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continued on the back inside cover

Cover: *Saproamanita praeclara*: Sporocarp in habitat © Kantharaja. R.

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SHORT COMMUNICATION

Case report of hook worm *Grammocephalus hybridatus* and stomach bot *Cobboldia elephantis* infections in a free-ranging Asian Elephant *Elephas maximus* in Tamil Nadu, India

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Abstract: Elephants in the wild are susceptible to many gastrointestinal parasites. In the present study, necropsy was conducted on a free-ranging Asian Elephant *Elephas maximus* female aged about 15 years which died at Coimbatore forest range, Tamil Nadu state, India. The necropsy revealed that the liver was infected with round worms and the stomach was heavily infested with dipteran larvae. These round worms and larvae were collected and processed by dehydrating in ascending grades of alcohol and then cleared in carbolic acid. The cleared samples were mounted and examined under light microscopy for species identification. Faecal samples collected from the rectum were analysed by sedimentation for the presence of helminth eggs. On microscopic examination, the head end of the round worms showed a buccal capsule which possessed a pair of semilunar ventral cutting plates. Male worms showed well-developed bursa at the posterior end. The anterior end of the dipteran larvae showed two powerful oral hooks with cephalopharyngeal skeleton. Anterior spiracle appeared as a short club-shaped tube with 12 lobes. The abdominal segments of the larvae had a row of belt-like triangular spines. The posterior spiracles of the larvae had three longitudinal parallel slits in each spiracle with closed peritreme. Based on the above morphological characters, the round worms and larvae were identified as *Grammocephalus hybridatus* and *Cobboldia elephantis*, respectively. Strongyle eggs were identified in the faecal sample based on the morphology of thin shell and segmented yolk. This appears to be the first report of *G. hybridatus* infection in a free-ranging elephant in Tamil Nadu state, India.

Keywords: Dipteran larvae, faecal samples, gastrointestinal parasites, helminth eggs, infection, morphological characters, necropsy, strongyle egg, round worms.

The life of the wild animals is threatened by many factors such as cannibalism, infighting injuries, accidents, habitat loss & fragmentation, poaching & hunting, as well as different diseases (Riddle et al. 2010). Furthermore, parasitism has an impact on host species evolution and ecology through sexual selection (Hamilton & Zuk 1982), and parasite-mediated competition results in reduced population size or extinction (Price et al. 1986). The Asian Elephant *Elephas maximus* is an 'Endangered' species as per the IUCN Red List of Threatened Species (Williams et al. 2020). Free-living wild animals are generally infected with numerous parasites, but adverse effects occur only in the animals that are physiologically or nutritionally stressed (Gaur et al. 1979; Fowler & Mikota 2006). Elephants in the wild are susceptible to many gastrointestinal (GI) parasites (Vidya & Sukumar 2002). According to investigations, many gastrointestinal parasites have been found in Indian and Sri Lankan elephants: *Murshidia murshidia, M. falcifera, M. indica, M. longicaudata, Quilonia renniei, Equinurbia sipunculiformis, Decrusia aditicta, Amira pileata* (Seneviratna 1955; Fernando & Fernando 1961;

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Sundaram et al. 1971), *Choniangium epistomum* (Datta et al. 1972), *Bathmostomum sangeri* (Sathianesan et al. 1979), *Grammocephalus hybridatus* (Islam & Talukdar 2014), and larvae of *Cobboldia elephantis* (Seneviratna & Jayasinghe 1968). Strongyles are the most frequent GI parasites in African (Scott & Dobson 1989) and wild Asian Elephants (Watve 1995; Vidya & Sukumar 2002; Dharmarajan et al. 2005; Nishanth et al. 2012; Abeysekara et al. 2018).

Parasitic infections can cause disease and death in wild animals and they can also infect domestic animals and vice versa. Parasites also alter the host's behaviour, health and reproduction, and make parasite transmission easier (Abhijit et al. 2018). Understanding infections in wild animals is critical because illnesses can cause elephants to die under extremely stressful situations. Hence, it is important to monitor the mortalities of wild elephants and carry out post-mortem sampling to determine whether the cause of death was due to GI parasitic infections (Vidya & Sukumar 2002; Lynsdale et al. 2017). This study reports the occurrence of *Grammocephalus hybridatus* worms in the liver and *Cobboldia elephantis* larvae in the stomach collected during the post mortem of a free-range elephant in Tamil Nadu.

