

Prevalence, intensity and mean abundance of digenean parasites of the frog *Fejervarya* sp. (Anura: Dicroglossidae) of the South Western Ghats

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Abstract. Four species of digenean parasites, *Ganeo glottoides*, *Pleurogenoides cyanophlycti*, *Tremiorchis ranarum* and *Encyclometra colubrimurorum* infecting the frog *Fejervarya* sp. (Anura: Dicroglossidae) of the Wayanad Region of the South Western Ghats are reported in this paper. Of the trematode parasites recovered, three, *G. glottoides*, *P. cyanophlycti* and *T. ranarum*, were adults and one, *E. colubrimurorum*, a metacercaria. Multiple infections were also recorded during the study. Prevalence of infection of *G. glottoides*, *P. cyanophlycti*, *T. ranarum* and *E. colubrimurorum* were 1.27%, 0.84%, 0.84% and 0.84%, respectively, the intensity of infection 5, 1, 4.5 and 1.5 and the mean abundance 0.063, 0.008, 0.038 and 0.012, respectively. Mean abundance is a clear indication of the dispersion of parasites among hosts. The mean abundance, prevalence of infection and the intensity were at the maximum level in *G. glottoides* infection.

Keywords: Digenean parasites; Frog; *Fejervarya*; Western Ghats; Prevalence.

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Introduction

Frogs harbour a variety of adult and larval trematodes where freshwater gastropods act as first intermediate hosts in their life-cycles. The digenetic

trematodes of amphibians of India have been studied by many investigations like Mehra and Negi (1926a, b; 1928), Bhalerao (1926, 1936a, b, c; 1937), Srivastava (1933a, b; 1934a, b), Pande (1937), Chauhan (1954), Gupta and

Agrawal (1966, 1967), Pandey (1968, 1969a, b; 1981), Gupta, (1970, 1977), Mukherjee and Ghosh (1970, 1972), Singh (1977) Janardanan et al. (1987), Janardanan and Prasadani (1991), Brinsh and Janardanan (2014) and Shinad and Prasadani (2017a, b; 2018a, b, c, d). Singh (1977) made the first contribution to the trematode fauna of amphibians in Kerala. From among the major contributors, Janardanan et al. (1987) recorded *Pleurogenoides ovatus* Rao, 1977 from *Rana tigrina* (*Hoplobatrachus tigerinus*) and later (1991) they elucidated its life cycle. The life cycle of a new species *P. malampuzhaensis* was established by Brinsh and Janardanan (2014). Recently four new species of digenetic trematodes from amphibian hosts were reported from the Western Ghats by Shinad and Prasadani (2017a, b; 2018a).

Fejervarya is an Asian frog belonging to the family Dicroglossidae. These are one of the common frogs of the Western Ghats (Frost, 2014) and are often found resting in shallow water and mud in wet and shaded areas. They are normally seen partly buried in mud or sand beside small streams and seepages where it is hard to locate due to their camouflaging colour. It is widely distributed throughout China, Nepal, Bhutan, western and central Myanmar through Bangladesh and India to the northern Pakistan and south to the Western Ghats (Frost, 2014). During our studies on the digenetic parasites of frogs of the Western Ghats, Wayanad region, an analysis was made on the species composition of digenetic parasites of *Fejervarya* sp.

This paper reports the digenetic parasites infecting *Fejervarya* sp. of the Western Ghats with their prevalence, intensity of infection and mean abundance.

Materials and methods

The investigations were made in the South Western Ghats, Wayanad Region (latitudes 11° 27' and 15° 58' N and longitudes 75° 47' and 70° 27' E). Western Ghats is recognized as one of the hottest hotspots of biodiversity due to its very rich fauna and flora and the highest level of endemism.

Two hundred and thirty six specimens of *Fejervarya* sp. collected during the period from January 2016 to March 2019 from various water bodies using sweep hand net were brought to the laboratory, maintained in cement cisterns and fed occasionally with insects. The specimens were narcotized with chloroform, dissected, their skin were removed, and the muscle tissues were macerated to detect the presence of metacercariae. Internal organs of each frog were dissected out, transferred to separate Petri dishes containing 0.75% saline, macerated and examined under the Labomed Luxeo4Z stereozoom microscope.

