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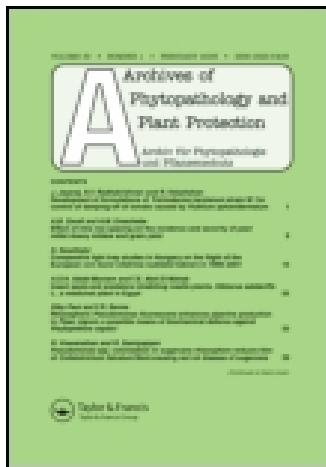


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## A new species of entomopathogenic nematode *Oscheius gingeri* sp. n. (Nematoda: Rhabditidae) from ginger rhizosphere

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A new species of entomopathogenic nematode (EPN) of the genus *Oscheius* was isolated from the rhizosphere of ginger (*Zingiber officinale* Rosc.) cultivated in Indian Institute of Spices Research Experimental Farm, Peruvannamuzhi, Calicut District, Kerala, India. This species is described as a new species on the basis of morphometrics study. Entomopathogenicity of this nematode was also tested against greater wax moth *Galleria mellonella* larvae. *O. gingeri* sp. n. is diagnosed by the presence of leptoderan bursa and crochet needle-shaped spicules, presence of 10 lateral lines, didelphic female reproductive system, double-flapped epipyigma and nine genital papillae. The characteristics of the new species are: male; body ventrally curved, “C”-shaped upon fixation, leptoderan bursa, tail short rounded conoid and nine pairs of genital papillae, female; body almost straight upon fixation, female reproductive system is didelphic, double-flapped epipyigma present on the vulval opening and tail is short conoid with pointed tip and infective and juvenile; body thin elongate, tail elongate attenuated, gradually tapering at tip and spine-like projection present. It closely resembles with *O. carolinensis* but differs in smaller length (vs. L=1728 µm), larger “a” value (vs. a=18.2); smaller “b” value (vs. b=7.0); and larger “c” value (vs. c=11.0), vulval opening (vs. V=50.3%), number of lateral lines (vs.=4) and male body posture “C” shaped (vs. elongate). This new species also closely resembles with *O. columbiana* but differs in higher body length (vs. L=1288 µm), smaller “b” value (vs. b=6.5), smaller stoma length (vs. SL=23) and number of lateral lines (vs.=4). *O. gingeri* sp. n. also resembles with *O. andrassyi* but differs in size of the body (vs. L=1601 µm), larger “b” value (vs. b=8.2), smaller “c” value (vs. c=14.4), vulval opening (vs. V=50.3%) and number of lateral lines (vs.=6). This new species is capable to kill ginger shoot borer larva *Conogethes punctiferalis* and *G. mellonella* within 24–72 h under laboratory conditions. This opens a new hope of utilising *Oscheius gingeri* sp. n. as a biopesticide for management of the shoot borer and other insect pests of ginger.

**Keywords:** Entomopathogenic nematodes; morphology; taxonomy; *Oscheius*; ginger

### Introduction

Entomopathogenic nematodes (EPNs) are emerging as a potent biocontrol agent against a variety of insect pests infesting different crops. The interest in using EPN as a biopesticide for pest control has increased exponentially over last decade. Globally, many

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researchers are exploring the potential of EPN to manage harmful insects infesting various crops.

Andrassy (1976) erected genus *Oscheius* with type species *O. insectivorus* as a sub genus of *Rhabditis*. Sudhaus (1976) placed *Oscheius* in the family Rhabditidae and divided into two groups on the basis of the bursa. The first group has a leptoderan bursa and the other group has a peloderan bursa, which comes under insectivora and dolichura group, respectively.

Tabassum and Shahina (2002), Tahseen and Nisha (2006), Weinin et al. (2010) and Ali et al. (2011) recognised *Oscheius* as an independent genus and described *O. maqbooli*, *O. shamimi*, *O. carlianensis* and *O. amsactae*, respectively. Among the *Oscheius* species, only four species such as *O. carlianensis* (Weinin et al. 2010), *O. siddiqii*, *O. niazii* (Tabassum and Shahina 2010) and *O. amsactae* (Ali et al. 2011) were reported as EPN.

