Studies on nematodes in *Fejervarya cancrivora* (Anura: Ranidae) from Leuwiliang District, Bogor Regency, West Java

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Abstract. Paddy frog (*Fejervarya cancrivora*) is one of the leading choices as export commodities in aquaculture businesses in Indonesia. However, the increasing consumption of frog has raised concerns regarding the presence of parasitic worms in frogs. Unfortunately, the studies on helminth parasites in Indonesia, especially nematodes in frogs are still limited. We conducted a study to identify the species of nematodes parasitizing *F. cancrivora*. In this research, 100 individuals of *F. cancrivora* from Leuwiliang, Bogor were observed for the presence of nematodes. The nematodes found were preserved in 70% alcohol and identified using a light microscope and a scanning electron microscope. Data on morphology, prevalence, and intensity of each nematode species was recorded. The Spearman correlation test was used to assess the correlation between sex and host body weight with the number of nematodes. In this study, we recorded four nematode species, i.e. *Chabaudus* sp. (68%), *Rhabdias* sp. (19%), *Ascarididae* gen. sp. (15%), and *Cosmocerca ornata* (2%). The results showed that there was no correlation between sex and host body weight with the number of nematodes.
Key Words: frog, intensity, morphological data, parasites, prevalence.

Introduction. Paddy frog (*Fejervarya cancrivora*) is one popular export commodity for Indonesian aquaculture business. The smooth texture and mild flavor of frog legs make them a popular food around the world (Winarno 2020). Since 1969, Indonesia has exported frog legs to various countries, primarily in Europe, America, and a small number of Asian countries (Andaruisworo 2015). Most of the products are shipped frozen (Arie 1999). In 2014, Indonesia's frog meat exports increased, and it was recorded as the largest frog-exporting country (Winarno 2020). The significant domestic consumption of frog meat occurs in various areas, including Riau, North Sumatra, South Sumatra, Batam, Jakarta, Central Java, East Java, and Kalimantan (Candrawati & Widani 2019; Widayanti & Pujiyono 2022).

The increasing consumption of frog meat has sparked new worries about the existence of zoonotic parasitic helminths in frogs (Chai 2015). One of them is sparganum or tapeworm larvae causing sparganosis disease in humans (Mukherjee et al 2007; Li et al 2019). Humans can become infected with parasitic worms by eating raw or undercooked meat. The studies on parasitic helminths, particularly nematodes, from frogs in Indonesia are limited. Suzanna et al (2006) reported *Amplicaecum, Camallanus, Aplectana, Cosmocerca, Cosmocercella*, and *Spinicauda* from Bogor, West Java. Furthermore, Purwaningsih & Dewi (2013) reported *Gendria* sp., *Raillietnema rhacophory*, *Meteterakis* sp., and *Cosmocerca ornata* from forest and rice fields in West Java. Eventually, *Gendria* sp. was revised by Purwaningsih et al (2015) as *Chabaudus* sp. In their research in Central Java, Purwaningsih et al (2015) also discovered *Meteterakis wonosoboensis, M. japonica,* and *Chabaudus* sp. Later, Purwaningsih et al (2016) also conducted research on Lombok Island and reported *Camallanus senaruensis* and *M.*

lombokensis. *C. senaruensis* and *M. wonosoboensis* are new species of nematodes described from Indonesian frogs.

This research was conducted in the Leuwiliang District, Bogor Regency, West Java because the area has large rice fields covering 1458 ha (Badan Pusat Statistik 2019), which serve as the primary habitat for *F. cancrivora*. Additionally, a number of residents in Leuwiliang district are employed as frog catchers, and there is no information regarding nematodes on *F. cancrivora* in this subdistrict.

This article aimed to identify the nematode species infecting *F. cancriviora,* determine their prevalence and intensity, and investigate the correlation between the host sex and the host weight with the number of nematodes. The data can be used to raise public awareness, especially among those whose livelihoods depend on frogs, about safe handling of frog meat and proper disposal of frog waste. This will help ensure safe frog consumption and prevent the transmission of parasitic worms to other hosts. This discovery contributes to the new information on the nematodes parasitizing frogs in Indonesia.

Material and Method. The materials examined were 100 individuals of *F. cancrivora* purchased from a dealer, who captured frogs in the Leuwiliang subdistrict of Bogor Regency, West Java, in January 2024. Before observing the nematode, the hosts were anesthetized to death with alcohol 96%. The observation was conducted in the Widyasatwaloka Building, BRIN.

