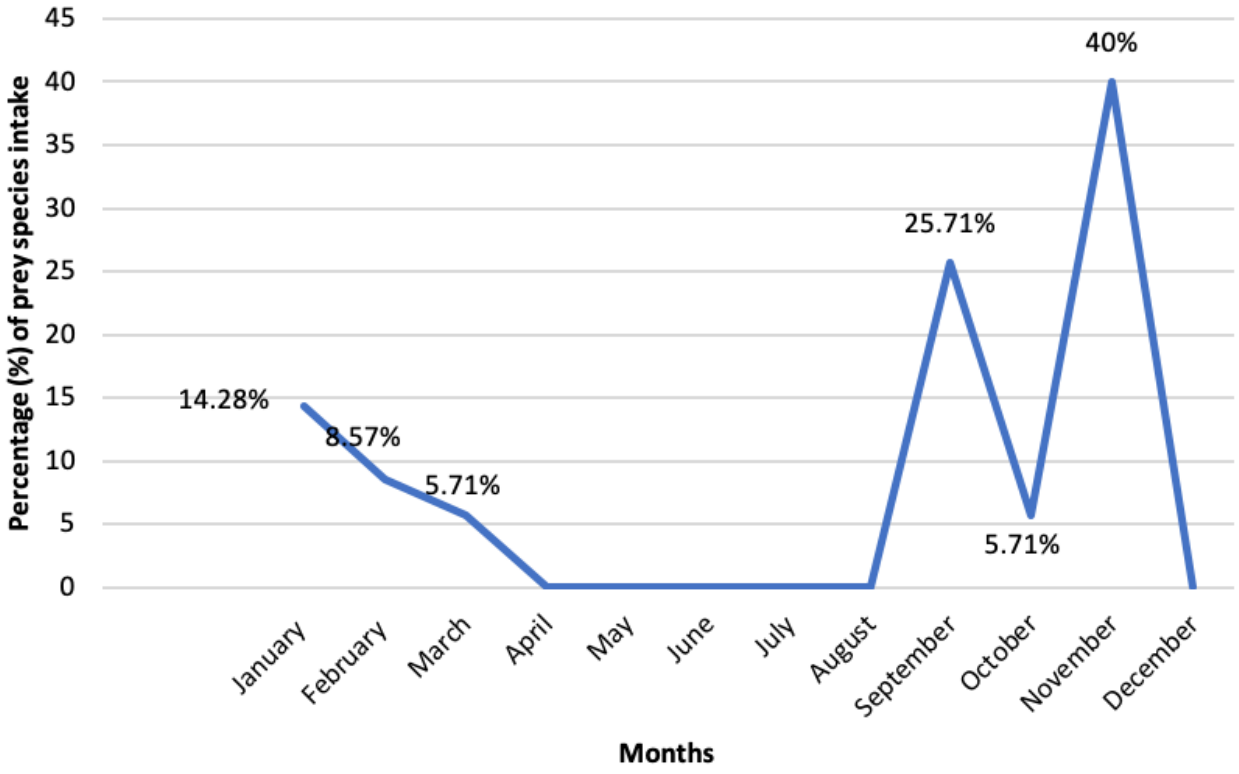

Figure 2. Monthly feeding trends of *P. molurus* as noted through frequency of faecal samples.

Table 2. Month- and species-wise break-up of prey intake of *Python molurus* studied in Moyar River Valley, southern India.

Month	No. of samples	Counts / sample	*Axis axis*	*Rusa unicolor*	*Semnopithecus hypoleucos*	*Felis chaus*	*Herpestes edwardsii*	*Melursus ursinus*	*Sus scrofa*	*Funambulus palmarum*
January	2	5	1	1	0	0	1	0	0	2
February	1	3	0	1	0	0	1	0	0	1
March	1	2	0	1	0	0	1	0	0	0
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0
August	0	0	0	0	0	0	0	0	0	0
September	2	9	1	1	1	2	2	1	0	1
October	1	2	1	0	0	0	1	0	0	0
November	4	14	2	2	1	1	1	2	1	4
December	0	0	0	0	0	0	0	0	0	0
Total	**11**	**35**	**5**	**6**	**2**	**3**	**7**	**3**	**1**	**8**

1993). Feeding may resume to a limited extent during the mating months of January and February (Ramesh et al. 2019), as few faecal samples were recorded during these months, suggesting some individual variation in reproductive or foraging behaviour (Figure 2). The absence of samples during the summer months (April–June) is consistent with reduced feeding, possibly due to warmer and drier conditions, partial aestivation, and breeding activities (Ramesh & Bhupathy 2010).

Cessation of feeding is recorded in different python species during the breeding season, demonstrating a behavioural trade-off, as foraging is incompatible with incubation and egg development (Slip & Shine 1988; Ramesh & Bhupathy 2010). These activities may also reflect adaptive strategies for temperature regulation and reproductive success. Occasional reports of fruit remains (e.g., Indian Jujube *Ziziphus jujuba*) in python's stomach contents may result from secondary ingestion via herbivorous prey and not direct frugivory (Wall 1912; Pope 1961; Bhupathy et al. 2014). Our data shows that mammals are the key prey of the Indian Rock Python in southern India, which is consistent with similar studies by Ernst & Zug (1966) and Snow et al. (2007). Pythons here prefer mammals of different sizes, from Sambar Deer to Three-striped Palm Squirrels. This variation could be due to different prey distributions in the different habitat types, seasonal variations and population abundance of the prey species (Smith & Vrieze 1993; Madsen & Shine 1999; Snow et al. 2007). Studies on the feeding habits of large snakes like pythons are crucial to determine their preying technique, energy intake, feeding habits, prey ecology and ecological value. Thus, detailed studies on the foraging ecology and prey size range for the different python species are important.

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