Nematodes of five species of Dicroglossid frogs (Anura: Dicroglossidae) from Southeast Asia


Abstract
Five species of dicroglossid frogs (Dicroglossidae) \( (n = 29) \) from Southeast Asia were examined for helminths: Limnonectes blythii, L. hascheanus, L. khasianus, L. kohchangae, L. macrognathus. We found 8 species of Nematoda, consisting of gravid individuals of Aplectana macintoshii, Cosmocerca ornata, Foleyellides malayensis, Icosiella innominata, Oswaldocruzia rohdei, Seuratascaris numidica and larvae of Abbreviata sp. and Physalopteridae gen. sp. Dicroglossid frogs from southeast Asia are infected by generalist helminths that infect other species of frogs. Twelve 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), including members of the Dicroglossidae which range from sub-Saharan Africa, South Asia to the Philippines and New Guinea, into the southwest Pacific Islands (Vitt and Caldwell 2014). The dicroglossid species in Peninsular Malaysia are small to very large, forest floor frogs (Grismer 2011). Despite having a very rich amphibian fauna, there are few reports of helminths from Southeast Asian frogs. Amphibian biodiversity from Southeast Asia is in peril due to factors that include the spread of agriculture, hunting, burning, invasive species, air pollution and urbanization (Cortlett 2010). Other threats include human consumption of large species, the pet trade and the pathogen *Batrachochytrium dendrobatidis* which causes amphibian chytridomycosis (Rowley et al. 2010). Because of these environmental threats it is important to document both invertebrate and vertebrate diversity of Southeast Asian fauna while it is still possible (Rowley et al. 2010). With the above in mind, a helminthological survey of dicroglossid frogs from Southeast Asia was undertaken to increase information of invertebrate biodiversity in frogs from a threatened area.

Blyth’s giant frog, *Limnonectes blythii* (Boulenger, 1920) ranges from southern Thailand south through Peninsular Malaysia to Singapore (Grismer 2011); The hill forest frog *Limnonectes hascheanus* (Stoliczka, 1870) ranges from India east to Vietnam, south through the Malay Peninsular (Grismer 2011); the Khasi hills frog, *Limnonectes khasianus* (Anderson, 1871) is distributed from Meghalaya State, and Assam State, India to peninsular Thailand, and Sumatra (Frost 2016); The Koh Chang frog, *Limnonectes kohchangae* (Smith, 1922) is known from Thailand, southern Vietnam, Cambodia and northern Laos (Frost 2016); the green swollen-headed frog, *Limnonectes macrognathus* (Boulenger, 1917) is known from Myanmar, through northwestern and southern Peninsular Thailand to northern
Malaya and Malaysia (Frost 2016). Previous helminth records on the above dicroglossid frogs are limited to microfilariae in *L. blythii*, (Chutmongkonkul et al. 2006).

MATERIALS AND METHODS

Twenty-nine dicroglossid frogs from Southeast Asia were borrowed from the herpetology collection of La Sierra University (LSUHC), Riverside, California and examined for helminths (Appendix 1): *L. blythii* (*n* = 5; mean snout length (SVL) = 66.4 mm ± 8.7 SD, range = 56-79 mm); *L. hascheanus* (*n* = 4; mean snout length (SVL) = 21.0 mm ± 1.8 SD, range = 19-23 mm); *L. khasianus* (*n* = 13; mean snout length (SVL) = 37.2 mm ± 5.5 SD, range = 28-47 mm); *L. kohchangae* (*n* = 6; mean snout length (SVL) = 38.1 mm ± 3.7 SD, range = 31-41 mm); *L. macrognathus* (*n* = 1; snout length (SVL) = 47.0 mm). Utilization of museum collections to obtain parasite data avoids removing additional animals from the wild.

