

## Nematoda of Eleven Species of Ranid Frogs (Anura: Ranidae) from Southeast Asia

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### Abstract

Eleven species of ranid frogs ( $N = 42$ ) from Southeast Asia were examined for gastrointestinal helminths: *Amolops torrentis*, *Chalcorana labialis*, *Hylarana erythraea*, *Hylarana taipehensis*, *Indosylvirana milleti*, *Pulchrana banjarana*, *Pulchrana baramica*, *Pulchrana glandulosa*, *Pulchrana picturata*, *Sylvirana cubitalis*, *Sylvirana mortensi*. The helminth community consisted of eight species of Nematoda, represented by *Aplectana macintoshii*, *Cosmocerca ornata*, *Cosmocercoides pulcher*, *Icosiella inominata*, *Meteterakis japonica*, *Seuratascaris numidica*, *Abbreviata* sp., and an Acuariidae. Ranid frogs from Southeast Asia are infected by generalist helminths that also infect other frogs. Twenty new host records are reported..

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## Introduction

The Oriental Region (which includes Southeast Asia) has an extremely diverse amphibian fauna with approximately 1534 species recorded (Das 2002). This biodiversity is threatened by a number of factors including growth of human populations, habitat loss, deforestation, habitat fragmentation, mining, logging, urbanization, fires, invasive species, diseases and air pollution (Corlett 2016). In view of these threats to the environment, it is crucial to document both invertebrate and vertebrate diversity of fauna from Southeast Asia while it is still possible. Frogs are particularly sensitive to environmental degradation and many species have suffered serious population declines (Lips and Donnelly 2005). It may be expected that the helminth faunas of those host frogs have also declined, in some cases, perhaps to the point of extinction. With the above in mind, an examination of frogs from Southeast Asia will add to our knowledge of invertebrate biodiversity from a threatened area.

The family Ranidae is cosmopolitan in distribution except for southern South America and most of Australia with approximately 347 known species (Vitt and Caldwell 2014). In this paper we report Nematoda from eleven species of Ranidae from Southeast Asia. The torrent sucker frog, *Amolops torrentis* (Smith, 1923) is known from southwestern and central Hainan Island, China (Frost 2016). The white-lipped frog, *Chalcorana labialis* (Boulenger, 1887) ranges from southern Thailand south through Peninsular Malaysia to Singapore and Sumatra and also occurs in the lowlands of Borneo (Grismer 2011). The red-eared frog, *Hylarana erythraea* (Schlegel, 1837) ranges from eastern India to Vietnam south through Indochina and the Malay Peninsula to Singapore and Sumatra; it also occurs in Java, Sulawesi and the Phillipines (Grismer 2011). The Taipei frog, *Hylarana taipehensis* (Van Denburgh, 1909) occurs in southern

China to Vietnam, Cambodia, through Laos to northeastern Thailand and Myanmar to India and Bangladesh (Frost 2016). The dalat frog, *Indosylvirana milleti* (Smith, 1921) occurs in southern Vietnam, southern and eastern Thailand and southwestern Cambodia (Frost 2016). *Pulchrana banjarana* (Leong and Lim, 2003) occurs in highlands of the Malay Peninsular, Malaysia and southern Thailand (Frost 2016). The masked rough-sided frog, *Pulchrana baramica* (Boettger, 1900) occurs in Cambodia, Laos, Indonesia, Brunei and Sabah, West and East Malaysia, Singapore, Thailand, Vietnam and introduced on the Philippines (Frost 2016). The Sarawak frog, *Pulchrana glandulosa* (Boulenger, 1882) occurs in Peninsular Thailand to Malaya, Sumatra, Natuna Island, Borneo, Singapore, southern Vietnam (Frost 2016). The spotted stream frog, *Pulchrana picturata* (Boulenger, 1920) ranges throughout Peninsular Malaysia and Borneo (Grismer 2011). The Siam frog, *Sylvirana cubitalis* (Smith, 1917) occurs in Myanmar into Thailand, southern China, northern Laos and adjacent Vietnam (Frost 2016). The Koh Chang Island Frog, *Sylvirana mortensi* (Boulenger, 1903) occurs in southeastern Thailand and southwestern Cambodia (Frost 2016).