Materials and Methods

During August 2020, the death of a wild female elephant (approximately 15 years of age) was reported in the Coimbatore forest range, Tamil Nadu. Necropsy was carried out by the forest veterinary officer. On detailed examination, the liver was found to be infected with round worms and the stomach was heavily infested with dipteran larvae. These round worms and larvae were collected and sent in 10% formalin to the Department of Veterinary Parasitology, Madras Veterinary College for further species identification. Faecal sample collected directly from the rectum was also sent to analyse the sample for helminth eggs.

Processing of the samples

The round worms were washed in water, dehydrated in ascending grades of alcohol, and then cleared using carbolic acid. Cleared specimens were mounted using DPX and examined under light microscope. The species identification of the round worm was carried out based on van der Westhuysen (1938) and Kumar et al. (2011). The dipteran larvae were boiled in 10% sodium hydroxide solution. They were kept in the same solution for one week. Later, they were dehydrated and cleared. The larvae were identified based on the morphological features described by Zumpt (1965). The faecal sample was analyzed using sedimentation and flotation technique as per the standard protocol (Soulsby 1982) and examined under light microscope for the presence of helminth eggs.

Results

In the present study, the round worms recovered from the elephant liver were identified as *Grammocephalus hybridatus*. The male worms measured 35 mm and 1.5 mm while the females were 37 mm and 1.5 mm in length and breadth, respectively. Both the male and female worms were more or less equal in size (Image 1a,b).

Morphology of female worm

The head end of the worms was characteristically curved towards the dorsum. The buccal capsule was wide anteriorly and narrowed posteriorly and possessed a pair of small semilunar ventral cutting plates (Image 2a). At a depth of the buccal cavity, there was a dorsal cone. The dorsal cone did not reach half the length of the buccal capsule. It was slender and curved (Image 2b). The oesophagus was long with a caecal diverticulum extending towards the pharynx (Image 2c). The vulva was present close to the middle of the body (Image 2d). The width of the worm reached a maximum at the vulvar region, then tapered to both ends. The tail end of the female was slightly bent dorsad and tapered to the posterior extremity with a slight knob-like structure at the end (Image 2e).

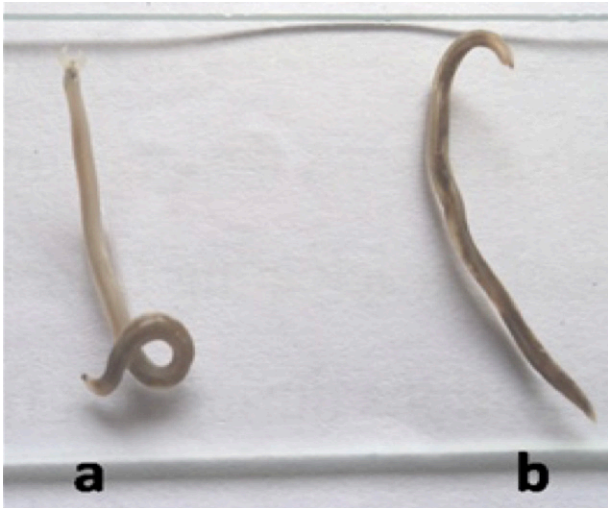

Image 1. Gross appearance of male (a) and female (b) worms of *Grammocephalus hybridatus* isolated from a free-ranging Asian Elephant in Tamil Nadu showing equal size. © Authors