To improve upon their biocontrol potential, it is important to isolate native strains or to detect species which can tolerate local climatic conditions. It is also important to identify the particular strain accurately for effective management. In view of this, the present study was undertaken to detect EPNs from the rhizosphere of ginger and identify them on the basis of morphometric study.

## **Materials and methods**

### *Isolation of EPN*

Soil samples were collected from the rhizosphere of ginger cultivated in Indian Institute of Spices Research Experimental Farm, Peruvannamuzhi, Calicut. The EPNs were isolated by insect-baiting (Bedding and Akhurst 1975) and modified white trap method (Kaya and Stock 1997).

### **Morphological characterisation**

Nematodes were killed in warm water at 60 °C and fixed in triethanolamine formaldehyde (Courtney 1955) and kept in this solution for 48 h. Fixed nematodes were transferred into glycerine-alcohol solution for dehydration according to Seinhorst rapid method (Seinhorst 1959) – and mounted on a glass slide using cover glass and glass rod supports to avoid flattening.

### *Light microscopy*

Light microscope photographs of males, females and IJs were taken by Leica microscope at different magnifications.

### **Measurements**

Holotype male, 10 paratype each of males, females and infective juveniles (IJs) were measured using an ocular µm. Selection of morphometric characters was done according to Hominick et al. (1997). The following ratios and abbreviations are used in the text and tables.

EPW	Width at excretory pore
NR	Distance from anterior end to nerve ring
D%	EP/ES × 100
EP	Distance from anterior end to excretory pore
ES	Oesophagus length
E%	EP/Tail length × 100
SW	SL/ABD
SL	Spicule length
ABD	Anal body width
GL	Gubernaculum length

### **Line diagrams**

Illustrations were prepared with the help of armed type camera lucida.

### **Nematode entomopathogenicity**

Entomopathogenicity of *O. gingeri* sp. n. was evaluated against *G. mellonella* larva by Petri dish method (Ali et al. 2005). Experiments were conducted at room temperature along with a control and replicated five times. Observations of the mortality of insects were recorded after 24 h interval.

### **Results and discussion**

#### **Measurements of *Oscheius gingeri* sp. n**

Measurements of various characters of the holotype male, paratype males, females and IJs of *O. gingeri* sp. n. are given in Table 1.

#### **Description**

Cuticle is smooth and finely striated, about 1 µm thick, as shown in (Figures 1 and 2). Lateral field pattern with nine ridges (10 lateral lines evenly spaced from each other) is visible from mid carpus to near phasmids in female or in bursal region of male. There are six unfused lips each bearing one terminal sensilla. Lip region diameter is about 8–12 µm. Amphidial pouch is pocket like and apertures are elliptical. Stoma is long, narrow and 5–6 times longer than the width. Cheilostom with indistinct cheilorrhadiions and stegostom (pharyngeal collar) comprises 60% of stoma length. Corpus is cylindrical, occupying 50–60% of pharynx length. Median bulb is absent. Isthmus forms 15–20% of pharynx length. Basal bulb pyriform has well-developed valve. Excretory pore is located posterior to nerve ring, before the basal bulb. Nerve ring is located at 50–60% of pharynx. Phasmids are conspicuous.

#### **Male**

Body is ventrally curved, “C”-shaped upon fixation and testis monarchic, situated to the left of intestine. Bursa is leptoderan with a short part of tail protruding beyond bursa. Nine pairs of genital papillae are present. Eight pairs of bursal rays are arranged as two pairs pre-cloacal and six pairs post-cloacal. First pair is almost at the level of spicule head, 3rd, 4th and 5th forming a continuous pattern and again 6th, 7th and 8th form a bench or continuous pattern. Pairs, 1st, 4th, 6th, 7th and 8th reach up to rim of bursa and 2nd and 3rd are curved dorsally not reaching up to the rim of bursa. Spicules are

Table 1. Morphometrics of *O. gingeri* sp. n. All measurements are in µm and in the form: mean ± SD (range).

