

**ENDOPARASITES OF TWO SPECIES OF EDIBLE FROGS,
Limnodynastes macrodon, Boie AND *Fejervarya cancrivora*, Gravenhorst,
FROM BOGOR, INDONESIA**

Mirza Dikari Kusri^{1,2}, Erna Suzanna¹ and Fajar Satrija³

¹Department of Forest Resources Conservation, Faculty of Forestry, Bogor Agricultural University. PO Box 168, Bogor 16000, Indonesia

²To whom correspondence should be addressed: mirza_kusri@yahoo.com

³Laboratory of Helminthology, Department of Parasitology and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia

Abstract: Seventy five adult *Limnodynastes macrodon* and 50 *Fejervarya cancrivora* collected from three subdistricts in Bogor were analysed for endoparasites. We found eleven species of parasites; seven species from the phylum Nematoda (Rhabditida, Spirurida, Heterokioidea, Oxyurida, *Oxyuris* spp., Cosmocercoidea, Ascaridida), one species from the class Cestoda (pleurocercoid larvae) and three species from the class Trematoda (*Haematoloechus*, Allocreadiidae, Paramphistomidae). Most species and individual parasites were found in the frogs' digestive tracts. In all the subdistricts *F. cancrivora* was more infected than *L. macrodon*. One type of parasite (pleurocercoid) was found in the leg muscles of both species of frogs. *F. cancrivora* had a higher prevalence of pleurocercoid infection (38%) than *L. macrodon* (5.3%). The differences of endoparasite infection is related to differences in habitat type and food.

Keywords: *Fejervarya cancrivora*; *Limnodynastes macrodon*, edible frogs, Bogor, Indonesia, endoparasite

Endoparasit pada dua jenis katak yang dikonsumsi, *Limnodynastes macrodon*, Boie and *Fejervarya cancrivora*, Gravenhorst, dari Bogor, Indonesia

Abstrak: Penelitian mengenai endoparasit dilakukan pada 75 ekor *Limnodynastes macrodon* dan 50 ekor *Fejervarya cancrivora* dewasa yang di ambil dari tiga kabupaten di Bogor (Caringin, Cimanggis and Cibatok). Hasil penelitian menemukan sebelas spesies parasit antara lain tujuh spesies dari filum Nematoda (Rhabditida, Spirurida, Heterokioidea, Oxyurida, *Oxyuris* spp., Cosmocercoidea, Ascaridida), satu spesies dari kelas Cestoda (larva pleurocercoid) dan tiga spesies dari kelas Trematoda (*Haematoloechus*, Allocreadiidae, Paramphistomidae). Umumnya parasit dijumpai di saluran pencernaan. *F. cancrivora* lebih banyak terinfeksi parasit dibandingkan *L. macrodon* di semua lokasi. Satu jenis parasit (pleurocercoid) dijumpai pada otot paha ke dua jenis katak. *F. cancrivora* memiliki prevalensi terinfeksi pleurocercoid lebih tinggi



(38%) daripada *L. macrodon* (5,3%). Perbedaan infeksi endoparasit pada ke dua jenis katak ini diduga berhubungan dengan perbedaan habitat serta makanan

Kata kunci: *Fejervarya cancrivora*; *Limnonectes macrodon*, katak yang dapat dikonsumsi, Bogor, Indonesia, endoparasit

INTRODUCTION

The Giant Java Frog, *Limnonectes macrodon* and the Crab-eating Frog, *Fejervarya cancrivora* are two species commonly caught for consumption in South East Asia (Berry, 1975; Church, 1960; Iskandar, 1998). There are only three reports of parasite communities associated with *F. cancrivora*, all from Indonesia (Purnomo & Bangs, 1996, 1999; Sunityoso et al., 1997) and no report on *L. macrodon* parasites. The only published report on *L. macrodon* parasites is that by Fusco and Palmieri (1979) from Malaysia. In this paper, we present the result of endoparasite surveys of the two species caught in Bogor, part of West Java province (Java Island), Indonesia.