The body cavity of the host was opened by a longitudinal incision and the gastrointestinal tract was removed, placed separately in a Petri-dish, and examined under a dissecting microscope. The helminth parasites were then preserved in 70% alcohol. For light microscopic examination, specimens were cleared in lactophenol solution. Morphometric data and optical micrographs of nematodes were taken by using a BX50 microscope (Olympus Corp., Tokyo, Japan), equipped with a DP26 digital camera (Olympus) and Olympus cellSens Entry. Specimens were fixed in glutaraldehyde and tannin acid, dehydrated through a graded series of alcohol and vacuum-dried using an aspirator (Luchi Seieido Co., Osaka, Japan) for 10 minutes prior to attaching to stubs with double sided cello-tape, coated with gold 400 Å thickness in an Eico I-B2 ion coater and observed with a JEOL JSM IT-200 scanning electron microscope. Measurements were presented as the average, followed by the range in parentheses, unless otherwise stated.

Data analysis. In this study we presented the prevalence data and the intensity of each species nematode. The prevalence, abundance and mean intensity of parasitism for each helminth species recovered were calculated according to Turgeon et al (2018). The nonparametric Spearman test was employed to determine possible correlations between the host sex and the abundance of nematodes, and for the host weight and the number of nematodes.

Results. Four species of nematodes belonging to four genera and four families were recorded.

Ascarididae gen. sp. (Larva)

Site in host: Liver and body cavity

Larva. The body is elongated and slender, 16.67 (8.48–23.27) mm long and 320 (224–409) µm wide at mid–length, whitish in color. The mouth is not fully developed (Figure 1). The esophagus is simple, 1.66 (1.05–2.21) mm long and 164 (123–193) µm wide (Figure 1A). The distance of the nerve ring and excretory pore (Figure 1E) from the anterior end is 270 (261–276) µm and 605 (437–748) µm, respectively. The tail is short, with a tapered tip, 121 (102–131) µm long (Figure 1F,G).

Remarks. These specimens were in the larval stage. They had undeveloped characteristics, such as lips and gonads, and very varied sizes. Some investigations have

documented the discovery of nematode larvae, specifically those belonging to the genus *Hexametra*, within the liver and body cavities of amphibians (Petter 1968; Hasegawa et al 2013; Sprent 1978). According to Petter (1968), the larvae of nematodes that infect frogs are in the second stage of the parasitic life cycle. The definitive host of this genus is snakes, and its intermediate hosts are amphibians. This specimen is similar to the second stage of *Hexametra quadricornis* larvae. In Indonesia, adult *H. quadricornis* was discovered in the snake *Psammodynastes pulverentulus* on Komodo Island (Sprent 1978). Since it is difficult to prove the validity of these specimens by morphological study only, DNA sequence analysis will be needed to prove their validity.