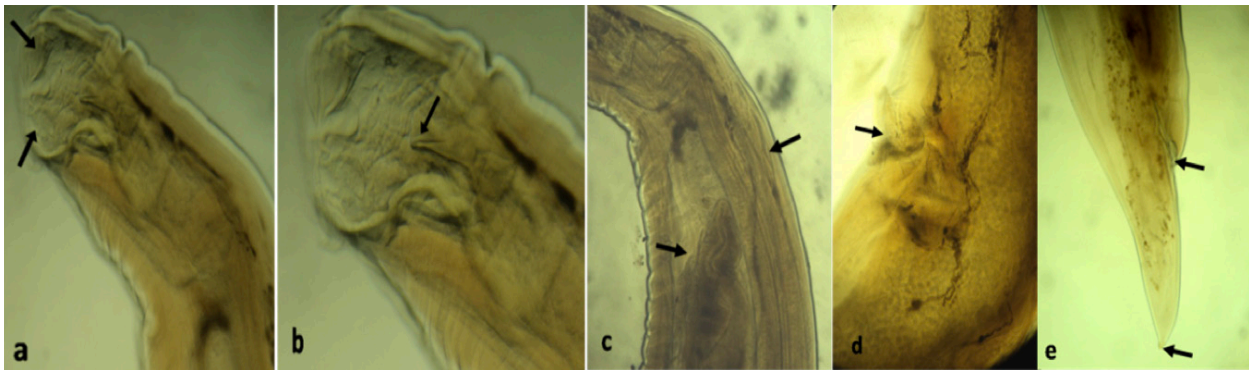

Image 2. Internal morphology of female worm: a—Lateral view of the anterior end of a female worm (10x), arrows indicate cutting plate | b—Lateral view of the buccal cavity (40x), arrow indicates dorsal cone | c—Lateral view of worm showing oesophagus (upper arrow) with caecal diverticulum extending towards pharynx (lower arrow) (10x) | d—Vulvar opening (Arrow) (10x) | e—Lateral view of posterior end female worm (10x) with knob (lower arrow) and anus (upper arrow). © Authors

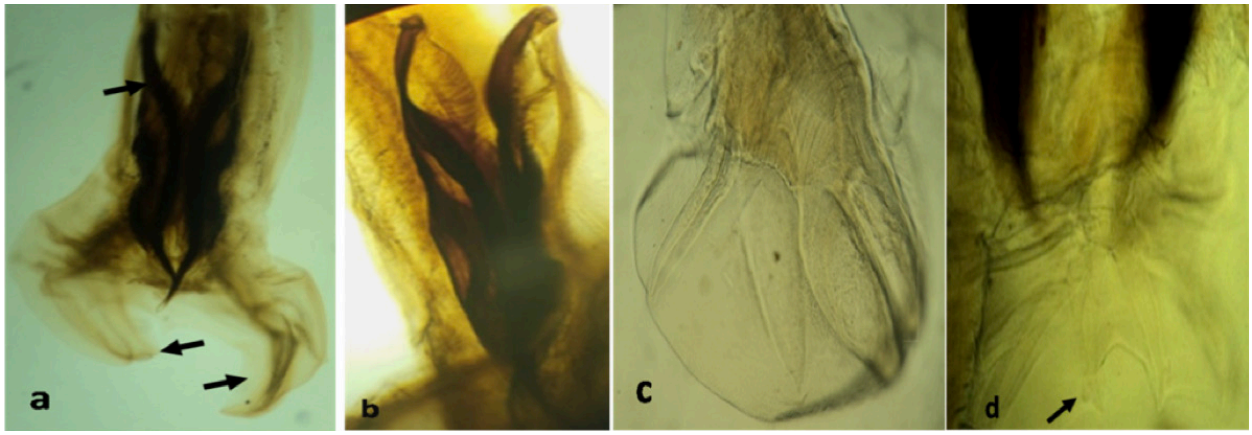

Image 3. Internal morphology of male worm: a—Ventral view of caudal end of worm showing well developed bursa with lateral lobes (opposing arrows) and spicules (upper arrow) (10x) | b—Pair of spicules (40x) | c—Lateral view of bursa (10x) | d—Dorsal view of bursa showing bifid tip of external branch of dorso-dorsal ray (Arrow) (40x). © Authors

Morphology of male worm

Male worms showed well-developed bursa at the posterior end (Image 3a). The spicules were equal in length (1.4 mm). They were stout, wavy, contained a dark core and were alate with ornamentations. Their tips were pointed and placed together (Image 3b). The lateral lobe was elongated when compared to the dorsal lobe; the bursal rays were relatively short and the lateral rays were quite stout (Image 3c). In the dorsal lobe, the external-dorsal rays were elongated; dorso-dorsal rays arose directly from the base of the dorsal lobe. Tip of the dorsal rays were bifurcated with the external branch ending in a cup-like structure (Image 3d).

Faecal examination

Strongyle eggs were identified in the faecal sample based on the morphology of egg showing thin shell and segmented yolk (Image 4); the same has been

Image 4. Strongyle eggs isolated from a free-ranging Asian Elephant in Tamil Nadu showing thin shell and segmented yolk (40x). © Authors

