

# The infection of trematodes on rice frogs (*Fejervarya cancrivora*) in Karawang Regency, West Java

Gloria Animalesto<sup>1,\*</sup>

<sup>1</sup>Zoology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Gedung Widiasatwaloka, Jl. Raya Jakarta-Bogor Km. 46, Cibinong, West Java, Indonesia 16911

**Abstract.** *Fejervarya cancrivora* is often used as raw material for food and export commodities. In 2014, it was recorded that Karawang Regency was the largest producer of frog's legs meat in West Java. Research on trematode worms of *F. cancrivora* from Karawang Regency was conducted to find out the diversity of trematodes worms and its infection patterns. The observation was carried out on 120 frogs. The lungs, stomach, intestine, and caecum were examined for trematodes. The results showed that the *F. cancrivora* infected by four species of trematodes i.e., *Glythelmins* sp., *Pleurogenoides* sp., *Haematoloechus* sp., and *Diplodiscus* sp. with a prevalence of 25.8%, 18.3%, 35.8%, and 23.3%, respectively, and parasite index 1 - 25, 1-268, 1-19, and 1-23 individual parasite in each host, respectively.

## 1 Introduction

Trematodes of frog in Indonesia have been investigated only by a few authors [1-2]. They reported trematode infection to the genus level from anurans. The negative impact of trematode presence in frogs intensively reported on two different genera of trematodes, *Ribeiroia* and *Echinostoma*. Both of them use tadpoles serve as a second intermediate host and cause limb malformation and tadpole mortalities, respectively [3].

*Fejervarya cancrivora* has widespread distribution throughout Indonesia, especially in Java [4]. Indonesia dedicated as the largest exporters of frog's legs for consumption as food, and predominantly from *F. cancrivora* caught in natural habitat in Java Island [5]. In 2014, it was recorded that Karawang Regency was the largest producer of frog's leg meat in West Java [6]. For amphibians, trematode dominates in ranids, tree frogs, and aquatic amphibians [7]. Therefore, the current study aimed to find out the diversity of trematode in frog viscera and investigate its infection pattern.

## 2 Material and Methods

### 2.1. Frog Collection

A total of 120 frogs were collected by hand on 16<sup>th</sup>-27<sup>th</sup> May 2018 by Dra. Hellen Kurniati and her team in the ricefield of East Karawang Regency, Sub-district of Klari Sukaresmi, Anggita village and Gintung village.

### 2.2 Parasite Collection and Preservation

The snout-vent length of all frogs was measured first. The lungs, stomach, intestine, and caecum were dissected and placed separately in a petri dish containing 70% alcohol. All organs were examined under a dissecting microscope. Any trematodes found were fixed in a vial containing 70% alcohol for morphology study.

### 2.3 Trematode Staining

Trematodes stained by acetocarmine for 5-10 minutes immersion, then continued with the destaining process. The worms were destained in ethyl acetate until the worms brighten (pinkish) and the organs are visible. After the destaining, the process of dehydration was applied gradually with graded alcohol as 70%, 80%, 90%, and 100%, then cleared in methyl salicylate and mounted in Canada balsam.

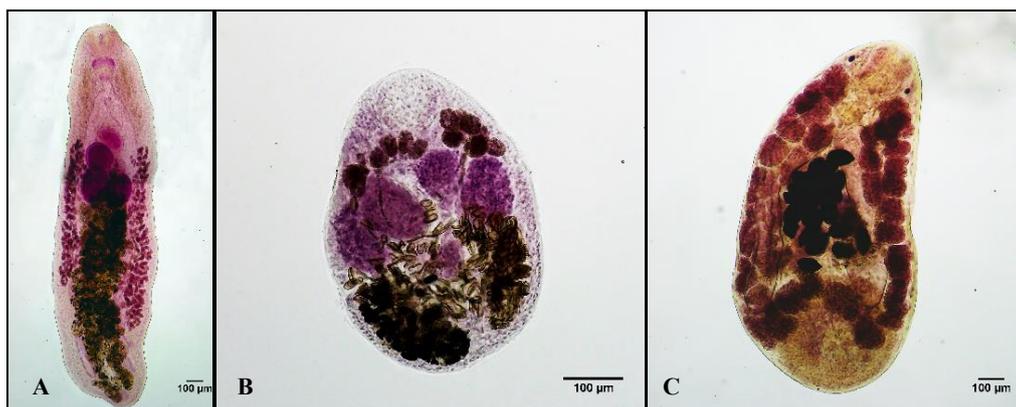
### 2.4 Trematode Identification

The morphology of trematodes was observed under a microscope and identified according to Bray *et al.* [8] and relevant journals. Each kind of species was photographed and measured by a micrometer scale.