MATERIALS AND METHODS

Collection and examination of frogs

All frogs were wild frogs caught by harvesters in selected areas of the Caringin, Cibatok and Cimanggis subdistricts in Bogor (West Java province) during July to September 2002 (Figure 1).

A total of 75 *L. macrodon* (32 from Caringin, 24 from Cibatok and 19 from Cimanggis) and 50 *F. cancrivora* (25 from Caringin, 10 from Cibatok and 15 from Cimanggis) were dissected and examined in the Laboratory of Helminthology, Department of Parasitology and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University. The sizes of the frogs examined are given in Table 1.

Table 1. Weight and Length (Snout Vent Length/SVL) of frogs examined

Location	<i>L. macrodon</i> (mean ± SD)		<i>F. cancrivora</i> (mean ± SD)	
	SVL (cm)	mass (gr)	SVL (cm)	mass (gr)
Caringin	7.69 ± 0.69	49.88 ± 14.00	7.76 ± 1.16	50.76 ± 24.24
Cimanggis	7.87 ± 0.84	51.22 ± 13.88	8.04 ± 0.71	56.36 ± 14.43
Cibatok	9.34 ± 1.16	75.89 ± 22.52	7.55 ± 0.83	42.04 ± 12.30

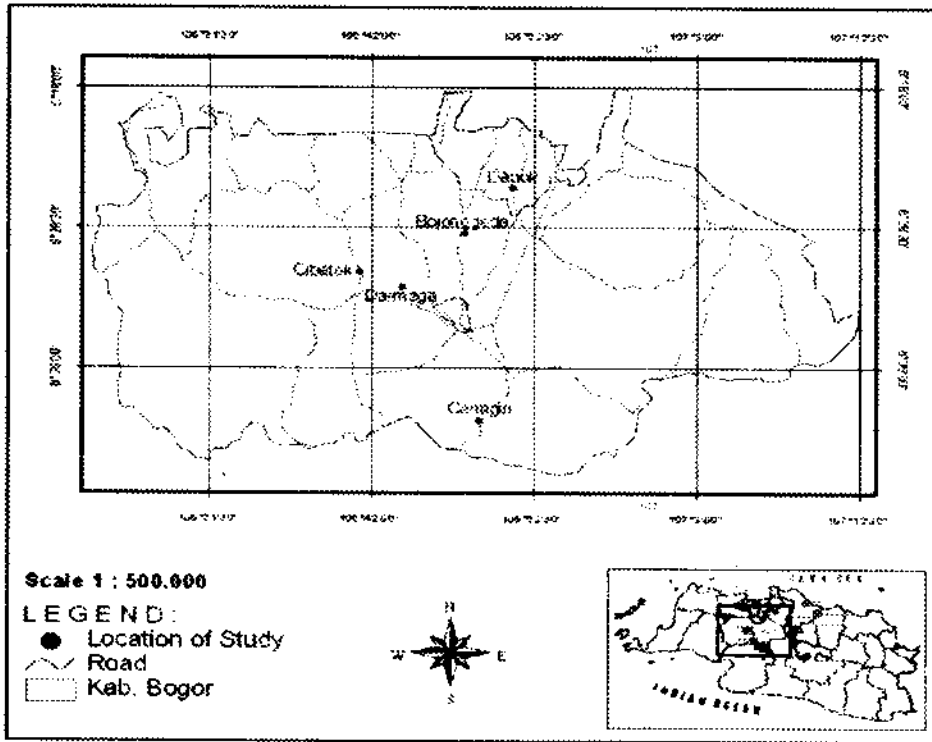


Figure 1. Location of study in Bogor, West Java province, Indonesia

Collection and preservation of parasites was carried out according to methods outlined by Pritchard and Kruse (1982). The frogs were killed and then weighed and skinned. The body cavity of each frog was opened and the viscera (liver, lungs, heart and digestive tract) were subsequently removed and placed in individual plastic containers with saline solution. Each organ was then torn open or cut apart with scissors to allow exposure of parasites. Digestive tract contents were then placed in individual petri dishes and examined for parasites, using a stereo microscope. Worms found in each organ were washed in saline solution and allowed to relax overnight in saline solution at 10°C, before being fixed and stored in 70% ethanol. We also examined the leg muscles of each frog for plerocercoids.