Previous reports of helminths in the above ranids include the nematode *Aplectana macintoshii* (Stewart, 1914) found in *Hylarana erythraea* by Yuen (1965), the acanthocephalan *Pseudoacanthocephalus nguyenthileae* Amin, Van Ha and Heckmann 2008 in *Hylarana taipehensis* from Vietnam by Amin et al. (2008) and the trematodes *Diplodiscus sacculosus* Yuen, 1962 and *Glypthelmins staffordi* Tubanguai, 1928 in *Hylarana erythraea* (Rahman and Shakinah, 2015).

#### MATERIALS AND METHODS

Forty-two individuals of ranid frogs from Southeast Asia were examined for helminths: *Amolops torrentis* ( $n = 3$ ; mean snout-vent length (SVL) = 31.0 mm  $\pm$  9.5 SD, range = 25-42 mm); *Chalcorana labialis* ( $n = 12$ ; mean SVL = 37.7 mm  $\pm$  5.4 SD, range = 30-48 mm); *Hylarana*

*erythraea* ( $n = 4$ ; mean SVL = 49.5 mm  $\pm$  8.5 SD, range = 37-56 mm); *Hylarana taipehensis* ( $n = 2$ ; mean SVL = 33.5 mm  $\pm$  7.8 SD, range = 28-39 mm); *Indosylvirana milleti* ( $n = 3$ ; mean SVL = 38.7 mm  $\pm$  1.2 SD, range = 38-40 mm); *Pulchrana banjarana* ( $n = 3$ ; mean SVL = 38.7 mm  $\pm$  5.8 SD, range = 32-42 mm); *Pulchrana baramica* ( $n = 2$ ; SVL = 56.0); *Pulchrana glandulosa* ( $n = 3$ ; mean SVL = 70.3 mm  $\pm$  6.4 SD, range = 63-75 mm); *Pulchrana picturata* ( $n = 5$ ; mean SVL = 44.2 mm  $\pm$  10.1 SD, range = 32-59 mm); *Sylvirana cubitalis* ( $n = 2$ ; mean SVL = 43.5 mm  $\pm$  2.1 SD, range = 42-45 mm); *Sylvirana mortensi* ( $n = 3$ ; mean SVL = 52.7 mm  $\pm$  3.9 SD, range = 46-57 mm).

Ranid frogs were borrowed from the herpetology collection of La Sierra University (LSUHC), Riverside, California (Appendix 1). Specimens had been previously fixed in 10% formalin and later stored in 70% ethanol. The body cavity was opened by a longitudinal incision and the gastrointestinal tract was removed by cutting across the esophagus and rectum. Nematodes were cleared in lactophenol and examined under a compound microscope and were identified to genus using keys in Anderson et al. (2009) and Gibbons (2010) and to species by comparison with the original descriptions. Helminths were deposited in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln (Appendix 2). Parasite terminology is in accordance with Bush et al. (1997).

## RESULTS

A total of 222 nematodes representing eight species were found: *Aplectana macintoshii* (Stewart, 1914) (in small and large intestines), *Cosmocerca ornata* Diesing, 1861 (in small and large intestines), *Cosmocercoides pulcher* Wilkie, 1930, (in small intestines), *Icosiella innominata* Yuen, 1962, (in body cavities), *Meteterakis japonica* (Wilkie, 1930) (in large intestines), *Seuratascaris numidica* Seurat, 1917 (in stomachs and small intestines), *Abbreviata* sp. (as stomach cysts), Acuariidae (as stomach cysts). Frog sample size, helminth number,

prevalence, mean intensity of infection and range are presented in Table 1. Mean number of helminth species per host was  $1.9 \pm 1.1$  SD, range = 1-4. Twenty new host records are reported (Table 1).

## DISCUSSION

### Nematoda

Cosmocercidae. *Aplectana macintoshii* is a widely ranging nematode which is known from amphibians and reptiles of five biogeographical realms: Afrotropical, Indomalayan, Neotropical, Oceanian and Palearctic (McAllister et al. 2010). It occurred in eight of eleven (73%) ranid frog species in this paper (Table 1). Molecular studies are warranted to ascertain if the various worldwide populations of *A. macintoshii* constitute a single species. The life cycle of *A. macintoshii* is not known.

*Cosmocerca ornata* occurred in four of the eleven ranid frog species (36%) in this paper. It is widespread (Baker 1987) and occurs in Europe, Africa, Malaysia, China, India and South America. Krillova and Krillov (2015) reported *C. inornata* larvae infected tadpoles of the marsh frog, *Pelophylax ridibundus* entering through the mouth or by skin penetration; however, they did not persist. A recent list of *Cosmocerca inornata* hosts is in Yildirimhan et al. (2009).