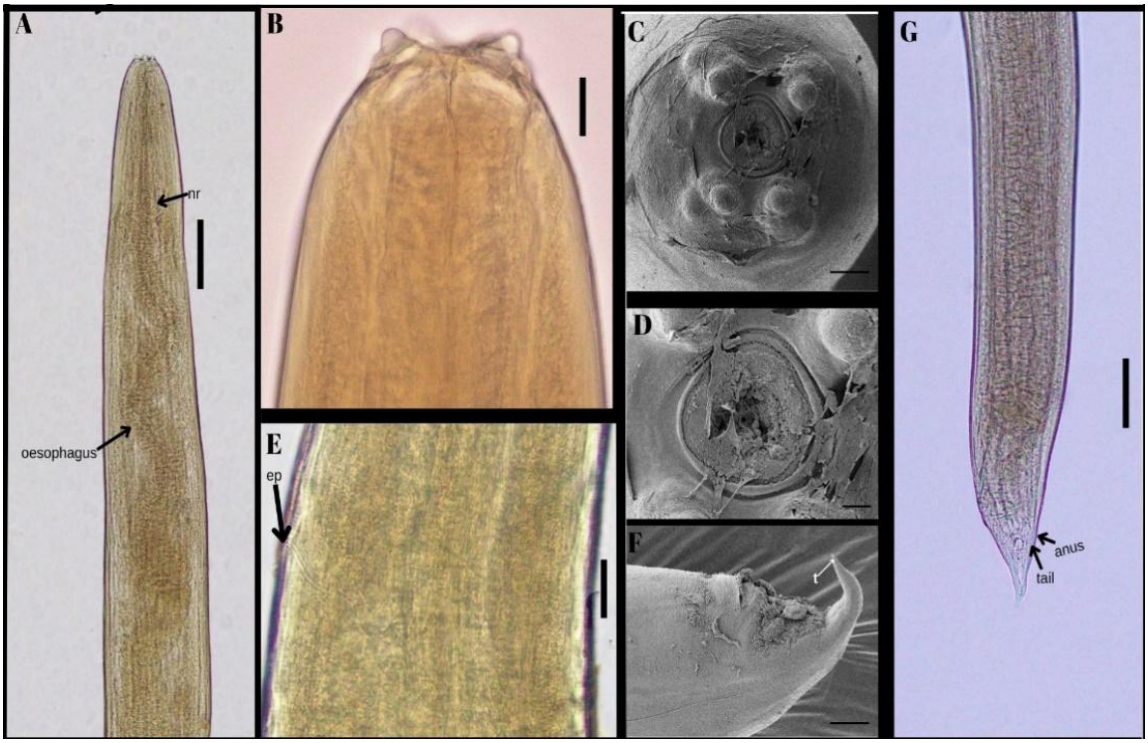

Figure 1. *Ascarididae* gen. sp.; A - anterior, esophagus and nerve ring, ventral view; B - head, lateral view; C - anterior end, face view; D - anterior end, mouth, face view; E - excretory pore, lateral view; F - posterior, lateral view; G - posterior, tail and anus, lateral view; scale bars: A,G=100 µm; B,E=50 µm; D=5 µm; C,F=10 µm.

Rhabdias sp. (Rhabditida: Rhabdiasidae)

Site of host: Lungs

Female (parasitic worm). The bodies of gravid females are elongated and slender, whitish in color. The anterior end is rounded. The mouth is surrounded by six small lips. The papillae are not seen (Figure 2B). The total body length is 4.32 (3.26–5.20) mm, maximum width is 275 (240–320) µm. Mouth has six lips (Figure 2B). Oral capsule is 17 (17–24) µm long and 21 (18–25) µm wide. The esophagus is club–shaped, 342 (318–384) µm long and 52 (50– 59) µm wide (Figure 2C). The distance of nerve ring, excretory pore, and vulva from the anterior end was 178 (165–235) µm, 256 (218–292) µm, and 1.76 (1.33–2.15) mm, respectively (Figure 2E,F). Vulva was lip–shaped, in the middle of the body, near the anterior end (Figure 2F). The genital system was typical of Rhabdiasidae, amphidelphic with anterior and posterior ovaries. The posterior end was extremely pointed (Figure 2D), and the tail was 235 (198–268) µm long (Figure 2D). The uterine branch is opposite and filled with eggs. The egg is thin–walled, few and large, embryonated, 82 (78–92) µm x 46 (31–54) µm (Figure 2G).

Male. Not found.

Remarks. The genus *Rhabdias* Stiles & Hassall, 1905 is common, being globally distributed lung parasites of amphibians and some reptiles (Teles et al 2014; Kuzmin et al 2016). This genus includes approximately 94 species distributed worldwide (Kuzmin et al 2012). Some studies had reported the occurrence of this genus parasitizing frogs in Europe, Siberia, Canada, the United States, Africa, China, Siam, and Argentina. For instance, *R. rotundata* was discovered in *Bufo viridis* in Europe, *R. bdellophis* in *Bdellophis villatus* in Africa, *R. bicornis* in *Bufo bufo asiaticus* in China, and *R. elegans* in *Bufo arenarum* in Argentina (Yamaguti 1961). In Indonesia, *Rhabdias* sp. has only been found in *R. hosii* from West Java, Indonesia (Purwaningsih & Dewi 2013). Unfortunately, the record did not provide the description of the specimen.