Character N	Holotype (Male)	Infective Juvenile		Paratype	
		10	Female 10	Male 10	Female 10
Total length	678.1	442.1 ± 77.8 (331.1–520.2)	1638.6 ± 145.8 (1418.3–1813.1)	739.5 ± 62.69 (673.1–821.5)	—
Greatest width	32.6	17.2 ± 0.78 (16.23–18.2)	81.6 ± 5.15 (75.1–89.1)	35.2 ± 3.07 (31.6–38.9)	—
Stoma length	17.9	14.4 ± 0.53 (13.9–15.2)	19.5 ± 0.86 (18.7–20.8)	17.9 ± 0.92 (16.8–19.2)	—
Stoma width	3.6	1.7 ± 0.22 (1.61–2.13)	3.1 ± 0.09 (2.99–3.2)	3.6 ± 0.33 (3.1–3.9)	—
EP	116.7	92.3 ± 3.83 (87.36–96.17)	204.1 ± 13.85 (187.1–223.2)	126.1 ± 13.27 (110.2–141.8)	—
EPW	31.6	17.2 ± 1.32 (15.3–18.9)	51.2 ± 1.55 (49.16–53.16)	32.2 ± 1.20 (30.5–33.6)	—
NR	110.8	68.9 ± 5.54 (60.1–74.1)	195.8 ± 10.1 (178.1–203.1)	105.2 ± 10.38 (90.5–114.3)	—
ES	171.7	95.9 ± 7.3 (83.1–101.5)	253.2 ± 37.48 (189.1–283.5)	171.4 ± 17.57 (142.5–187.5)	—
Testis ratio	10.8	—	—	11.6 ± 0.84 (10.1–12.2)	—
Vulval opening	—	—	—	—	—
ABW	16.8	12.1 ± 1.58 (10.1–14.2)	54.1 ± 3.48 (51.3–60.2)	17.7 ± 1.47 (15.5–19.2)	—
Tail	45.8	52.1 ± 6.99 (43.5–60.1)	26.4 ± 1.20 (25.2–28.1)	51.8 ± 6.24 (43.5–59.3)	—
SL	24.6	—	121.3 ± 5.31 (115.2–129.1)	25.7 ± 1.31 (24.1–27.3)	—
GL	9.6	—	—	9.3 ± 0.56 (8.69–9.86)	—
a	20.8	24.6 ± 1.90 (22.81–27.18)	20.3 ± 1.13 (18.5–21.23)	20.6 ± 2.14 (18.3–24.0)	—
b	3.9	4.3 ± 0.62 (3.61–5.1)	6.2 ± 0.08 (5.15–5.35)	4.7 ± 0.36 (4.3–5.3)	—
c	14.8	7.2 ± 0.63 (6.4–7.9)	13.8 ± 0.42 (12.1–13.2)	14.6 ± 1.92 (11.5–16.7)	—
SW	1.46	—	—	1.4 ± 0.14 (1.3–1.6)	—
D (%)	67.9	96.8 ± 10.67 (88.9–115.6)	80.9 ± 6.61 (64.7–81.7)	74.2 ± 11.31 (61.1–90.4)	—
E (%)	254.8	179.6 ± 10.6 (173.5–191.3)	168.5 ± 17.23 (151.1–193.8)	243.8 ± 52.11 (191.9–312.2)	—