The parasites found from each organ were examined using a compound microscope to determine the parasite's order, family and genus. Trematode specimens were stained with Mayer's carmalum to display their internal organs for identification (Pritchard and Kruse, 1982). Identification was made based on those used by Smyth and Smyth (1980) and Yamaguti (1961).

We calculated prevalence (percentage of hosts infected with the parasites) and parasite abundance (number of parasites per examined host). Differences between the number of frogs infected between location and between species were tested using a chi-square test. For female *F. cancrivora* the relationship between intensity of infections and host snout vent length (SVL) was determined by correlation analysis and tested using one way ANOVA for Oxyurida, Paramphistomidae and Pleuroceroid.. No tests on the relationships between intensity of infections and host SVL were made for male *F. cancrivora* because of lack of sample size (n=4), nor for *L. macrodon* since the frequency of infection was too small to allow statistical analysis .

RESULT

Eleven species of parasites, including seven species from the phylum Nematoda (Rhabditida, Spirurida, Heterakiodea, Oxyurida, *Oxyuris* spp., Cosmocercoidea, Ascaridida), one species from the class Cestoda (pleuroceroid larvae) and three species from the class Trematoda (*Haematoloechus*, Allocreadidae, Paramphistomidae) were recovered.

A total of seven species of nematode were found in *L. macrodon* and *F. cancrivora*. All seven were found in *L. macrodon*, but Ascaridida and *Oxyuris* spp were not found in *F. cancrivora*. Pleuroceroid larvae were found in both *L. macrodon* and *F. cancrivora*, as were all three species of trematode (*Haematoloechus*, Allocreadidae, Paramphistomidae).

Table 2 shows the infection prevalence of parasites in both species. Oxyurida and Heterakoidea were the most common species found in *L.*



macrodon, while pleuroceroid larvae and Oxyurida were the most common parasites found in *F. cancrivora*.

Table 2. Prevalence, mean intensity \pm standard deviation and maximum intensity of parasite infection on *L. macrodon* (75) and *F. cancrivora* (50) from Bogor, Indonesia caught between July-September 2002

Type of Parasite	Site of infection	<i>L. macrodon</i>			<i>F. cancrivora</i>		
		Prevalence (%)	Mean Intensity	Max Intensity	Prevalence (%)	Mean Intensity	Max Intensity
Nematoda							
Rhabditida	DI	13.33	6.58 \pm 1.30	13	16	12.75 \pm 5.42	45
Spirurida	D1	1.33	2.00 \pm 0.00	2	6	19.00 \pm 4.00	23
Heterakoidae	D1	17.33	6.31 \pm 1.88	20	12	19.00 \pm 10.61	60
Oxyurida	DI, LV	17.33	6.58 \pm 1.58	17	32	23.56 \pm 9.46	155
<i>Oxyuris</i> sp		3.99	9.00 \pm 1.73	12	0	0	0
Cosmocercoidea	DI	9.33	8.71 \pm 4.05	27	8	11.75 \pm 5.14	24
Ascaridida	HR	1.67	1.00 \pm 0.00	1	0	0	0
Cestoda							
Pleuroceroids larvae	LM	10.67	1.71 \pm 0.18	2	40	2.8 \pm 0.92	20
Trematoda							
Haematoloechus	LU	1.33	1.00 \pm 0.00	1	22	1.00 \pm 0.00	1
Allocreadiidae	DI	2.67	7.00 \pm 5.00	12	6	10.33 \pm 7.84	26
Paramphistomidae	DI	4	7.67 \pm 4.18	16	30	23.53 \pm 7.05	104