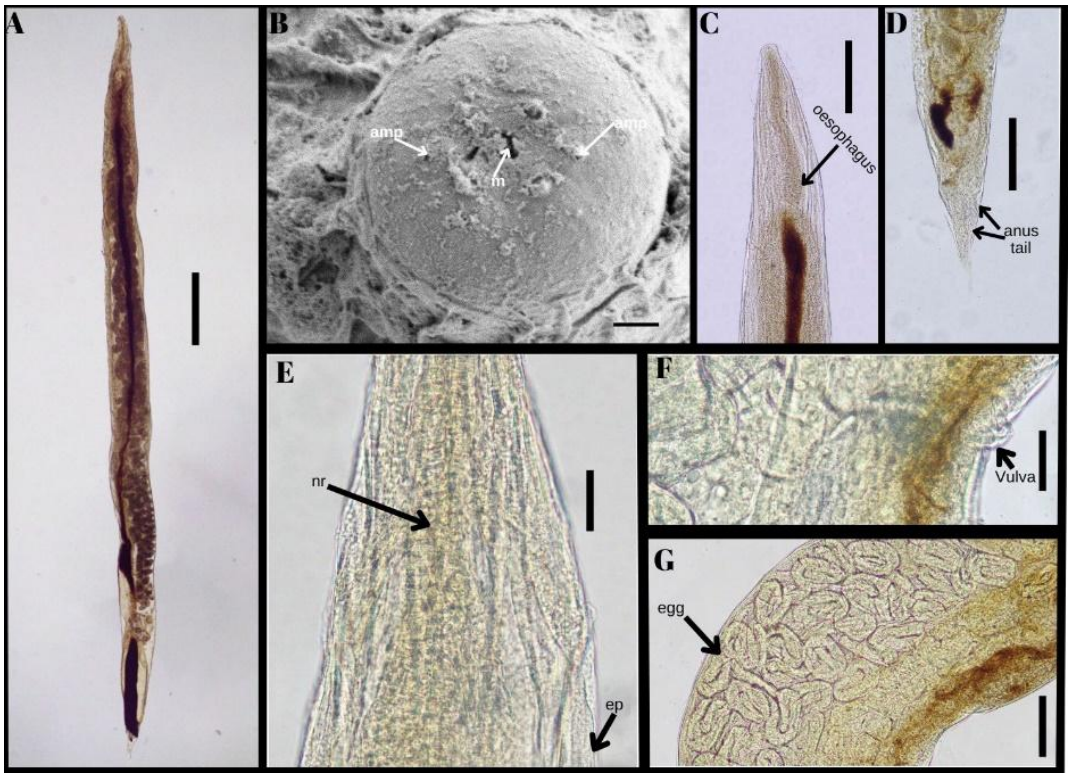

Figure 2. *Rhabdias* sp.: A - complete body; B - anterior end, enface view (m=mouth, amp=amphid); C – anterior; D - posterior female; E - nr=nerve ring and ep=excretory pore; F – vulva; G – egg; scale bars: A=200 µm; B=5 µm; C,D=100 µm; E,G=50 µm; F=20 µm; m=mouth; amp=amphid.

Chabaudus sp.

Site of infection: intestines, stomach, cecum.

General morphology. The body is medium-sized and whitish. Cuticles have transverse striations. Mouth is triradiate with 3 lips, each divided into two lobes. The nerve ring is located in the anterior third of the esophagus (Figure 3A). The face has 2 pairs of cephalic papillae and 2 amphids (Figure 4J). The esophagus is short, widens anteriorly, and ends in an elongated bulb (Figure 3A). Cervical papilla has a blunt tip (Figure 4L). Lateral alae emerge at the level of the cephalic vesicle to the posterior of the deirids (Figure 3A,B, 4I).

Male (n=10). Total body length is 18.15 (13.83–22.53) mm, width 331 (293–373) µm. Length and width of esophagus are 520 (485–556) µm and 111 (97–148) µm, respectively. Head width is 24 (16–33) µm. The distance of the nerve ring, excretory pore, cephalic papilla from the anterior end was 203 (188–217) µm, 602 (558–658) µm, and 442 (341–571) µm, respectively (Figure 3A,C). Spicules are complex, alate, have a

pointed tip, 309 (274–346) µm long (Figure 3H). Gubernaculum length is 55.75 (50–63) µm, is V–shaped with small and slender dimensions. Tail length is 264 (230–314) µm; it curves ventrally (Figure 4O). Caudal papillae are sessile, consisting of 12 paired and 1 unpaired papilla, arranged as follows: 7 pairs are preanal (ventrolateral position) (Figure 4M), 1 pair is anterior to the precloacal sucker, 1 pair is just in front of the sucker, 1 pair is just behind the sucker, 4 pairs are between the sucker and the cloacal opening; unpaired papilla is anterior to the anus; 5 pairs are postanal (3 pairs ventrolateral, 2 pairs paired lateral) (Figure 4N). Preanal pseudosucker is simple, without a chitinous rim, 718 (600–870) µm.