Adults and metacercariae were carefully taken out, transferred to 0.75% saline in separate watch glasses and studied under phase contrast research microscope (Nikon ECLIPSE Ni-U) without supravital staining or after staining with neutral red. Permanent preparations of adult parasites were made after fixing them in 5% formalin under slight cover glass pressure and staining with acetocarmine, following the procedure outlined by Cantwell (1981).

Nikon NIS Elements Imaging software was used for the morphometric studies. All measurements are in micrometers (μm), as range followed by mean in parentheses. Figures were drawn using the Nikon Y-IDT drawing

tube attached to the Nikon ECLIPSE Ni-U microscope and the details were added free hand from observations made on live specimens. Photomicrographs were taken with the Nikon Y-TV55 digital camera attached to the phase contrast microscope. **Results**

Three species of adult digenetic trematodes *G. glottoides*, *P. cyanophlycti*, *T. ranarum* and one metacercaria of *E. colubrimurorum* were found infecting the frog *Fejervarya* sp. (Tabela 1, Figures 1 to 3).

Collection localities: Muthireri, Kalpetta, Peechangode, Kurumbalak

kotta, Idikkara, Thalappuzha Engineering College, Valliyoorkavu, Atturkundu (Pulpally), Thalappuzha, Pakkam, Chandhanathodu, Kannur University Campus Mananthavady, Korom, Sugandhagiri, Neerattadi (Panamaram), Chakadi, Kalluvayal (Batheri), Banasura Chirappullumala, Ondayangadi, Panamaram, Pupally, Birakuppa, Munderi, Batheri, Kambamala, Ellumandam, Karappuzha, Koileri, Ambalavayal, Manivayal, Moolithode, Koolivayal and Pottan kolli Wayanad District.

Period of collection: January 2016 to March 2019.

Table 1. Summary of prevalence, intensity of infection and mean abundance of trematodes under study.

Name of the parasite	Prevalence of infection	Intensity of infection	Mean abundance
<i>Ganeo glottoides</i>	3 out of 236 frogs (1.27%)	15 from 3 infected frogs (5)	15 in 236 frogs (0.063)
<i>Pleurogenoides cyanophlycti</i>	2 out of 236 frogs (0.84%)	2 from 2 infected frogs (1)	2 in 236 frogs (0.008)
<i>Tremiorchis ranarum</i>	2 out of 236 frogs (0.84%)	9 from 2 infected frogs (4.5)	9 in 236 frogs (0.038)
<i>Encyclometra colubrimurorum</i>	2 out of 236 frogs (0.84%)	3 from 2 infected frogs (1.5)	3 in 236 frogs (0.012)

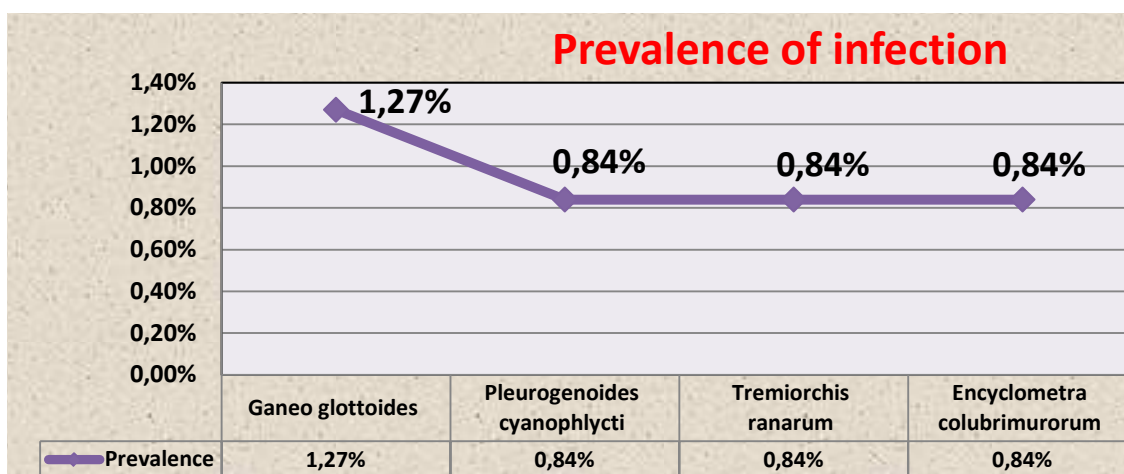


Figure 1. Prevalence of infection of the parasites.