*Cosmocercoides pulcher* was found in one of eleven (9%) of the ranid species reported herein (Table 1). It has been found in anurans from China, Japan, Borneo, Okinawa, Papua New Guinea, Taiwan, Siberia (Goldberg et al. 2013). The life cycle of *C. pulcher* is not known but eggs of the congener *C. variabilis* molt twice to the infective third stage larvae before infecting a new host by skin penetration (Anderson 2000).

Onchocercidae. *Icosiella inominate* was found in two of eleven (18%) ranid species from Southeast Asia (Table 1). *Icosiella inominate* was previously found in *Phrynomantis asper* (as *Bufo asper*) and *Fejervarya cancrivora* (as *Rana cancrivora*) from Perak and Johor states, West

Malaysia by Yuen (1962). Although the life cycle of *I. inominata* is not known, Desportes (1941) reported a biting midge (Ceratopogonidae) and sand fly (Psychodidae) as likely vectors of the congener *Icosiella neglecta*.

Heterakidae. *Meteterakis japonica* was found in one of eleven (9%) of the eleven ranid frog species from Southeast Asia we examined (Table 1). *Meteterakis japonica* has been found in several anuran species from Japan, China and Borneo (Baker 1987) and the skink *Plestiodon japonicus* from Japan (Sata 2015). Heterakids are monoxenous; eggs containing the infective stage are ingested by the host (Anderson 2000).

Ascarididae. *Seuratascaris numidica* was found in three of eleven (27%) of the eleven ranid species we examined from Southeast Asia (Table 1). *Seuratascaris numidica* is known from a variety of anurans from Europe, southeast Asia, New Guinea and Australia (Baker 1987). The life cycle of *S. numidica* is not known but ascaridoids are basically heteroxenous; in various species transmitted in aquatic habitats, paratenic hosts such as crustaceans, oligochaetes and larval insects ingest free larvae or eggs and transmit larvae via the food chain (Anderson 2000).

Physalopteridae. *Abbreviata* sp. were found in one of eleven (9%) of the eleven species of ranid frogs examined from Southeast Asia (Table 1). Cysts containing physalopterid larvae (*Abbreviata* sp.) *sensu* (Jones 1995) are commonly found in the stomach walls of snakes, lizards and anurans (McAllister et al. 1995). As no further development occurs, reptiles are likely paratenic (= transport) hosts. Development to the adult physalopteran occurs when the reptile or amphibian carrying the cysts is eaten by a carnivore (definitive host).

Acuarididae are parasites of aquatic birds and utilize arthropods as intermediate hosts (Anderson 2000). Reptiles and amphibians serve as paratenic (transport) hosts with development to the adult occurring when the reptile is eaten by a carnivorous bird. Larvae of Acuariidae were found in one of eleven (9%) of the eleven ranid species reported in this paper.

We have no explanation as to why only nematodes were found in our samples of ranid frogs. It is plausible that if additional samples of ranids were examined for parasites, other kinds of helminths (i.e. cestodes, digeneans, acanthocephalans) would be found.

Because frogs are ectotherms, environmental conditions limit helminth recruitment potential by affecting feeding rates and foraging behavior. Amphibians are mainly opportunistic, generalist feeders (Toft 1985). Thus the helminth community of anurans is the result of chance and rather than consisting of species that infect specific anurans, they harbor generalist helminths that infect a variety of anurans. Furthermore terrestrial amphibians are limited in distribution to moist microhabitats as they are subject to desiccation (Duellman and Trueb 1986). Our small sample sizes and wide geographic distribution of the frogs prohibit meaningful discussion as to the distribution of helminths in our samples.

The data presented herein suggest that Southeast Asian frogs are infected by helminth generalists. All helminths examined in this study are known to infect at least two host species. This finding parallels findings of Goldberg et al. (2015) in which 12 species of *Cnemaspis* from Southeast Asia, six species of *Gonocephalus* (Goldberg et al. 2016) and eight species of *Cyrtodactylus* from Southeast Asia (Goldberg et al. 2017) were infected by generalist helminths. Nevertheless, helminths infecting anurans and those infecting squamate reptiles differ, indicating host preference at the order level.

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Table 1. Number, prevalence (P) in %, mean intensity (MI) and range (R) for eleven species of ranid frogs from Southeast Asia; \* = new host record.