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, examined under a compound microscope and identified to species utilizing Anderson et al. (2009), Gibbons (2010) and by comparisons with the original descriptions. Nematodes 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 86 nematodes representing eight species were found: *Aplectana macintoshii* (Stewart, 1914) (infection sites: small, large intestines); *Cosmocerca ornata* Diesing, 1861 (large intestine); *Foleyellides malayensis* (Petit and Yen, 1979) (body cavity); *Icosiella innominata* Yuen, 1962 (body cavity); *Oswaldocruzia rohdei* Yuen, 1963 (small intestine); *Seuratascaris numidica* Seurat, 1917 (small intestine); *Abbreviata* sp. (stomach) and Physalopteridae gen. sp.
(stomach). Dicroglossid frog sample size, nematode number, prevalence, mean intensity of infection are presented in Table 1. Mean number of helminth species per host was $2.4 \pm 1.7$ SD, range = 1-5. All helminth findings $(n = 12)$ are new host records.

DISCUSSION

Nematoda

Ascarididae. *Seuratascaris numidica* was found in one of the five dicroglossid species (Table 1). It is known to occur in a variety of anurans from Europe, Southeast Asia, New Guinea and Australia (Baker 1987). The dicroglossid *Limnonectes macrodon* (Sprent 1985) and the microhylid *Copiula fistulans* from Papua New Guinea should be added to that list (Goldberg et al. 2009a). *Seuratascaris numidica* was reported to occur in frogs from Malaysia (as Malaya) by Sprent (1985). *Seuratascaris numidica* in *L. kohchangae* from Cambodia (Table 1) is a new locality record. The genus *Seuratascaris* was proposed by Sprent (1985). The type and only species is *S. numidica*. The life cycle of *S. numidica* is not known.

Cosmocercidae. *Aplectana macintoshii* is a widely distributed nematode with representatives in the following biogeographical regions (*sensu* Holt et al. 2013): Afrotropical, Neotropical, Oceanian, Oriental, Palearctic. A host list for *A. macintoshii* is in McAllister et al. (2010). *A. macintoshii* was previously reported in Malaysia (as Malaya) by Baker (1980) and has recently been found in eight ranid species (Goldberg et al. 2017a) and two bufonid species (Goldberg et al. 2017b) from Peninsular Malaysia. *Aplectana macintoshii* in *L. kohchangae* from Cambodia (Table 1) is a new locality record. Molecular studies are warranted to ascertain if the worldwide populations of *A. macintoshii* consist of a single species. The life cycle of *A. macintoshii* is not known. *Aplectana macintoshii* was found in two of five dicroglossid species (Table 1).

*Cosmocerca ornata* is widespread and occurs in the Afrotropical, Neotropical, Oceanian, Oriental, and Palearctic Regions (Baker 1987). A host list is in Yildiririmhan et al. (2009).
Cosmocerca ornata was recently reported in the gecko Cnemaspis mcquirei, four ranid species, and the toads Ingerophrynus parvus and Phrynoidis aspera from West Malaysia (Bursey et al. 2014, Goldberg et al. 2017a, 2017b). Cosmocerca ornata in L. kohchangae from Cambodia (Table 1) is a new locality record. While the life cycle of C. ornata is not known, Fotedar and Tikoo (1968) reported larvae of the congener C. kashmirensis penetrated the skin of Bufo viridis (as Bufo viridis) and adults were recovered 10 to 14 days post-infection. Cosmocerca ornata was found in three of five dicroglossid species (Table 1).

Molineidae. Oswaldocruzia rohdei was described from L. macrodon from Pahang State, Peninsular Malaysia by Yuen (1963). Limnonectes blythii is the second host found to harbor O. rohdei. Oswaldocruzia occur in the intestinal tract of amphibians and reptiles throughout the world (Anderson 2000). The life cycle of O. rohdei is not known, but Oswaldocruzia filiformis and O. pipiens have been shown to have direct life cycles (Anderson 2000). Oswaldocruzia rohdei was found in one of the five dicroglossid species (Table 1).