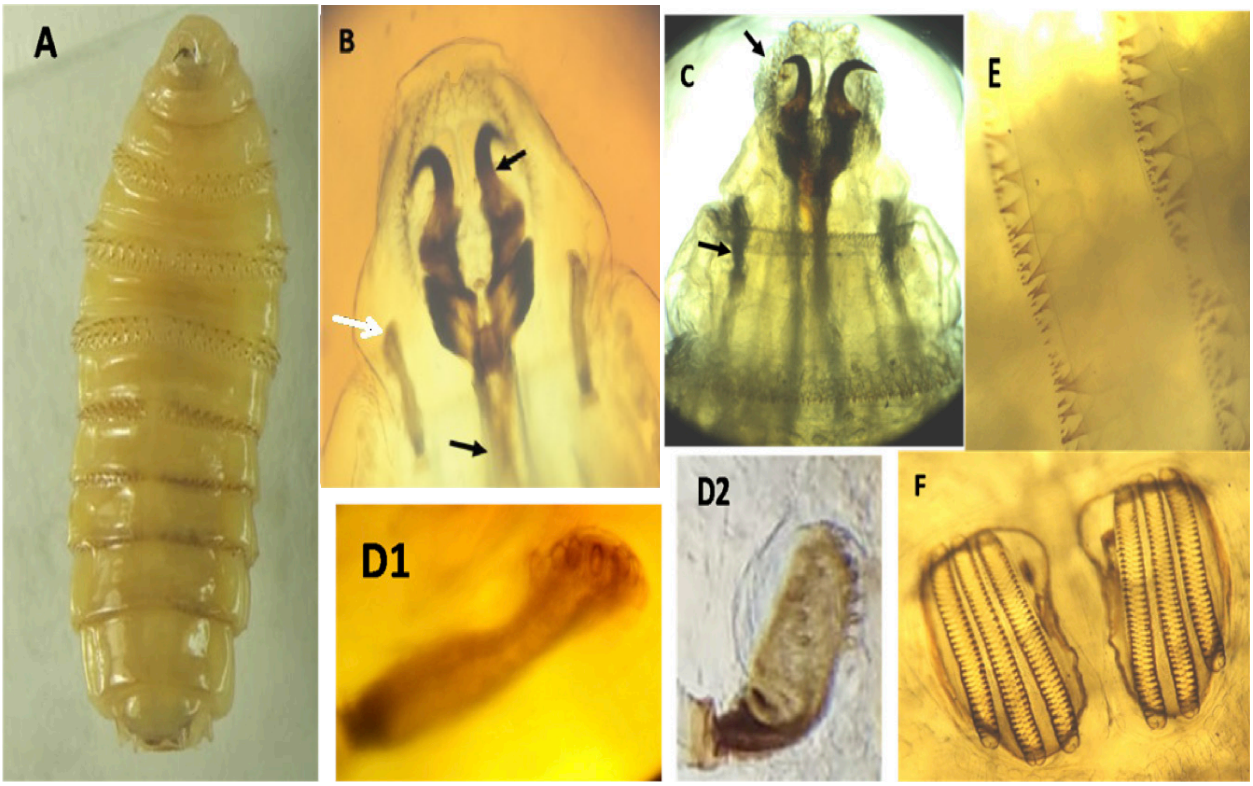

Image 5. A—Larva of *Cobboldia elephantis* (stomach bot) under stereozoom microscope | B—The anterior end showing two powerful oral hooks with cephalopharyngeal skeleton (black arrows) and anterior spiracle (white arrow) (40x) | C—The mouth cavity is surrounded by a crown of teeth (upper arrow) which are somewhat shorter ventrally, the anterior spiracles (lower arrow) are also seen (40x) | D1—Lateral view of anterior spiracle showing a short, club shaped tube (100x) | D2—Ventral view of anterior spiracle with 12 lobes (100x) | E—Abdominal segments of the bot with a row of belt like triangular shaped spines (40x) | F—The posterior spiracles showing three longitudinal parallel slits in each spiracle with completely closed peritreme (40x). © Authors

earlier described by van der Westhuysen (1938). Many investigators made similar observations in Asian Elephants from various places (Nishant et al. 2012; Abeysinghe et al. 2017; Abeysekara et al. 2018; Abhijit et al. 2018).

Morphology of dipteran larvae

In this study, the dipteran larvae collected from the elephant stomach were identified as stomach bot *Cobboldia elephantis* (Cobbold, 1866) based on the morphological characters of the anterior end, body spines and posterior spiracles as per the descriptions of Zumpt (1965). A total of 400 stomach bots (2–2.8 cm long) were collected from the stomach (Image 5A). The anterior end of the larva showed two powerful oral hooks with cephalopharyngeal skeleton (Image 5B). The mouth cavity was surrounded by a crown of teeth which was somewhat shorter ventrally (Image 5C). Anterior spiracle appeared as a short club-shaped tube with 12 lobes (Image 5D1,D2). The abdominal segments of the larvae had a row of belt-like triangular shaped spines (Image 5E). The posterior spiracles of the larvae showed three longitudinal parallel slits in each spiracle with closed peritreme following processing (Image 5F).