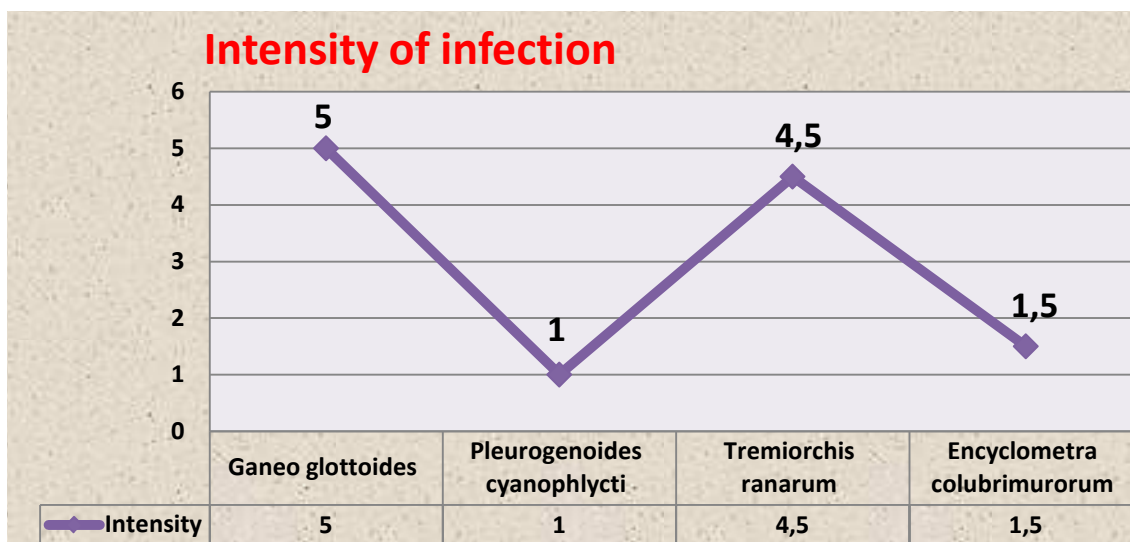


Figure 2. Intensity of infection of the parasites.

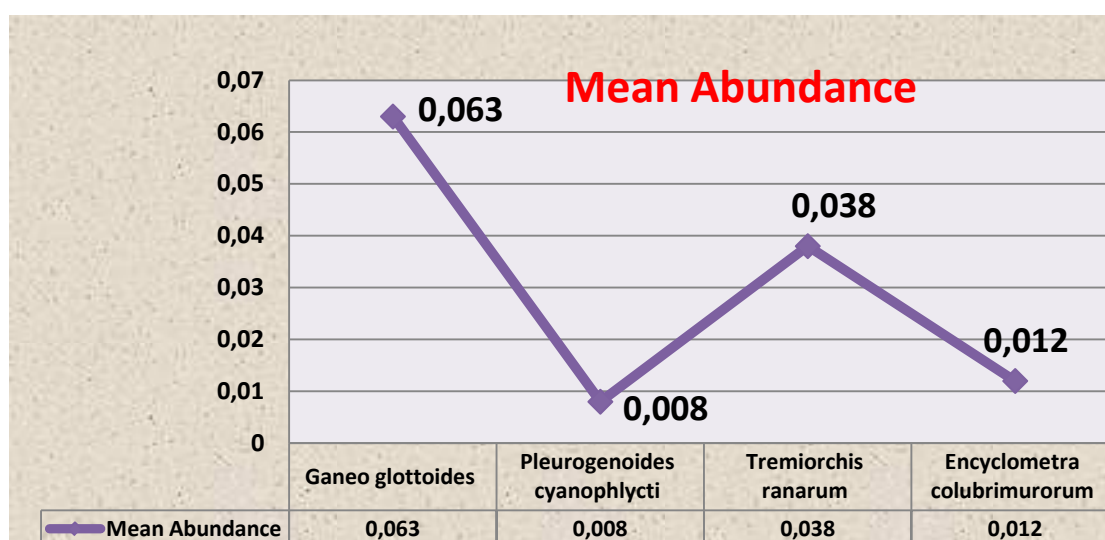


Figure 3. Mean abundance of parasites.