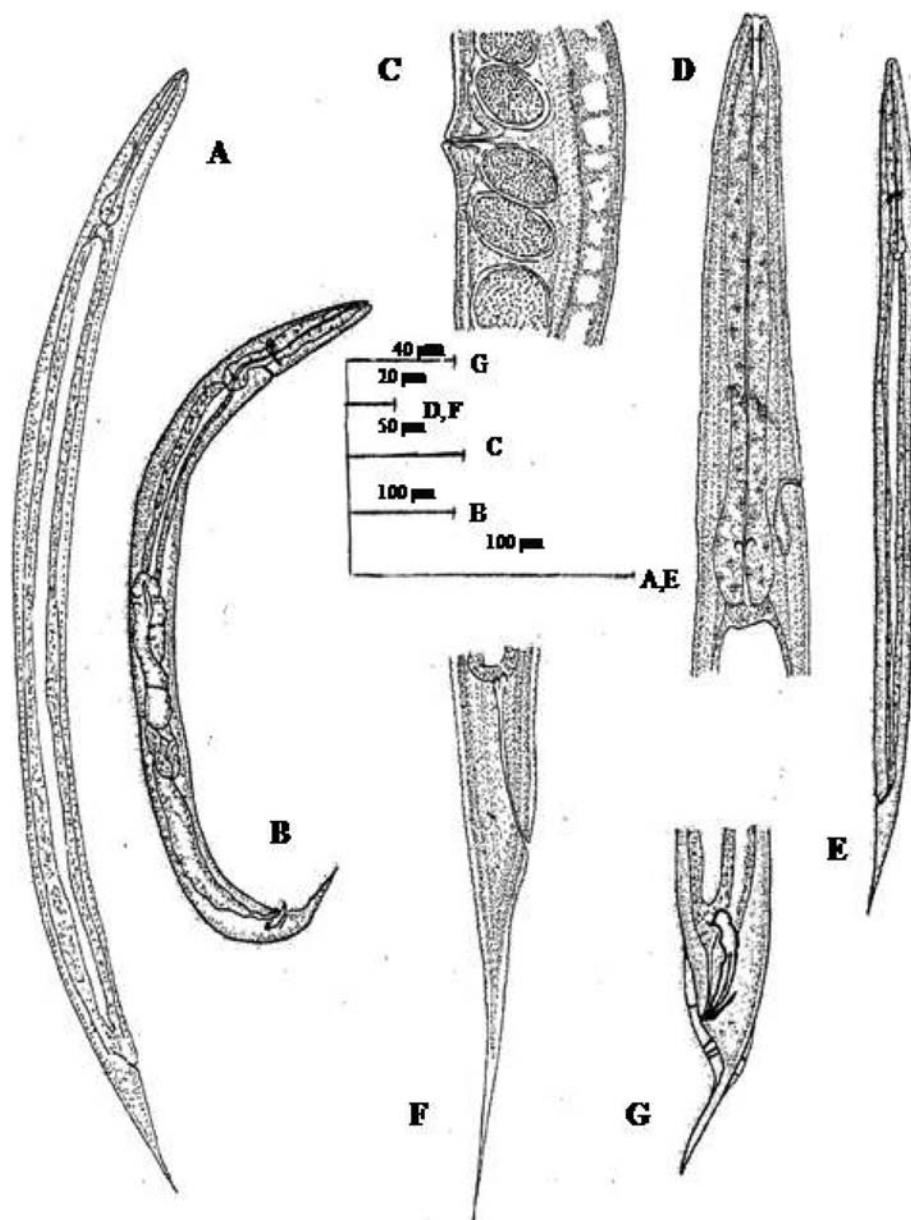


Figure 1. Line diagram of the *O. ginneri* sp. n.: A – entire female; B – entire male; C – vulval opening; D – anterior region; E – infective juvenile; F – female tail; G – male tail.

slender, with crochet needle-shaped tip of head. Lamina is with one internal rib. Gubernaculum is thin boat-shaped following contour of spicule.

#### *Female*

Body is almost straight upon fixation. Female reproductive system is didelphic. Anterior branch is situated on the right of intestine, posterior on the left side and dorsally



Figure 2. Light photograph of the *O. gingeri* sp. n.: A – infective juvenile; B – anterior region; C – vulval region; D – entire female; E – female tail; F – male tail. Photographs A, D at 20X; B, C, E at 40X and F at 100X.

reflexed ovaries often extending as far as vulva in form of a transverse slit. Double-flapped epipygma is present on the vulval opening. Phasmids are adanal and tail is short conoid with pointed tip.

#### *Juvenile*

Body is slender. Cuticle is with transverse striae. Lip region is smooth; mouth closed. Stoma is long and narrow, more than four times longer than diameter. Stoma length

forms 19–20% of pharynx length. Pharynx and isthmus are both long and narrow. Basal bulb is valvate. Nerve ring is located at isthmus level. Excretory pore is located at middle pharynx level. Tail elongates attenuated, gradually tapering at tip, and spine-like projection is present.

#### Type host and locality

The natural host of *O. gingeri* sp. n. is unknown. The nematode was trapped by baiting with *G. mellonella* from the soil. Soil samples were obtained from rhizosphere of ginger cultivated in Indian Institute of Spices Research Experimental Farm, Peruvannamuzhi, Calicut ( $11^{\circ}35'52.34''$  N,  $75^{\circ}49'20.48''$  E, elev 58 m), Kerala, India.