	<i>Amolops</i>	<i>Chalcorana</i>	<i>Hylarana</i>	<i>Hylarana</i>	<i>Indosylvirana</i>	<i>Pulchrana</i>	<i>Pulchrana</i>	<i>Pulchrana</i>	<i>Pulchrana</i>	<i>Sylvirana</i>	<i>Sylvirana</i>
	<i>torrentis</i>	<i>labialis</i>	<i>erythraea</i>	<i>taipehensis</i>	<i>milleti</i>	<i>banjarana</i>	<i>baramica</i>	<i>glandulosa</i>	<i>picturata</i>	<i>cubitalis</i>	<i>mortensi</i>
	N = 3	N = 12	N = 4	N = 2	N = 3	N = 3	N = 2	N = 3	N = 5	N = 2	N = 3
	N P MI R	N P MI R	N P MI R	N P MI R	N P MI R	N P MI R	N P MI R	N P MI R	N P MI R	N P MI R	N P MI R
<i>Aplectana macintoshii</i>	---	*23 42 4.6 1-14	14 100 3.5 2-8	*150 1.0	*6 67 3.0 1-5	---	*18 100 7.5 4-11	*62 67 21.0 12-30	---	*5 50 5.0	*15 67 7.5 1-14
<i>Cosmocerca ornata</i>	---	*9 50 1.7 1-2	*1 25 1.0	---	---	---	---	---	*1 20 1.0	---	*5 33 5.0
<i>Cosmocercoides pulcher</i>	---	---	---	---	---	---	---	*5 66 2.5 1-4	---	---	---
<i>Icosiella innominata</i>	---	---	---	---	---	---	*2 50 2.0	---	---	---	*1 33 1.0
<i>Meteterakis japonica</i>	---	---	---	---	---	---	---	*7 33 7.0	---	---	---
<i>Seuratascaris numidica</i>	---	*1 8 1.0	*2 25 2.0	---	---	*1 33 1.0	---	---	---	---	---
<i>Abbreviata</i> sp.	---	---	---	---	---	---	---	*41 33 41.0	---	---	---
<i>Acuariidae</i>	*2 33 2.0	---	---	---	---	---	---	---	---	---	---

## Appendix 1

Ranid frogs from Southeast Asia examined from the herpetology collection of La Sierra University (LSUHC), Riverside, California.

*Amolops torrentis* ( $n = 3$ ): LSUHC 4121, 4123, September 2001, China, Hainan Island, Diao Luo Shan (20°1'2"N, 110°20'11"E); LSUHC 4192, September 2001, China, Hainan Island, Wuzhi Shan (19°14'12"N, 109°2'36"E).

*Chalcorana labialis* ( $n = 12$ ): LSUHC 7084, March 2005, West Malaysia, Kedah State, Pulau Langawi (5°47'3"N 103°0'24"E); LSUHC 7652, August 2005, West Malaysia, Johor State, Endau Rompin (2°25'13"N, 103°15'41"E); LSUHC 7692, August 2007, West Malaysia, Johor State, Endau Rompin (2°25'13"N, 103°15'41"E); LSUHC 8125, August 2006, West Malaysia, Johor State, Selai, (2°13'44"N, 103°25'40"E); LSUHC 8128, August 2006, West Malaysia, Johor State, Selai, Lubok Tapah (6°1'0"N, 102°9'0"E); LSUHC 8266, June 2012 Cambodia, Pursat Province, Base of Gunung Lawit (5°25'11"N, 102°35'24"E); LSUHC 9585, March 2010, West Malaysia, Penang State, Pulau Pinang, Telok Bahang (5°24'51"N, 102°35'24"E); LSUHC 9638, March, 2010, West Malaysia, Kedah State, Sungai Sedim (5°25'16"N, 100°49'52"E); LSUHC 10190, 10222-10224, September 2011, West Malaysia, Johor State, Guning Berlumut, (2°3'N, 103°30'E).

*Hylarana erythraea* ( $n = 4$ ): LSUHC 10298, September 2011, West Malaysia, Kedah State, Ulu Muda, (6°0'0" N, 100°58'0"E); LSUHC 9607, March, 2010, West Malaysia, Penang State, Pulau Jerejak, (3°58'60"N. 100°6'0"); LSUHC 7107, 7108 March 2015, West Malaysia, Kedah State, Pulau Langkawi, Lubuk Semilang, (6°17'15"N, 99°43'14"E).