Family: Lecithodendriidae
(Luhe, 1901) Odhner, 1901

***Ganeo glottoides* Klein, 1905**
(Figure 4)

The genus *Ganeo* of the family Lecithodendriidae (Luhe, 1901) Odhner, 1901 was proposed by Klein (1905) with *G. glottoides* as its type from *E. hexadactylus* from Tamil Nadu. Since then 17 species have been added to the genus. Fotedar (1959) while reviewing the genus synonymised *G. kumaonensis*

with *G. tigrinum*. Mukherjee and Ghosh (1970) synonymised *G. attenuates* with *G. tigrinum*. *G. kawi* and *G. gazipurensis* were synonymised with *G. bufonis* by Rao (1974) and Gupta and Jahan (1976) respectively. Later Gupta (1977) considered *G. bufonis* as a synonym of *G. tigrinum*. Rao and Kameswari (1976) synonymised *G. korkei* and *G. punjabensis* with *G. tigrinum*.

Remarks: The species under study has an elongate oval body with

bluntly pointed anterior end and broadly round posterior end. Body is covered with small spines which are closely set at the pre-acetabular zone and gradually decrease in number behind ventral sucker. It has a small, circular, sub terminal oral sucker and a slightly larger equatorial ventral sucker. Its mouth is sub terminal and pharynx is thick walled and globular. It has long, narrow oesophagus and intestinal caeca. Testes are obliquely placed, one behind the other, in front of ventral sucker and ovary is round, placed just below the posterior testis. Uterus is coiled, filled with oval operculate eggs. Urinary bladder is V-shaped and vitellaria on the lateral margin up to the posterior one-fourth. Cirrus sac is above the ventral sucker. Its gonopore opens on the lateral margin just above the position of caecal bifurcation.

Three out of 236 *Fejervarya* sp. were infected with *G. glottoides* and the prevalence of infection was 1.27%. Fifteen *G. glottoides* were recovered from three frogs and the intensity of infection was 5 and 15 *G. glottoides* were recovered from a total of 236 frogs so the mean abundance was 0.063 (Table 1).

Multiple infection was observed in few frogs with *P. cyanophlycti*, *T. ranarum* and *Clinostomum complanatum*.

Collection localities: Peechan-gode and Ambalavayal, Wayanad District.

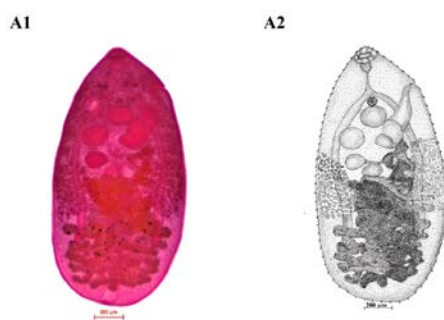


Figure 4. *Ganeo glottoides* (A1 and A2).

Family: Lecithodendriidae
(Luhe, 1901) Odhner, 1901

Pleurogenoides cyanophlycti
Shinad and Prasad, 2017 (Figure 5)

Based on the length of the intestinal caeca Travassos (1921) established the genus *Pleurogenoides* of the family Pleurogenidae Looss, 1899 to accommodate those species of the genus, *Pleurogenes* Looss, 1896, and considered *P. tener* as its type species. On the basis of the length of intestinal caeca and position of genital pore, Mehra and Negi dropped the genus *Pleurogenoides* into two sub genera, *P. (Pleurogenes)* and *P. (Telogonella)*. Srivastava, (1934a) also dropped the genus *Pleurogenoides* and transferred the species under it to *Pleurogenes*. Macy (1936) retained the genus *Pleurogenoides* and the same were accepted by Kaw (1943) and Mukherjee and Ghosh (1970). Of the 32 species of *Pleurogenoides* recorded worldwide from amphibians, 16 species were recorded from India. Life cycles of two species of *Pleurogenoides*, *P. ovatus* and *P. malampuzhaensis* have been elucidated from Kerala (Janardanan and Prasad, 1991; Brinesh and Janardanan, 2014). Recently three new species of *Pleurogenoides*, *P. cyanophlycti*, *P. euphlycti* and *P. wayanadensis* were described by Shinad and Prasad (2017b, 2018a) from *E. cyanophlyctis* of the Wayanad region of the Western Ghats.