Onchocercidae. The Onchocercidae have evolved blood or skin-inhabiting microfilariae and are transmitted by blood sucking arthropods (Anderson 2000). The onchocercid Foleyella brachyoptera is transmitted by mosquitoes (Causey 1939). Foleyellides malayensis, was found in one of five dicroglossids (Table 1). It was originally described as Waltonella malayensis by Petit and Yen (1979) but was moved to Foleyellides by Esslinger (1986). It was previously reported from Malaysia in Pulchrana glandulosa (as Rana glandulosa) by Petit and Yen (1979) and Limnonetes macrodon (as Rana macrodon) and Amolops larutensis by Mak and Yong (1981). Icosiella innominata was previously reported in Phrynoidis asper (as Bufo asper) and Fejervarya cancrivora (as Rana cancrivora) from Perak and Johor States, West Malaysia by Yuen (1962). The life cycle of Icosiella are similar to those of Foleyella (Baker 2007). Desportes (1941) reported a biting midge (Ceratopogonidae) and sand fly (Psychodidae) as probable
vectors of the congener *Icosiella neglecta*. *Icosiella innominata* was found in one of five Dicroglossidae (Table 1).

Physalopteridae. *Abbreviata* sp., and Physalopteridae gen. sp. larvae are commonly found in the stomachs of snakes and lizards (Jones 1995). Insects have been shown to serve as intermediate hosts for species of *Physaloptera* (Anderson 2000). The final (definitive host) can become infected directly by ingesting insects containing larvae or through paratenesis in which a temporary host harbors the larva, with no further development occurring. As we found no adult *Abbreviata* or *Physaloptera* in any frogs from Southeast Asia in this study or other studies from Southeast Asia (Goldberg et al. 2017a, b), Papua New Guinea (Goldberg et al. 2009b, c) or elsewhere (Goldberg and Bursey 1991, 2010; Goldberg et al. 1996), we suspect those anurans with cysts containing larvae may act as paratelic hosts for members of the Physalopteridae while those without cysts represent incidental infections through diet. *Abbreviata* sp. has previously been reported from the frog *Pulcharana glandulosa* and *Physaloptera* sp. from the toad *Phrynoidis asper* from Peninsular Malaysia (Goldberg et al. 2017a, 2017b).

The data presented herein indicate that Southeast Asian dicroglossids are infected by nematode generalists. All nematodes found in the current study are known to occur in other species of frogs. As further representatives of *L. blythii*, *L. hascheanus*, *L. khasianus*, *L. kohchangae*, and *L. macrognathus* as well as other species are examined, we anticipate the helminth list for dicroglossids from Southeast Asia will grow. Further investigations should also document presence of helminths of other categories (Cestoda, Digenea, Acanthocephala) in dicroglossid frogs. With at least 15 species of dicroglossid frogs known from Peninsular Malaysia alone (sensu Chan et al. 2010), further work is needed to document the helminths occurring in this family in Southeast Asia.
LITERATURE CITED


Appendix 1

The dicroglossid frogs from Southeast Asia from the herpetology collection of La Sierra University (LSUHC), Riverside, California examined for this study.


*Limnonectes khasianus* (*n* = 13): LSUHC 7674, August 2005, West Malaysia, Johor State, Endau-Rompin, 2°25'13.08"N, 103°25'40.7994"E; LSUHC 8062, August 2006, West Malaysia, Pahang State, Fraser's Hill, 3°42'42.84"N, 101°44'11.76"E; LSUHC 8183, September 2006, West Malaysia, Johor State, Selai, 2°13'44.00394"N, 103°25'40.7994"E; LSUHC 8273, LSUHC 8274, June 2012, West Malaysia, Terengganu State, Gunung Lawit, 5°25'0.01"N, 105°34'59.99"E; LSUHC 9396 LSUHC 9397 September 2009, West Malaysia, Terengganu State, Pulau Redang, 5°47'3.83"N, 103°0'24.84"E; LSUHC 9680, March 2010, West Malaysia, Kedah State, Hutan Lipur, Sungai Tupah, 5°37'36.2166"N, 100°27'54.5904"E, LSUHC 9710, LSUHC 9711, March 2010, West Malaysia, Perak State, Bukit Larut, 4°51'44.28"N, 100°47'4.8"E.; LSUHC 9943, West Malaysia, Johor State, Gunung
Belumut, 2°2'32.99"N, 103°33'39.95"E; LSUHC 10193, September 2011, West Malaysia,  
Johor State, Gunung Belumut, 2°2'32.9994"N, 103°33'39.9594"E; LSUHC 10252,  
September, 2011, West Malaysia, Pahang State, Cameron highlands, 4°28'19.56"N,  
101°22'4836"E.