#### Type material

Holotype male (permanent mounted in glycerine), 10 paratypes each of females, males and IJs were deposited in Nematology lab, Indian Institute of Spices Research, Calicut and three paratype each of females, males and IJs were deposited at CAB, International Institute of Parasitology, St. Albans, UK.

#### Etymology

This species is named after its isolation from ginger (*Zingiber officinale* Rosc.) rhizosphere.

#### Diagnosis and relationships

The leptoderan bursa and crochet needle-shaped spicules place *O. gingeri* sp. n. in the insectivora group of *Oscheius* (Sudhaus and Hooper 1994; Sudhaus and Fitch 2001). Species comparisons in the insectivora group are compiled in Table 2 updated by Stock et al. (2005). *O. gingeri* sp. n. is characterised by the length of the male (739 µm) which is the smallest nematode among the *Oscheius* species of insectivora group, presence of leptoderan bursa and crochet needle-shaped spicules, presence of 10 incisures in the lateral field, didelphic female reproductive system, presence of double-flapped epipygma on the vulval opening and presence of nine genital papillae.

*O. gingeri* sp. n. is morphologically closest to *O. carolinensis* (Weimin et al. 2010), *O. columbiana* (Stock et al. 2005) and *O. andrassyi* (Tabassum and Shahina 2008) (Table 2).

It closely resembles with *O. carolinensis* but differs in smaller length (vs. L=1728 µm), larger “a” value (vs. a=18.2); smaller “b” value (vs. b=7.0); and larger “c” value (vs. c=11.0), vulval opening (vs. V=50.3%), number of lateral lines (vs.=4) and male body posture “C” shaped (vs. elongate).

This new species also closely resembles with *O. columbiana* but differs in higher body length (vs. L=1288 µm), smaller “b” value (vs. b=6.5), smaller stoma length (vs. SL=23) and number of lateral lines (vs.=4).

*O. gingeri* sp. n. also has similarity with *O. andrassyi* but differs in size of the body (vs. L=1601 µm), larger “b” value (vs. b=8.2), smaller “c” value (vs. c=14.4), vulval opening (vs. V=50.3%), female reproductive system (vs.=amphidelphic) and number of lateral lines (vs.=6).

Table 2. Comparison of species in the insectivora group of *Oscheius* (all measurements are in µm and in the form of mean (range).