### 2.5 Data Analysis

Every individual of trematode species was counted to reveal the prevalence, mean intensity and mean abundance of trematode by Microsoft Excel 2013. The collected species were classified into genus level. The infection pattern based on host size and host sex were estimated using IBM SPSS Statistics 23.

\* Corresponding author: [glorianimalesto@gmail.com](mailto:glorianimalesto@gmail.com)



**Fig. 1.** A. *Glypthelmins* sp., B. *Pleurogenoides* sp., C. *Diplodiscus* sp.

**Table 1.** Summary of trematode infection in each site of viscera

Trematode species	Site of Infection								Infection rate (Range)
	Lungs		Stomach		Intestine		Caecum		
	Pr%	MI	Pr%	MI	Pr %	MI	Pr %	MI	
<i>Haematoloechus</i> sp.	35.8	2.5	-	-	-	-	-	-	1-19
<i>Diplodiscus</i> sp.	-	-	-	-	6.7	3.1	19.2	4.6	1-23
<i>Pleurogenoides</i> sp.	-	-	1.7	1.5	17.5	48.4	0.8	1	1-268
<i>Glypthelmins</i> sp.	-	-	1.7	3	25	4.3	1.7	1	1-25

Pr% = Prevalensi, MI = Mean Intensity

**Table 2.** Number of parasitized host and prevalence (%), mean intensity (MI), mean abundance (MA) number of trematode recovered and occupied microhabitat in the host

Helminth species	Number of individuals parasitized (prevalence %)	MI ± SD	MA ± SD	Number recovered	Site of Infection
<i>Haematoloechus</i> sp.	43 (35.8%)	2.5 ± 2.3	0.9 ± 2.2	109	Lungs
<i>Diplodiscus</i> sp.	28 (23.3%)	4.7 ± 4.2	1.1 ± 4.0	131	Intestine Caecum
<i>Pleurogenoides</i> sp.	22 (18.3%)	46.4 ± 44.7	8.5 ± 39.0	1021	Stomach Intestine Caecum
<i>Glypthelmins</i> sp.	31 (25.8%)	4.3 ± 4.4	1.2 ± 3.9	138	Stomach Intestine Caecum

**Table 3.** Correlation between total infection of each trematode species with host size and host sex

Helminth species	Host Size		Host Sex	
	P	R <sup>2</sup>	P	R <sup>2</sup>
<i>Haematoloechus</i> sp.	0.434	-	0.109	-
<i>Diplodiscus</i> sp.	0.020	0.45	0.449	-
<i>Pleurogenoides</i> sp.	0.000	0.142	0.143	-
<i>Glypthelmins</i> sp.	0.000	0.155	0.311	-

### 3 Results

#### 3.1 Trematode Fauna

The trematode infection consisted of four species. The identification only reported to the genus level because of the inappropriate fixation. In the beginning, rice frogs caught by hand and directly injected and immersed by 96% alcohol. This treatment caused the specimens

wrinkled and folded in which difficult to ensure to the species level. However, the genus character still recognized.

The specific group in which infected frog lungs belong to the genus *Haematoloechus* Looss, 1899. *Haematoloechus* sp. had an elongate body and vitellarium extends into the hind body. The trematode infection was also dominated by common species such as *Pleurogenoides* sp., *Diplodiscus* sp., and *Glypthelmins*

sp. (Figure 1). Member of genus *Pleurogenoides* Travassos, 1921 predominantly found in intestine (Table 1). As same as the key [8], *Pleurogenoides* had a small and oval body; characterized by moderately long caeca, terminate near the anterior margin of testes with symmetrical testes and postcaecal.

The other intestinal flukes belonging to *Glypthelmins* sp. was characterized by an elongate body with spines extending from anterior end to varying level of the hind body. Oral sucker subterminal, larger than ventral sucker. Prepharynx short, and pharynx well developed. Caeca long extend to near posterior end of the body, but do not reach it. Testes are symmetrical and intercaecal. Genital pore median, in forebody. Ovary submedian, and anterior to testes. Lastly, *Diplodiscus* sp. mostly occurred on frogs' caecum with a conical body and characterized by a single testis and intercaecal. In addition, vitelline follicles are extending along caeca.

### 3.2 Level of Infection

Of the 120 frogs examined, 41 frogs were infected by trematodes, and 79 frogs were not infected. This showed that the prevalence of positively infected was 65.8%. *Haematoloechus* sp. had the highest prevalence (35.8%), while the *Pleurogenoides* sp. was the lowest (18.3%). In contrast, *Pleurogenoides* sp. had the highest abundance (8.5) and *Haematoloechus* sp. had the lowest abundance (Table 2).