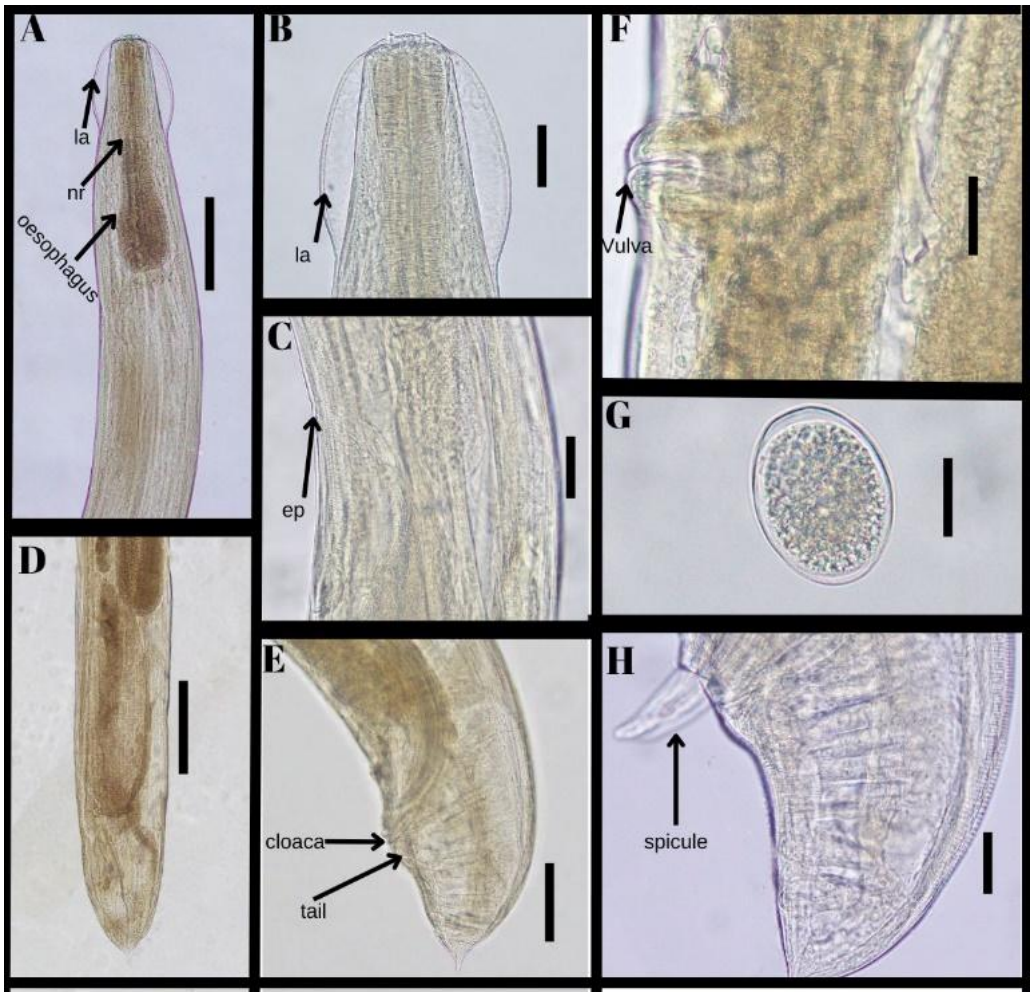

Figure 3. *Chabaudus* sp.: A – anterior; B - head with la=lateral alae; C - excretory pore; D – posterior; E - posterior female, cloaca and tail; F – vulva; G – egg; H - posterior male with spicule; scale bars: A,D=100 µm; B,C,H=20 µm; E,F,G=50 µm; ep=excretory pore; nr=nerve ring.

Female (n=10). The total body length is 22.85 (14.20–28.52) mm, with maximum width of 426 (266–586) µm. The esophagus is club-shaped, 545 (454–597) µm long and 125 (96–166) µm wide. The distances of the nerve ring, excretory pore, and cervical papilla from the anterior end are 222 (211–235) µm, 623 (596–672) µm, and 701 (654–777) µm, respectively. The vulva is in the middle, close to the posterior end; the distance of the vulva from the posterior end is 10.99 (9.46–12.70) mm (Figure 3F). The tail has a spike, 265 (225–380) µm long (Figure 3D,E). The egg is round, has a thin shell, and has no embryo; the measurement of eggs (length and width) is 52 (47–57) µm and 43 (38–47) µm (Figure 3G).

Remarks. *Chabaudus* sp. from *F. cancrivora* was reported in Central Java and West Java, Indonesia (Purwaningsih & Dewi 2013; Purwaningsih et al 2015). The specimens of this research have similar characters including the size, the shape of the cephalic end, the spicules, and the number and position of the caudal papilla with specimens described by Purwaningsih et al (2015). In this research, *Chabaudus* sp. has measured 16.15 (15.83-18.53) mm for the males and 22.85 (17.20-26.52) mm for the females (total body length). The total length of *Chabaudus* sp. described by Purwaningsih et al (2015) measured 15.64 (14.03–17.65) mm for the males and 22.51 (18.66–24.4) mm for the females. According to Purwaningsih et al (2015), the morphology of the specimens is close to that of *C. alaini*. However, the specimen of *C. alaini* is not accessible, so the final identification could not be done.