DI = digestive tract, HR = Heart, LU = Lung, LM = Leg muscle, LV = liver

Most of the parasites were found in the digestive tract. More than two third (69%) of infected *L. macrodon* were infected in one body part, under one third (28.57%) were infected in two body parts and a small number (2.38%) were infected in three parts. Just over half (53%) of infected *F. cancrivora* were infected in one body part, just under a third (31%) were infected in two body parts, and 16% were infected in three body parts. Most frogs (55.8% for *L. macrodon* and 44.4% for *F. cancrivora*) were only infected by one species of parasite, approximately a third (34.8% for *L. macrodon* and 28.9% for *F. cancrivora*) were infected by two species of parasite and only a small number (~9% for *L. macrodon* and ~28% for *F.*



cancrivora) were infected with three or four species of parasite (Figures 2, 3, and 4).

The difference in infection rates between species of frogs is highly significant (Pearson Chiquare test, $df = 1$, $P < 0.001$). The difference in infection rates for *L. macrodon* and *F. cancrivora* between locations is also significant (Pearson Chisquare test, $df = 1$, $P < 0.05$). Figure 2 shows the infection rates for each species in the different locations.

The difference in parasite infection between the sexes of each species is not significant (Pearson Chiquare test, $df = 1$, $P > 0.5$). However, the difference in parasite infection between female *L. macrodon* and *F. cancrivora* is highly significant (Pearson Chiquare test, $df = 1$, $P < 0.001$). No test were made for between female *F. cancrivora* and male *F. cancrivora* because of the lack of sample size for male ($n=4$).

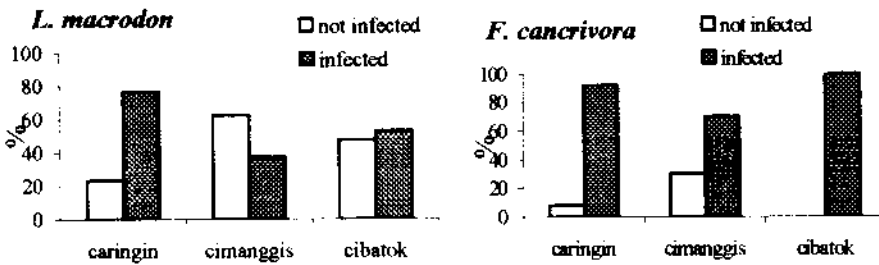


Figure 2. Parasite infection rates (%) of each species caught in three locations in Bogor between July – September 2002.

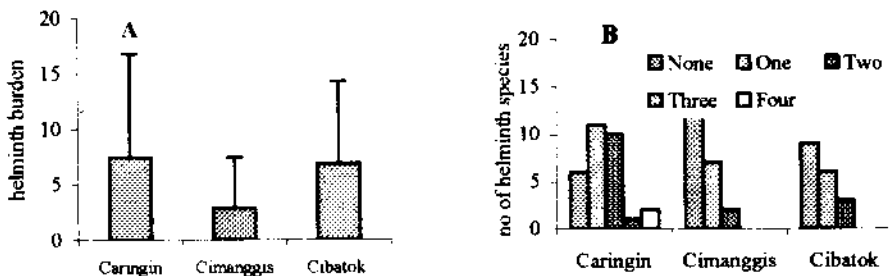


Figure 3. Mean Helminth Burden (standard error; A) and number of helminth infected (B) on *Limnonectes macrodon* in three location