*Hylarana taipehensis* ( $n = 2$ ): LSUHC 10155, 10159, August 2011, Cambodia, Pursat Province, O'Som, Marsh, (02°03'49"N, 103°32'0.0"E).

*Indosylvirana milleti* ( $n = 3$ ): LSUHC 7893, 7895, August 2006, Cambodia, Pursat Province, (12°8'48"N, 103°1'07"E); LSUHC 10152, August 2011, Cambodia, Pursat Province, O'Som Marsh (02°03'00"N, 103°31'00"E).

*Pulchrana banjarana* ( $n = 3$ ): LSUHC 9740, March 2010, West Malaysia, Cameron Highlands (4°28'19"N, 101°22'48"E); LSUHC 9766, March, 2010, West Malaysia, Pahang State, Fraser's Hill, Bishop Trail (3°41'42"N, 101°44'11"E); LSUHC 10782, August 2012, West Malaysia, Perak State, Bukit Larut (4°47'58"N, 101°3'30"E).

*Pulchrana baramica* ( $n = 2$ ): LSUHC 4070, 4071, August 2001, East Malaysia, Sarawak, Niah Cave, (3°39'12"N, 113°40'5"E).

*Pulchrana glandulosa* ( $n = 3$ ): LSUHC 9194, March 2008, West Malaysia, Perak State, Pulau Pangkor, (4°13'39"N, 100°33'71"E); LSUHC 9592, March 2010, West Malaysia, Johor State, Pulau Pinang, Telok Bahang (5°27'74"N, 100°12'71"E); LSUHC 10946, July 2012, West Malaysia, Perak State, Lengong Gua Peteri, (5°06'0"N, 100°58'0"E).

*Pulchrana picturata* ( $n = 5$ ): LSUHC 4040, August 2001, East Malaysia, Sarawak, Ranchan Pool on Sadong River, (13°54'0"N, 110°20'0"E); LSUHC 4638, 4639, July 2002, West Malaysia, Pulau Tioman, Sungai Mentawak (2°47'24"N, 104°10'11"E), LSUHC 7690, August 2007, West Malaysia, Johor, Endau-Rompin, (2°25'13"N, 103°15'41"E); LSUHC 10045 March 2011, West Malaysia, Terengganu, Sekaryu (4°57'40"N, 102°58'15"E).

*Sylvirana cubitalis* ( $n = 2$ ): LSUHC 4184, September 2001, China, Hainan Island, Wuzhi Shan, (19°14'12"N, 109°2'36"E); LSUHC 4168, September 2001, China, Hainan Island, 21 km NW of Shiyun (18°59'35"N, 109°36'2"E).

*Sylvirana mortensi* ( $n = 3$ ): LSUHC 7925, August 2006, Cambodia, Pursat Province, 912°21'68"N, 103°38'10"E); LSUHC 8557, 8562, July 2007, Cambodia, Kampot Province, Bokor National Park, (10°38'31"N, 104°1'64"E).

## Appendix 2

Harold W. Manter Laboratory (HWML) accession numbers for helminths from ranid frogs collected in Southeast Asia taken from the herpetology collection of La Sierra University (LSUHC), Riverside, California

*Amolops torrentis* Acuariidae (HWML 99973);

*Chalcorana labialis* *Aplectana macintoshii* (HWML 99976), *Cosmocerca ornata* (HWML 99977),

*Seuratascaris numidica* (HWML 99978);

*Hylarana erythraea* *Aplectana macintoshii* (HWML 99957), *Cosmocerca ornata* (HWML 99958),

*Seuratascaris numidica* (HWML 99959);

*Hylarana taipehensis* *Aplectana macintoshii* (HWML 99971);

*Indosylvirana milleti* *Aplectana macintoshii* (HWML 99976);

*Pulchrana banjarana* *Seuratascaris numidica* (HWML 99952);

*Pulchrana baramica* *Aplectana macintoshii* (HWML 99950), *Icosiella innominata* (HWML 99951);

*Pulchrana glandulosa* *Aplectana macintoshii* (HWML 99953), *Cosmocercoides pulcher* (HWML 99954), *Meteterakis japonica* (HWML 99955), *Abbreviata* sp. (HWML 99956);

*Pulchrana picturata* *Cosmocerca ornata* (HWML 99960);

*Sylvirana cubitalis* *Aplectana macintoshii* (HWML 99974);

*Sylvirana mortensi* *Aplectana macintoshii* (HWML 99961), *Cosmocerca ornata* (HWML 99962),

*Icosiella inominata* (HWML 99963).