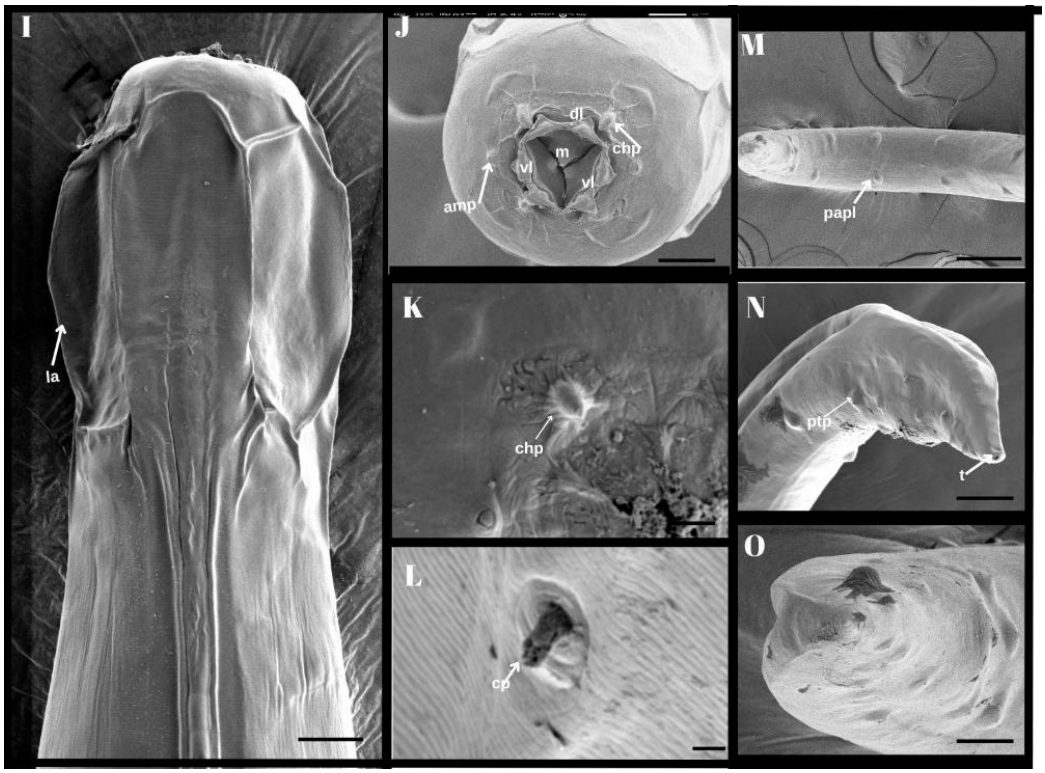

Figure 4. *Chabaudus* sp.: I - anterior portion showing small lateral alae; J - anterior end; K - chepalic papilla; L - cervical papilla; M - male posterior (ventral view); N - male posterior (lateral view); O - male posterior tip; I,N=50 µm; J=10 µm; K=5 µm; L=2 µm; M=100 µm; O=20 µm; la=lateral alae; dl=dorsal lips; vl=ventral lips; m=mouth; amp=amphid; chp=chepalic papilla; cp=cervical papilla; papl=preanal papilla; ptp=postanal papilla; t=tail.

Cosmocerca ornata (Dujardin, 1845)

Site of infection: cecum.

General morphology. The body is small and stout (Figure 5A). Cuticle bears fine transverse striations. The mouth has three developed lips: one dorsal lip and two sub-ventral lips. The dorsal lip has a pair of large sub-median cephalic papilla, and each sub-ventral lip has one lateral amphid and one sub-median cephalic papilla (Figure 5C). The mouth opening is triangular, with the end of each lip bordered by free thick cuticularized margin. The esophagus consists of a small pharynx, corpus, and bulbus. The nerve ring is located in the middle of the corpus. The excretory pore is located anterior to the esophageal bulb. The lateral alae are well developed, extending from the nerve ring to the anus.

Female (n=2). The body length is 4.15 (4.09–4.21) mm, and the width is 275 (258–293) µm. The cervical papilla was not seen. The pharynx is 38 (38–38) µm long and 43 (41–46) µm wide. The total length of esophagus is 449 (425–473) µm (Figure 5B). Lateral alae are well–developed. The distance of the nerve ring, excretory pore, and vulva from the anterior end is 172 (158–187) µm, 279 (271–287) µm, and 1.98 (1.90–2.07) mm, respectively. The vulva is in the middle of the body close to the anterior (Figure 5E). The tail is 208 (192–224) µm long, with the tip gradually tapering to a long filament point (Figure 5D). Prodelphic female has two ovaries. The vagina runs anteriorly. The eggs are oval–shaped, with a thin wall, and embryonated when laid; their measurements are 116 (104–124) µm x 72 (62–80) µm (Figure 5F).

Male. Not found.

Remarks. *Cosmocerca ornata* is widely distributed and occurs in the Afrotropical, Neotropical, Oceanian, Oriental, and Palearctic regions (Baker 1987; Sou et al 2018). Our specimens bear a resemblance to the female specimen of *C. ornata* described by Sou et al (2018) and Baker & Vaucher (1984), including the characters and the measurements. The presence of *C. ornata* in Indonesia was reported by Purwaningsih & Dewi (2013) in *Fejervarya chalconota* and *F. cancrivora*. In addition, Suzanna et al (2006) discovered that *Cosmocerca* sp. infected *F. cancrivora* and *L. macrodon*. Both records are also from West Java. Unfortunately, these records also were not accompanied by the description of the specimens.