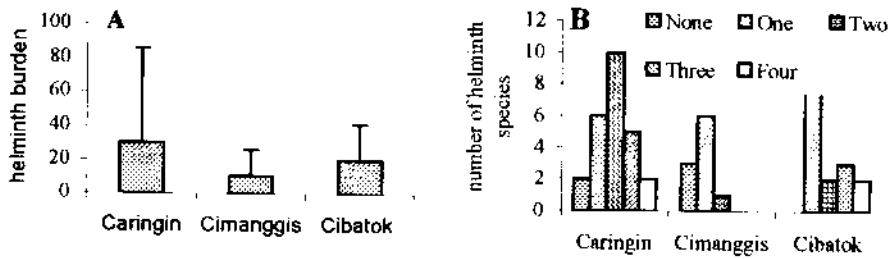


Figure 4. Mean Helminth Burden (A) and number of helminth infected (B) on *Fejervarya cancrivora* in three location

There was a significant positive correlation between female *F. cancrivora* SVL and the intensity of Oxyurida infection ($r_{14}=0.52$, $P<0.05$) (Figure 5). There was no significant relationship between female *F. cancrivora* SVL and intensity of pleuroceroid infection ($F_{1,16}=0.58$, $P=0.83$) nor with intensity of Paramphistomidae infection ($F_{1,11}=1.68$, $P=0.22$).

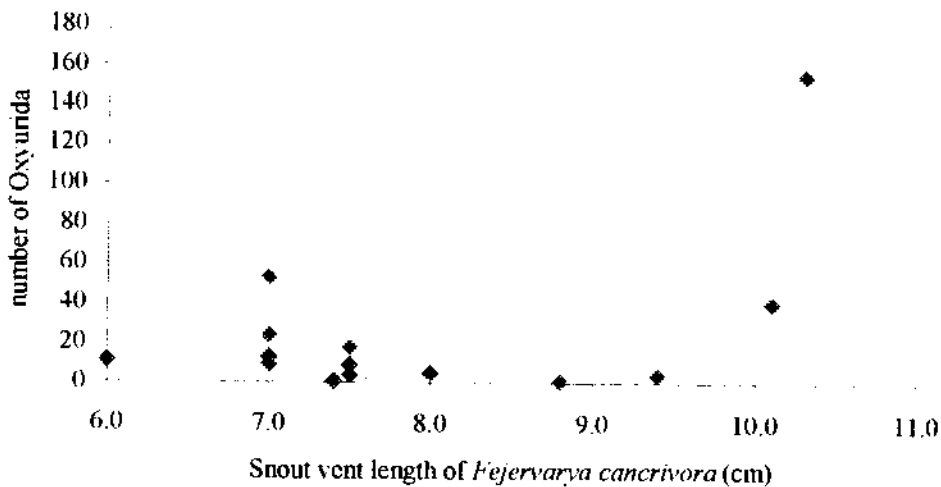


Figure 5. Relationship between number of Oxyurida and snout-vent length (cm) of female *Fejervarya cancrivora* collected from Bogor, West Java, Indonesia

DISCUSSION

Terrestrial frogs usually have a higher prevalence of nematodes infection compared to stream dwelling frogs. On the other hand, stream dwelling



frogs are usually more susceptible to trematode infections (Smyth and Smyth, 1980). Nematode parasites of amphibian have a direct life cycle, whereas trematodes and cestodes usually have complex life cycles, which include various intermediate hosts such as snails, insects and vertebrates (Smyth and Smyth, 1980).

F. cancrivora showed a higher prevalence of parasite infection than *L. macrodon*. *Fejervarya cancrivora* is an aquatic frog, although they are most abundant in low-land rice fields (Church, 1960; Iskandar, 1998) and eat various insects, invertebrates (crustacea, myriopod, arachnid, gastropod), amphibians and fish (Nurmainis, 2000). The water level in wet rice fields was regulated according to the growth of the rice plants, and it has been suggested that the behavior of *F. cancrivora* is related to the water level in these fields. Research by Kurais (1995) in Malaysia indicated that the breeding periods of this species is related to the rice field irrigation phase. Most rice fields in our study had low water levels or no water at all during harvest time, and frogs needed to adapt to dry periods during fallow periods (personal observation). This will enable parasite growth in various intermediate hosts such as molluscs, insects, or vertebrates host, for example snakes.