### 3.3 The composition of trematode infection in each site of viscera

*Haematoloechus* sp. only restricted on lung with 35.8% prevalence. *Diplodiscus* sp. infected two parts of viscera, i.e., intestine and caecum, wherein the highest prevalence was in the caecum (19.2%). *Pleurogenoides* sp. and *Glypthelmins* sp. were found in three sites of viscera (stomach, intestine, and caecum), and both of them mostly found in the intestine (Table 1).

### 3.4 Correlation between host body size and host sex with the number of parasites

The observed frogs consist of 64 males and 56 females with varies between 15.8 mm and 69.3 mm. Based on differences in host body size and host sex, there is a significant correlation between host body size and total infection of trematode ( $R^2 = 0.180$ ,  $P = 0.00$ ). On the contrary, the correlation between sex and parasite number was not significant ( $P = 0.173$ ).

There was no significant correlation found between all trematodes species and host sex (Table 3). Correlation of *Haematoloechus* sp. is not significant with host size ( $P = 0.434$ ), while the other three types are significant.

## 4 Discussion

All trematodes obtained in this research are common as anuran parasites. Anurans have an important role as hosts, both definitive and intermediate hosts, and also as paratenic hosts in the transmission of parasitic worms

[9]. The transmission related to the ecology and feeding behaviour of the host. According to Nurmainis [10], the groups of rice frog's diet in Bogor district consist of insect, crustacean, myriapod, arachnid, gastropod, small amphibian, and fish.

Our result showed the highest prevalence of trematodes reached by *Haematoloechus* sp. The species belong to *Haematoloechus* are known in every continent except Antarctica [11]. More than 50 species of this genus have been described around the world [12]. In Southeast Asia, *Haematoloechus singaporensis* recorded as a new species in Singapore [13]. *Haematoloechus* sp. also found in Shanghai with high prevalence, counted at 24.44% [14]. In general, the frog becomes infected with *Haematoloechus* spp. by ingested odonate as its intermediate host [15].

*Diplodiscus* sp. is part of the Family Diplodiscidae which is predominantly in amphibians but also found representative in reptile and fish [16]. The species of genus *Diplodiscus* may either infect tadpole and frog with a snail as its first intermediate host [17]. This statement explains how the small frog of this research (20.7 mm) could be infected by *Diplodiscus* sp.

The highest infection rate and mean intensity were occupied by *Pleurogenoides* sp. (1-268). More than 26 valid species of genus *Pleurogenoides* from frogs have been reported around the world. The intermediate host of genus *Pleurogenoides* consist of gastropod, dragonflies, caddisflies, beetles, mayflies, alderflies, isopod, and amphipod crustacean [18]. The chance of ingesting those gastropods and aquatic insect mention above could explain the infection rate comes from.

The Genus *Glypthelmins* is the single genus of Family Glypthelminthidae and comprises 19 to 28 species worldwide which is parasitizing the intestine of amphibians [19-20]. Transmission of *Glypthelmins* transferred by consuming cysts that found in their own shed skin and develops to adults in the intestine [7, 21].

Our result shows a significant increase in helminth intensity related to host size. Consequently, the bigger size of the host is more parasitized. The large-body host provides more space of food ingested, more space of surface area [22]. Also, the larger host has a longer time to live, so they have the possibility to be exposed by parasites longer [23].

Regarding the relationship between parasite number and host-sex, there was no significant correlation. It is similar to the research of Comas *et al.* [24] which showed no significant patterns between host-sex and prevalence. On the other side, there were significant differences between the intensity of infection and sexes in Gonzales and Hamann [25]. They found that mean intensity of female frogs was higher than males. Indeed, the sex-linked patterns of infection is still unclear.

In the special case of this research, *Haematoloechus* sp. did not correlate to the host size while all the other three species had significant differences. The smallest frog infected with *Haematoloechus* sp. has a size of 22.8 mm. It is relevant to Bolek and Janovy [26] which analyzed about small frogs infected with frog lung flukes. The mean of their northern leopard frogs was 30.85 mm. Bolek and Janovy [26] indicates that the

small frogs infected with frog lung flukes by nonodonate arthropods such as Coleoptera, Ephemeroptera, Hemiptera, and Amphopoda. Those nonodonate arthropods infected with metacercariae located in the head, legs, and hemocoel.

## 5 Conclusion

To sum up, there were four species of trematodes infected rice frogs *F. cancrivora*, i.e. *Haematoloechus* sp., *Diplodiscus* sp., *Pleurogenoides* sp., and *Glypthelmins* sp. The transmission depends on the host ecology and feeding behaviour. It affects the calculation of prevalence, mean intensity, means abundance, and infection rate. This research also found that the infection related to the host size.

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