Remarks: The species is characterized by an oval body with a blunt anterior and broadly round posterior ends and the body is covered with minute, backwardly directed spines which are closely set at the anterior region but widely spaced at the posterior end. It has a sub-terminal and almost round oral sucker which is larger than the ventral sucker. Its ventral sucker is spherical and equatorial. Mouth is sub-terminal with an ovoid and muscular pharynx and short oesophagus. Intestinal caeca bifurcates anterior to ventral sucker into short, blunt, laterally inclined, unequal caecum and ends

slightly behind testes. It has two slightly spherical or oval, symmetrical testes, at the equatorial or post-equatorial region and one on either side of the ventral sucker in the lateral body margin. Its cirrus sac is claviform extending from the anterior margin of the ventral sucker to genital pore, at the left body margin near the level of oral sucker; it encloses bipartite seminal vesicle, well developed pars-prostatica, long and narrow ejaculatory duct and an un-spinosed cirrus. It has a spherical ovary which is intercaecal and located anterior to right testis. Its uterus is post-acetabular and winding mostly in the hind body region. Its eggs are ovoid, operculate; vitellaria are well developed, extending from the mid-level of oral sucker to the anterior margin of testes, on either side, converging medially, but not meeting together.

Two out of 236 *Fejervarya sp.* were infected with *P. cyanophlycti* so the prevalence of infection was 0.84%. Two *P. cyanophlycti* were recovered from two frogs and the intensity of infection was one. Its mean abundance was 0.008 as two individual *P. cyanophlycti* were recovered from the 236 *Fejervarya sp.* examined (Table 1).

A few hosts infected with *P. cyanophlycti* exhibited multiple infections with *G. glottoides*.

Collection localities: Peechangode, Wayanad District.

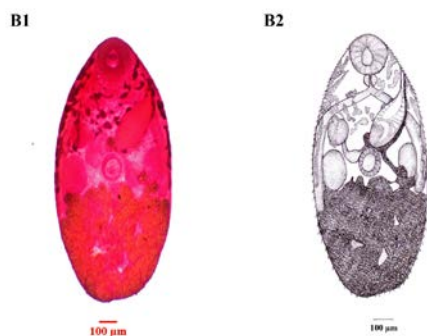


Figure 5. *Pleurogenoides cyanophlycti* (B1 and B2).

Family: Brachycoelidae
Johnston, 1912

***Tremiorchis ranarum* Mehra and Negi, 1926 (Figure 6)**

Mehra and Negi (1926a) proposed the genus *Tremiorchis* of the family Brachycoeliidae Looss, 1899 with *T. ranarum* from *H. tigerinus* as the type species. Ten species of this genus have been reported so far. Pandey and Agarwal (1981) with due justification, considered *T. mehrai*, *T. vitelloconfluentum*, *T. tigrinarum*, *T. mathurensis* and *T. spiniphlyctis* as synonyms of *T. ranarum*. Characters of *T. attenuates*, *T. jamshedpurensis* and *T. jaini* do not validate them as distinct species and they are, therefore, synonyms of *T. ranarum* (Rajendran and Janardanan, 1993). The genus *Tremiorchis* is therefore monospecific and is restricted to the Indian subcontinent. Rajendran and Janardanan (1993) elucidated the life cycle of *T. ranarum*.

Remarks: The species is characterized by elongate-oval body with spines on the surface, except at the posterior third. It has a sub terminal and roughly spherical oral sucker and a ventral sucker at about one-third distance from the anterior end of body. Mouth is sub terminal, pharynx is small and thick walled, oesophagus is long narrow and intestinal caeca terminates just behind the anterior margin of anterior testis. It has a pair of round or transversely ovoid testes and a round ovary. Numerous small eggs are present.