Limnonectes macrodon is an endemic frog on Java island and is found mostly in streams (Iskandar, 1998). Distributional studies by one of the authors on *L. macrodon* indicate that adult frogs are not only found in the streams, but also in natural ponds in forested areas or in rice fields near densely vegetated irrigation channels. The ecology of this species is poorly understood, although the species has been known to eat various insects, invertebrates (chilopod, diplopod, arachnid, annelid), amphibians, even small birds (Sugiri, 1979).

Because humans eat the legs of both of these species of frogs, special attention needs to be focused on parasites found in the legs. *F. cancrivora* legs have shown to be more prevalent to infection by pleurocercoid larvae than those of *L. macrodon*. The prevalence of pleurocercoid infection in our study is comparable with Sunityoso et al. (1997) findings,



which recorded infection prevalence of 42.4 % from *F. cancrivora* legs in Depok (a subdistrict of the Bogor area).

Both species of frogs from the subdistrict of Caringin showed higher infection rates compared to the other locations. One female *F. cancrivora* from Caringin was not only infected by three species of helminthes but also had the highest burden of helminthes, especially Paramphistomidae (Trematoda, n= 104) and Oxyurida (Nematoda, n= 155). Deformities have been reported in some frogs from this area (Kusrini, 2003) but no cause has as yet been investigated. Recent research has indicated that deformities are caused by various factors such as parasites, environmental pollution or traumatic injuries (Ouellet, 2000). Trematodes are linked to deformities in frogs in northern America (Kaiser, 1999; Johnson et al., 1999, Stopper et al., 2002), however parasitic copepods are also connected with *Rana chalconota* metamorph deformities in Sukabumi, another district in West Java (Leong, 2001). However, all frogs examined in this study were adults (since harvesters usually collected only big frogs), in normal condition. Future research on parasites in juvenile frogs is needed.

The differences in parasite infection between the two host in this study indicate different life habits and also the effect of their habitat. This report is the first on parasite infection in Indonesian *Limnonectes macrodon*. Although we were unable to classify parasites to species level because of specimen condition, we were able to determine the parasitic structure for *L. macrodon* and *F. cancrivora*.

ACKNOWLEDGMENTS

Field research was made possible by funding from the Higher Education Directorate of the Ministry of Education (Indonesia) through its Basic Biological Research project number 016/P2IPT/DPPM/IV/ 2002. The principal author was supported by the Australian Development Scholarship for postgraduate study in James Cook University, Australia, during this research. We would like to thank the head of Department of



Forest Resources Conservation, Bogor Agricultural University, Dr. Ani Mardistuti and research students and technicians of the Helminthology Laboratory, Department of Parasitology and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University (Mr. Sulerman, Ena, Eko, Devy, Fania and Lina) who had conducted collection and identification of the parasites.

We also appreciate the help of Anisa Fitri, Vivien Lestari and Hijrah Utama who contacted and organized the harvesters. We thank Dr. Diane Barton for advice and comments during the preparation of this project and throughout the completion of this manuscript.

REFERENCES

- Berry, P.Y. 1975. The amphibian fauna of peninsular Malaysia. Kuala Lumpur, Tropical Press. 127 p.
- Church, G. 1960. The effects of seasonal and lunar changes on the breeding pattern of the edible Javanese frog, *Rana cancrivora* Gravenhorst. *Treubia* **25**:215-233
- Fusco, A.C. and J.R. Palmieri. 1979. The nematode fauna of *Rana macrodon* Dumeril and Bibron with supplementary data on *Batrachonema synaptospicula* Yuen, 1965 (Nematoda: Amidostomatidae). *Proc. Helminthol. Soc. Wash.* **46**(2): 298-292.
- Iskandar, D.T. 1998. Amfibi Jawa dan Bali. Bogor, Puslitbang Biologi-LIPI. 132 p.
- Johnson, P.T. J., K.B. Lunde, E.G. Ritchie and A.E. Launer. 1999. The effect of trematode infection on amphibian limb development and survivorship. *Science* **284**: 802.
- Kaiser, J. 1999. A trematode parasite causes some frog deformities. *Science* **284**: 731-732.