Discussion

The present communication records the morphological features with specific characters that assisted in the identification of *G. hybridatus* and *C. elephantis*. *Grammocephalus hybridatus* is a hookworm inhabiting the bile duct of elephants and immature stages are present in the intestine causing nodules which is similar to *Bunostomum* sp. (Fowler & Mikota 2006). Elephants get infected through skin penetration or by direct ingestion of larvae. They are the largest hook worms under the family Ancylostomidae (Sundaram 1966). *G. clathratus, G. hybridatus, G. varedatus*, and *G. intermedius* are the four species in the genus *Grammocephalus*, each of which is unique to a single host (Obanda et al. 2011). *G. clathratus* was observed in the liver and bile duct of African Elephants in Kenya by Obanda et al. (2011). *G. varedatus* and *G. hybridatus* infect Indian Elephants, while *G. intermedius* lives in the large intestine of African Rhinoceros (van der

Westhuysen 1938). The adult parasites are blood suckers which cause anaemia and weakness along with other signs of hepatic insufficiency when occurring as heavy infection (Fowler & Mikota 2006).

The study of the morphology of *G. hybridatus* was in accordance with the description of van der Westhuysen (1938) from Asian Elephant origin, with slight variations in morphometry. Bhalearo (1935) first recorded the hookworm (*G. clathratus*) from India, while Rajasekhariah et al. (1975) reported the occurrence of both the immature and mature *G. hybridatus* from the stomach nodule of a captive Asian Elephant. Previously, Pillay et al. (1976) reported the occurrence of *G. hybridatus* from an elephant in Mysore while Islam & Talukdar (2014) reported *G. hybridatus* infection in a free-ranging Asian Elephant from Assam. Apart from *G. hybridatus*, elephants are affected with a variety of intestinal strongyle worms (Vidya & Sukumar 2002), implying that the strongyle eggs found in the faeces likely represented a diversity of species.

Vidya & Sukumar (2002) analyzed the faecal samples from wild elephants in Nilgiris, southern India and found that 86.8% of the samples were positive for parasite eggs. Strongyles were found in 40%, 16%, and 8% of samples from Mudumalai, Anamalai, and Sathyamangalam forests in southern India, respectively (Nishant et al. 2012). In Sri Lanka, wild elephants (93.3%) had a greater prevalence of parasite infection than semi-captive (55.0%) and captive elephants (25.0%) (Abeysekara et al. 2018). Mixed infections were also reported to be more common (47.1%) than single infections (21.2%) (Abeysekara et al. 2018). The strongyle eggs were found in 100% of wild elephants in Sri Lanka, according to Abeysinghe et al.(2017). The majority of strongylid nematodes have eggs that are morphologically indistinguishable and is referred to as strongyles. Because of their direct life cycle, nematodes are the most common and numerous helminths in elephants, as evidenced by the high species richness (Elsheikha & Obanda 2010). Intestinal parasites in general and strongyles in particular, appear to be common in wild elephants, but the prevalence appears to vary greatly between locations.

The morphology of *C. elephantis* larvae (stomach bot) in this study was in accordance with that observed by Panda et al. (2005), Venu et al. (2015), and Ananda et al. (2017) who reported the larvae of *C. elephantis* from the stomach of free-ranging wild elephants from different parts of country. The anterior spiracle of this larvae, however, has yet to be described. The anterior spiracle and crown of teeth around their mouth were described for the first time in this study. Such characters were identified based on the descriptions of the larva of the subfamily *Platycobboldia loxodontis*, which affects African Elephants (Zumpt 1965).

The pathogenesis of *C. elephantis* larvae in elephants has not been well studied and they appear to be well tolerated by the animals even in large numbers; however, it has been reported that the infested animals show symptoms of gradual emaciation, disinclination to feed and loss of condition (Raquib 1970). In the present case, major area of the stomach was attached with 2–2.8 cm sized larvae of *C. elephantis* similar to the observations by Panda et al. (2005) and Kakkassery et al. (2011). The gastric wall revealed congestion with tiny ulcers and significant inflammation of the gastric mucosa after these larvae was removed. Panda et al. (2005) had previously reported similar findings.

This appears to be the first report of *G. hybridatus* infection in a free-range elephant in the state of Tamil Nadu. The anterior spiracle and crown of teeth around the mouth of *C. elephantis* larvae were morphologically described for the first time in this work, based on descriptions of larvae from the same subfamily. The morphological characteristics, along with supporting figures, will be helpful in diagnosis.

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