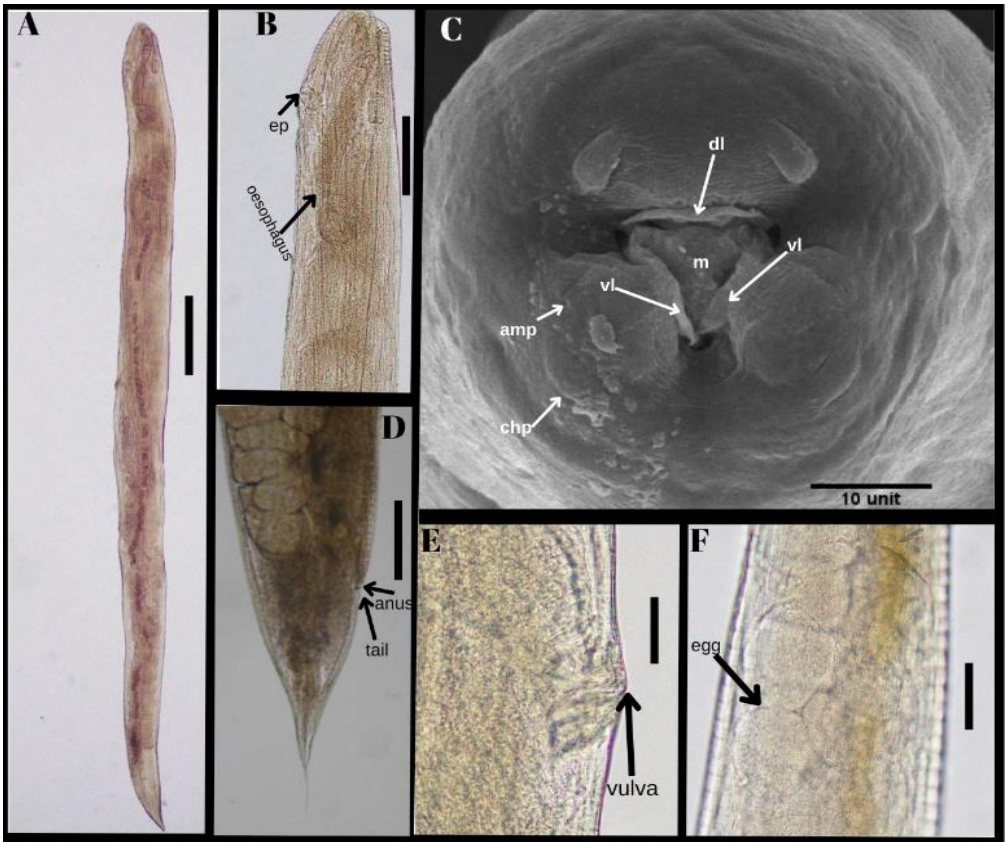

Figure 5. *Cosmocerca ornata*: A - complete body; B - anterior with esophagus and ep=excretory pore; C – head; D - posterior female (tail tip cut off); E – vulva; F – egg; A=200 µm; B=100 µm; C,D,F=50 µm; E=10 µm; chp=chepalic papilla; m=mouth; amp=amphid; dl=dorsal lips; vl=ventral lip.

Prevalence and intensity of nematodes. An examination of 100 *F. cancrivora* revealed that 68% of them were found to be positive for single and mixed infection with nematode parasites (Tables 1 and 2).

Table 1

Pattern of nematode infecting *Fejervarya cancrivora* in Leuwiliang sub-district, Bogor Regency, West Java

Family/species	*Number positive of host*		*Number of nematodes*			*Intensity*			*Habitat of nematodes*
	No. positive	*Prevalence (%)*	♂	♀	*Larva*	♀	♂	*Larva*	
Quimperiidae/ *Chabaudus* sp.	68	68	46	311	0	1-22	1-10	0	Intestines, stomach, cecum
Cosmocercidae/ *Cosmocerca ornata*	2	2	0	2	0	1	-	-	Caecum
Rhabdiasidae/ *Rhabdias* sp.	19	19	0	68	0	1-19	0	0	Lungs
Ascarididae/ *Ascarididae* gen. sp.	15	15	0	0	20	0	0	1-3	Liver, body cavity

Table 2

Single and mixed infections of parasitic worms infecting *Fejervarya cancrivora* frogs in Leuwiliang sub-district, Bogor Regency, West Java

Type of infection	*Number of hosts*	*Percentage of hosts*
Single infection		
Chabaudus sp.	48	48
Cosmocerca ornata	1	1%
Rhabdias sp.	6	6%
Ascarididae gen. sp.	2	2%
Mixed infection		
Ascarididae gen. sp.+ *Chabaudus* sp.	8	8%
Ascarididae gen. sp.+ *Rhabdias* sp.	1	1%
Rhabdias sp. + *Cosmocerca ornata*	1	1%
Rhabdias sp. + *Chabaudus* sp.	7	6%
Ascarididae gen. sp.+ *Rhabdias* sp. + *Chabaudus* sp.	4	4%
Negative	22	22%
Total	100	100%

Discussion. Research on the nematode parasites of *F. cancrivora* has been limited so far. In Indonesia, studies by Suzanna et al (2006), Purwaningsih & Dewi (2013), Purwaningsih et al (2015, 2016) have contributed to this field. Additionally, Bursey et al (2003) described a new species *Icosiella turgeocauda* from Luzon, Republic of the Philippines.