- Kurais, A.R. 1995. The Biology and Ecology of Rice Field Vertebrates. IRPA Research Programme Performance Report (1991-1995). Biology and Ecology of Animal Pest and their Controlling Organisms.
<http://www.fsas.upm.edu.my/~stshasan/ark.html>. Access on 27 August 2002.
- Kusrini, M.D. 2003. Keanekaragaman amfibia di Bogor dan kemungkinan infeksi endoparasit pada jenis yang dikonsumsi. Laporan Akhir Kegiatan Penelitian Perguruan Tinggi Kontrak Nomor 016/P2IPT/DPPM/IV/2002. Institut Pertanian Bogor. Bogor.
- Leong, T.M. 2001. Parasitic Copepods Responsible for Limb Abnormalities. *FROGLOG* 46(3).
- Nurmainis. 2000. Kebiasaan makanan kodok sawah *Rana cancrivora* di Kabupaten Bogor, Jawa Barat. Unpublished Thesis. Program Studi Manajemen Sumberdaya Perairan Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor. Bogor.
- Ouellet, M. 2000. Amphibian deformities: current status of knowledge. In D.W. Sparling, G. Linder and C.A. Bishop Ecotoxicology of amphibians and reptiles.. Pensacola, Society of Environmental Toxicology and Chemistry (SETAC): 617-696.
- Pritchard, M.H. and G.O.W. Kruse. 1982. The Collection and Preservation of Animal Parasites. University of Nebraska Press. Lincoln and London.
- Purnomo and M.J. Bangs. 1996. "*Isosiemella intani* sp. n. (Filarioidea: Onchocercidae), a parasite of *Rana cancrivora* from South Kalimantan, Indonesia." *J. Helminthol. Soc. Wash.* 63(1): 47-50.



- Purnomo and M.J. Bangs .1999. "*Paraochoterenella javanensis* gen. et sp. n. (Filarioidea: Onchocercidae) from *Rana cancrivora* (Amphibia: Anura) in West Java, Indonesia." *J. Helminthol. Soc. Wash.* **66**(2): 187-193.
- Smyth, J.D. and M.M. Smyth. 1980. Frogs as host-parasite systems I. London, The Macmillan Press Ltd.
- Stopper, G.F., L. Hecker, R.A. Franssen and S.K. Sessions. 2002. How Trematodes Cause Limb Deformities in Amphibians. *J. Exp. Zool. (Mol. Dev. Evol.)* **294**: 252-263.
- Sugiri, N. 1979. Beberapa aspek biologi kodok batu (*Rana blythii*) Boulenger, Ranidae, Anura-Amphibia di beberapa wilayah Indonesia dan kedudukan taksanya. Unpublished PhD Thesis. Fakultas Pasca Sarjana. Institut Pertanian Bogor. Bogor. 188 p.
- Sunityoso, S., E.I.M. Adil dan M.Werdhiyanti. 1997. Cacing Endoparasit pada Saluran Pencernaan dan Jaringan Otot Paha Kodok yang Tertangkap di Daerah Kampus UI Depok. *Hayati* **4**(1).
- Yamaguti, S. 1961. *Systema Helminthum*, Vol. II. The Trematodes of Vertebrates. Interscience. New York and London.
- Yamaguti, S. 1961. *Systema Helminthum*, Vol. III. The Nematodes of Vertebrates. Interscience. New York and London.