Character	<i>O. carinensis</i>	<i>O. colombiana</i>	<i>O. insectivorus</i>	<i>O. shamimi</i>	<i>O. andrassyi</i>	<i>O. maghooli</i>	<i>O. ansactae</i>	<i>O. gigneri</i> sp. n.
Type locality	North Carolina, USA	Colombia	Germany	Aligarh, India Soil around flower bud	Karachi, Pakistan Soil from sugarcane	Pakistan Soil from sugarcane	Kanpur, India Hairy caterpillar ( <i>Ansacaea mori</i> )	Calicut, India Soil from ginger
Type habitat/host	Vermicompost	Burrower bug ( <i>Cyrtomenus bergi</i> )	4	6	6	6	11	10
Lateral line	4							
Female	1728 (1360–2420)	1288 (923–1805)	– (2200–2912)	1114 (760–1524)	1601 (1322–1962)	1123 (942–1342)	– (658–786)	1638 (1418–1813)
Total length	23 (18–27)	23 (21–28)	–	20.5 (19–23)	15.7 (14–18)	15 (12–19.6)	– (15.8–18.1)	19.5 (13.9–15.2)
Stoma length	247 (228–264)	205 (176–225)	–	211 (181–241)	191 (175–215)	211 (182–237.6)	– (109.2–130.3)	253 (189.1–283.5)
Esophagus	231 (205–262)	155 (147–165)	–	157 (137–190)	192 (160–225)	169 (150–200)	– (109–130)	204 (187–223)
EP	36 (28–43)	31 (22–38)	–	24 (21–32)	32.4 (25–40)	23.7 (20.29–26)	– (15.8–17.4)	26 (25–28)
ABD	17 (15–19)	– (14.2–18.5)	–	17.7 (15–21)	16.4 (14.1–18)	– (19.7–22.9)	– (19.7–22.9)	20.3 (18.5–21.2)
a	18.2 (14.9–23.2)	–	–	8.2 (6.7–9.4)	5.2 (4.8–5.6)	– (4.1–4.8)	– (4.1–4.8)	5.2 (5.15–5.35)
b	6.5 (5.2–8)	6.5 (5.2–8)	– (9.3–10.5)	5.3 (4.2–6.3)	10 (6.8–13.8)	9 (6.7–11)	– (8.9–12.1)	12.8 (12.1–13.2)
c	11 (8.4–17.8)	9.2 (8.3–10)	– (12.4–15.1)	14.4 (11–17.9)	–	–	– (49.7–58.4)	54.1 (51.3–60.2)
V (%)	50.3 (47.6–55.6)	51 (47–57)	– (45.5–50.5)	49.5 (45.6–51.3)	50.3 (48.9–52.9)	51 (49.6–52.8)	–	
Male								
Total length	1499 (1000–2000)	915 (665–1163)	– (1618–1921)	1012 (938–1118)	1096 (1025–1132)	983 (720–1165)	– (804–1012)	739 (673–821)
Oesophagus	214 (165–245)	183 (168–213)	–	190 (181–203)	168 (158–178)	204 (184–248)	– (134.2–169)	171 (142.5–187.5)
SL	65 (50–81)	56 (43–68)	– (88–95)	60.3 (53–67)	46 (45–51)	54.8 (48–60)	– (30.8–35.5)	25.7 (24.1–27.3)
GL	29.4 (20–35)	20 (16–24)	– (38–54)	23.6 (22–28)	22 (20–25)	27.8 (25.6–29.6)	– (13.7–16.5)	9.3 (8.69–9.86)
a	20.4 (15.8–24.3)	18 (16–29)	– (20.7–24.8)	–	21.8 (20–25)	17.1 (12.8–19)	– (16.6–19.3)	20.6 (18.3–24)
b	7 (4.9–8.9)	4.9 (3.9–5.4)	– (7.3–9.6)	5.4 (5.1–5.5)	6.3 (6.2–7)	4.7 (3.9–5.3)	– (4.1–5.0)	4.7 (4.3–5.3)
c	32.3 (18.9–50)	14.5 (13–16)	– (21.1–24.8)	28.2 (25.1–31.1)	21 (17.9–24.9)	22.5 (18.6–25.9)	– (10.7–17.8)	13.6 (11.5–16.7)
Reference	Weinin et al. (2010)	Komer (1954), Stock et al. (2005)	Taliseen and Nisa (2006)	Tabassum and Shahina (2008)		Ali et al. (2011)	This paper	

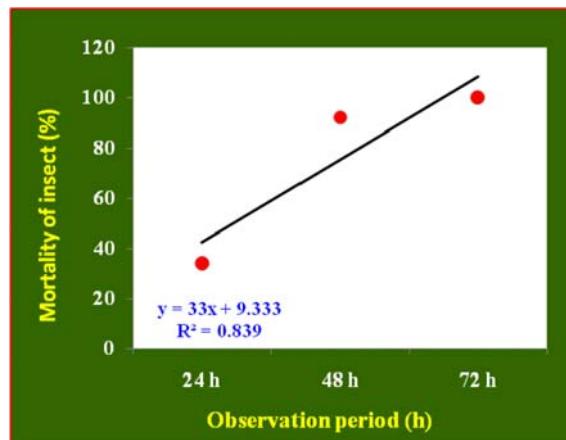


Figure 3. Infectivity of *O. gingeri* sp.n. against *G. mellonella* larva.

### Nematode entomopathogenicity

Results indicated that, *O. gingeri* is pathogenic to *G. mellonella* larva. They started killing insect larvae within 24 h (34%); whereas, it brought about 100% mortality within 72 h post-exposure (Figure 3).

Our study revealed that this newly encountered species is described as a new species based on morphometrics differences. This is the first report of *Oscheius* species from ginger rhizosphere. This new species is capable of killing *G. mellonella* and *C. punctiferalis* within 24–72 h under laboratory conditions (Pervez et al. 2012). This opens a new hope of utilising *O. gingeri* sp. n. as a biopesticide for management of the shoot borer and other insect pests of ginger because of their adaptation to local climate and population regulators.

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