This research observed 100 *F. cancrivora* individuals and recorded a total of 447 nematodes of four species. Among them, *Chabaudus* sp. had the highest prevalence and number of individuals, with a total of 357 individuals, 11 in the stomach, 338 in the intestines, and 8 in the cecum. It was found that 57 frogs (57%) had single infections

and 21 frogs (21%) had mixed infections (Table 2). The highest prevalence of single infection was caused by *Chabaudus* sp. (68%), while the lowest was caused by *C. ornata* (1%). The highest prevalence of mixed infection was with *Ascarididae* gen. sp. and *Chabaudus* sp. (8%).

Purwaningsih & Dewi (2013) also reported that *Chabaudus* sp. was most abundant compared to other species. *Chabaudus* sp. was found in *F. cancrivora* in Cibadak with a prevalence of 64%, with a total of 236 individual nematodes, consisting of 136 female worms and 100 male worms, with intensity for each host ranging from 1 to 32 male worms and 1 to 26 female worms.

In the previous research conducted by Purwaningsih & Dewi (2013), the prevalence of the frogs from rice fields and forests in West Java was 34.78%. Meanwhile, Suzanna et al (2006) reported that the prevalence of nematodes in *F. cancrivora* frogs from Caringin, Cibatok and Cimanggis, West Java was 66.67, 80, and 60%, respectively. Therefore, the prevalence in this research was greater than in that of Purwaningsih & Dewi (2013), and Suzanna et al (2006) in Caringin and Cimanggis, but lower than that in Cibatok (Suzanna et al 2006).

The second highest prevalence was obtained for *Rhabdias* sp. at 19%, with a total of 68 individuals found in the lungs with an intensity of 1-19 individual per host. Purwaningsih & Dewi (2013) reported that *Rhabdias* sp. Infected *Rana hosii* from Selabintana, West Java; among 7 individuals observed, 2 were infected with an intensity of 1-2 worms per host.

Ascarididae gen. sp. was found in the liver and body cavity, with a prevalence of 15% and a total number of 20, with an intensity of 1-3 individuals per frog. The occurrence of larvae in frogs has never been reported in Indonesia yet. Lastly, *C. ornata* had a prevalence of 2%, with an intensity of 1 individual per host. Purwaningsih & Dewi (2013) reported that *C. ornata* infected 1 individual of *R. chalconota* out of 3 animals examined, and the number of individual worms found was 11 females. Therefore, the prevalence of the *C. ornata* nematode in this study is smaller than the prevalence in the study by Purwaningsih & Dewi (2013), and the number found is less than in previous studies.

Correlation test between frog sex and frog body weight with the number of nematodes. Among 100 dissected frogs, 57 were male, and the remaining were female. The body weight of the males ranged from 10.0 to 40.3 g, while the female body weight ranged from 9.1 to 95.7 g. The Spearman test indicated that there was no correlation between frog body weight and the number of nematodes ($p>0.05$), and there was no correlation between frog sex and the number of nematodes ($p>0.05$). Animalesto (2020) conducted research on trematode infections in *F. cancrivora* in Karawang Regency, West Java, and the results showed that there was no significant correlation between the sex of *F. cancrivora* and the number of trematodes found. According to Dare & Forbes (2008), the relationship between sex and worm prevalence did not show a clear pattern in amphibians. In *Rana sylvatica*, the prevalence of *Rhabdias* sp. was found to be higher in males, as they generally require a longer time to metamorphose compared to females (Dare & Forbes 2008). Furthermore, Luque et al (2005) stated that host sex is not considered to be important in structuring parasite communities, and this is applicable to anurans. Luque et al (2005) also found that the total abundance of parasites did not correlate significantly with the sex of *Bufo ictericus*. The possible reason is due to the internal conditions of the frog, including a generalized opportunistic diet, intestinal system, low vagility and ectothermic metabolism (Luque et al 2005).

Conclusions. A survey on nematode parasites of the edible frog *Fejerfarya cancrivora,* in Leuwiliang District, West Java, recorded four species of nematodes belonging to four genera and four families: *Ascarididae* gen. sp., *Cosmocerca ornata*, *Chabaudus* sp and *Rhabdias* sp. Except *Chabaudus* sp., all species discovered were described for the first time from Indonesian specimens. This data contributes to understanding nematode diversity in Indonesia and can be used as a foundation for preventing diseases caused by these parasitic organisms.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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