Two out of 236 *Fejervarya sp.* were infected with *T. ranarum* so the prevalence of infection was 0.84%. Nine *T. ranarum* were recovered from two frogs and the intensity of infection was 4.5 and nine *T. ranarum* were recovered from a total of 236 frogs so the mean abundance was 0.038 (Table 1).

Few frogs infected with *T. ranarum* exhibited multiple infections with *G. glottoides* and *C. complanatum*.

Collection localities: Pulpally and Ambalavayal, Wayanad District.

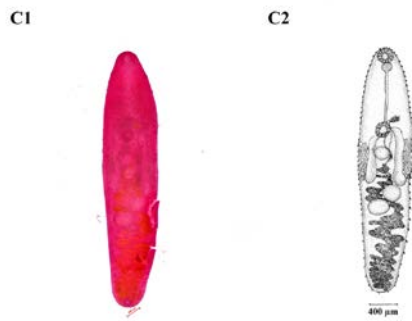


Figure 6. *Tremiorchis ranarum* (C1 and C2).

Family: Plagiorchiidae (Luhe, 1901) Ward, 1917

Metacercaria of *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 (Figure 7)

The genus *Encyclometra* was proposed by Baylis and Cannon in 1924 of the family Plagiorchiidae (Luhe, 1901) Ward, 1917. As far as is known, only four species of the genus *Encyclometra* have been reported. Adults of all the four species have been reported from India. Mehra (1931) from *Natrix piscator* and *N. mucosus* at Allahabad (U.P.); Bhalerao (1926, 1936) reported it from *Ptyas* (= *Zamensis*) *mucosus* at Calcutta; Gupta (1954) from *N. piscator* Ludhiana (Punjab); Srivastava and Ghosh (1968) from the snakes, namely *P. mucosus*, *N. piscator*, *N. stolata* and *Atrretium schistosum* from Calcutta (West Bengal) and Patna (Bihar); Dwivedi and Chauhan (1970). The adults of *E. colubrimurorum* were recorded from Turkey (Düsen and Oz, 2004; Capuse, 1971; Biserkov, 1996; Shimalov and Shimalov, 2000). Fischthal and Kuntz (1965, 1967) reported *E. colubrimurorum*, infecting *E. plumbea* from North Borneo and Korea. The life-cycle of the genus has not so far been elucidated. Metacercariae of *E. colubrimurorum* and *E. asymmetrica* have been reported by Yamaguti (1936) and Chiang (1951) respectively. Nature of the intestinal caeca is a constant and

reliable character to differentiate the various species of this genus. The first report of *E. colubrimurorum* metacercaria from India was made from the tadpole of *C. curtipes* and the adult frog (*Fejervarya* sp) and the morphological characters of the metacercaria is done by Shinad and Prasadani (2018b).

Remarks: This species has two structural variations, an elongate and a small ovate. Body is fusiform and aspinose. It has a small, oval, sub-terminal oral sucker and a slightly larger equatorial and oval to round ventral sucker. Mouth is sub terminal; pharynx is muscular thick walled and oval to round. It has short oesophagus. Pharynx and oesophagus are surrounded by gland cells. It has a long, asymmetrical and unequal intestinal caeca extended almost up to the posterior end of body. Testes are fairly developed, round to ovoid and tandemly placed in the posterior half of body and ovary is small ovoid located just behind the acetabulum. Excretory bladder is large, saccular filled with concretions and extending from the posterior end to just below the acetabulum. Excretory pore is terminal. Cirrus sac is poorly developed, immediately above the anterior border of acetabulum.

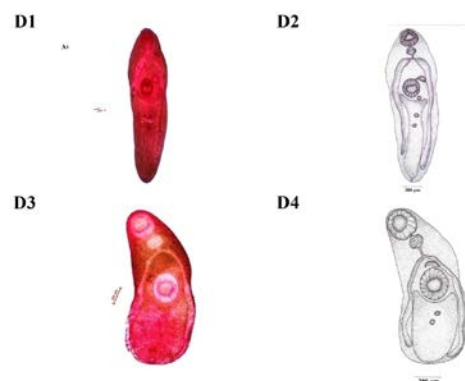


Figure 7. Metacercaria of *Encyclometra colubrimurorum* (D1, D2, D3 and D4).

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Conflict of interest

Authors declare that there are no conflicts of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

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