*Limnonectes kohchangae* (*n* = 6): LSUHC 7388, LSUHC 7433, LSUHC 7434, LSUHC 7436,  
LSUHC 7441, LSUHC 7449, August, 2005, Cambodia, Kampong Speu Province, Phnom Aural, 12°1'57"N, 104°10'14.87"E.

*Limnonectes macrognathus* (*n* = 1): LSUHC 7094, March, 2005, West Malaysia, Kedah  
State, Pulau Langawi, 6°20'53.99"N, 99°47'59.99"E.
Appendix 2

Harold W. Manter Laboratory (HWML) Accession Numbers for Helminths from dicroglossid frogs collected in Southeast Asia, taken from the herpetology collection of La Sierra University (LSUHC), Riverside, California.

_Limnonectes blythii:_ Aplectana macintoshii (HWML 99941), Cosmocerca ornata (HWML 99942), Foleyellides malayensis (HWML 99943), Icosiella inominata (HWML 99944), Oswaldocruzia rohdei (HWML 99945);

_Limnonectes hascheanus:_ Abbreviata sp. (larva) (HWML 99981);

_Limnonectes khasianus:_ Cosmocerca ornata (HWML 99979), Physalopteridae gen. sp. (larva) (HWML 99980);

_Limnonectes kohchangae:_ Aplectana macintoshii (HWML 99946), Cosmocerca ornata (HWML 99947), Seuratascaris numidica (HWML 99948);

_Limnonectes macrognathus:_ Physalopteridae gen. sp. (larva) (HWML 99949).
Table 1. Number (n), prevalence (%), mean intensity (X ± SD), and range (r) for Nematoda in 29 Microringiidae frogs (*Limnonectes*) from Southeast Asia: *Limnonectes blythii* (n = 5), *Limnonectes hascheanus* (n = 4), *Limnonectes khasianus* (n = 13), *Limnonectes kohchangae* (n = 6), and *Limnonectes macrognathus* (n = 1). All are new host records.

<table>
<thead>
<tr>
<th>Nematoda</th>
<th>L. blythii</th>
<th>L. hascheanus</th>
<th>L. khasianus</th>
<th>L. kohchangae</th>
<th>L. macrognathus</th>
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<tr>
<td></td>
<td>n  %</td>
<td>X ± SD</td>
<td>n  %</td>
<td>X ± SD</td>
<td>r  n  %</td>
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<td><em>Aplectana macintoshii</em></td>
<td>2 20</td>
<td>2.0</td>
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<td>11 50</td>
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<td><em>Cosmocerca ornata</em></td>
<td>7 40</td>
<td>2.5 ± 2.1</td>
<td>1 8</td>
<td>1.0</td>
<td>3 50</td>
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<td>(2-5)</td>
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<td><em>Foleyellides malayensis</em></td>
<td>3 40</td>
<td>3.0</td>
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<tr>
<td><em>Icosiella innominata</em></td>
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<td><em>Oswaldocruzia rohdei</em></td>
<td>28 20</td>
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<tr>
<td><em>Seuratascaris numida</em></td>
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<td><em>Abbreviata</em> sp.</td>
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<td>1 25</td>
<td>1.0</td>
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<tr>
<td><em>Physalopteridae</em> gen. sp.</td>
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