

Endoparasites in *Limnonectes magnus* (Anura, Dicroglossidae) from Samar Island, Philippines with description of a new species of *Aplectana* (Nematoda, Cosmocercidae)

Charles R. Bursey^{1*}, Stephen R. Goldberg², Cameron D. Siler³ and Rafe M. Brown⁴

¹Department of Biology, Pennsylvania State University, Shenango Campus, Sharon, Pennsylvania 16146, USA; ²Department of Biology, Whittier College, Whittier, California 90608, USA; ³Department of Biology and Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, Oklahoma 73072, USA; ⁴Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045, USA

Abstract

Aplectana samarensis sp. nov. (Ascaridida, Cosmocercidae) from the intestines of *Limnonectes magnus* (Anura, Dicroglossidae) is described and illustrated. *Aplectana samarensis* sp. nov. represents the 53rd species assigned to the genus and the 6th species reported from the Oriental region. It is easily separated from the 5 species previously reported from the region by the presence of a gubernaculum and the distribution pattern of male caudal papillae: *A. macintoshii* has an irregular pattern, no preloacal papillae in rows; both *A. akhrami* and *A. dubrajpuri* lack a gubernaculum; *A. duttaphryni* has 3 pairs of preloacal papillae forming 2 rows; *A. ranae* has 7 pairs of preloacal papillae forming 2 rows; *A. samarensis* sp. nov. has 4 pairs of preloacal papillae in 2 rows. In addition to the new species, individuals of *Falcaustra dubia* and *Pseudoacanthocephalus bufonis* as well as cestode plerocercoids were also found.

Keywords

Aplectana samarensis sp. nov., Nematoda, *Limnonectes magnus*, Anura, Samar Island, Philippines, *Falcaustra dubia*, *Pseudoacanthocephalus bufonis*, cestode plerocercoids

Introduction

The Mindanao Fanged Frog, *Limnonectes magnus* (Stejneger, 1910) (originally *Rana magna* Stejneger, 1910) is a large frog within the family Dicroglossidae found throughout much of the Mindano Pleistocene Aggregate Island Complex (Brown and Diesmos 2002, 2009) in the Philippines, where it is known from Basilan, Biliran, Bohol, Camiguin Sur, Dinagat, Leyte, Mindano, and Samar islands (Diesmos *et al.* 2015; Sanguila *et al.* 2016). The species is associated with forest stream systems, occurring from near sea level up to 1,800 m elevation and is active at night, hiding in rock crevices during the day (Brown and Alcalá 1970; Alcalá and Brown 1998). It is harvested heavily for food (Alcalá 1986). Fabricante and Nuñez (2012) previously reported the cestode, *Diphyllobothrium latum*, and unidentified nematodes in *L. magnus*. In addition, Vande Vusse (1976) described the monogenean, *Parapolytoma crooki*, from *L. magnus* (as *Rana magna*) collected

from Negros Island, Philippines. Earlier, Walton (1931) during the examination of material from Philippine amphibians, described *Oxysomatium ranae* (currently, *Aplectana ranae*) from what was thought to be *R. magna* (currently *L. magnus*). The genus *Aplectana* was erected as a replacement name for the preoccupied name *Aplecta* given by Railliet and Henry (1916) for the reassignment of *Ascaris acuminata* Schrank, 1788. Baker (1980) revised the genus and provided a key to 32 species; later he (1987) assigned 40 species to the genus; since then, 11 species have been added to bring the total to 52 (Bursey *et al.* 2018). In this paper we describe a new species of *Aplectana* and add to the helminth list for *L. magnus*.

Materials and Methods

Eight specimens of *L. magnus* were borrowed from the Department of Herpetology, Sam Noble Museum of Natural

*Corresponding author: cxb13@psu.edu

History (University of Oklahoma, Norman Oklahoma, USA,) and examined for helminths (OMHH 45068, 45069, 45073, 45074, 45077, 45080, 45081, 45082). The frogs were collected between March 1 and October 1, 2014 in Western Samar Province Samar Island, Philippine Islands, field-fixed in 10% formalin, and later transferred to storage in 70% ethanol. For this study, the body cavity of each frog was opened by an incision along the ventral midline and the gastrointestinal tract was removed by cutting across the esophagus and rectum. The esophagus, stomach, small intestine, and large intestine of each frog were examined separately for helminths with a dissecting microscope. The coelom was also searched. Nematodes, acanthocephalans, and cestodes were found and each parasite, fixed in situ and in good condition, was removed and placed in lactophenol, allowed to clear, then examined with a light microscope. Drawings were made with the aid of a microprojector. Measurements were made with an optical micrometer and are given in micrometers, unless otherwise stated, with mean \pm SD, followed by range in parentheses.

Results

One species of Cestoda represented by plerocercoids, 1 species of Acanthocephala, *Pseudoacanthocephalus bufonis* (Shiple, 1903), 2 species of Nematoda, *Falcaustra dubia* Yuen, 1963, and an undescribed species of *Aplectana* Railliet and Henry, 1916 were found (Table I). Selected nematode specimens were deposited in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, USA.

Description

Aplectana samarensis sp. nov. (Figs. 1–9)

General: Cosmocercoidea Railliet, 1926, Cosmocercoidea Travassos, 1925, *Aplectana* Railliet and Henry, 1916. Small, slender nematodes. Females of slightly greater length and girth than males. Cuticle with longitudinal striations from pharyngeal region to end of tail. Narrow lateral alae beginning at base of lip, ending on tail in both sexes. Somatic papillae absent. Mouth triangular, three lips, approximately 12 in length, each with a cuticular flap on anterior edge. Dorsal lip with 2 papillae, each ventrolateral lip with 1 ventrally situated papilla and 1 laterally situated amphid. Oesophagus divided into short an-

terior pharyngeal portion, elongate corpus, short isthmus and large valved bulb.

Male (*holotype and 11 paratypes*): Length 3.07 ± 0.45 mm (2.56–3.39 mm), width at midbody, 158 ± 25 (128–204). Total length of esophagus 657 ± 36 (612–740), pharynx 54 ± 2 (52–58) long; corpus 491 ± 28 (459–561) long; isthmus 36 ± 5 (24–43) long; bulb 74 ± 6 (67–85) long, 66 ± 5 (61–73) wide. Nerve ring 273 ± 19 (239–305) and excretory pore 516 ± 40 (459–587) from anterior end. Anus 146 ± 6 (140–153) from posterior end. Tail conical, typically bent ventrally producing a depression around cloaca and terminating in a slender filament approximately 30 in length. Testis flexed at midbody. Spicules slender, equal in length 173 ± 19 (158–220) long, 90° bend turning distal third toward ventral surface, diameter consistent throughout, distal extremity pointed; gubernaculum 60 ± 5 (49–67) long, "T"-shaped, width of proximal border not determined. Nine pairs of caudal papillae: 4 pairs precloacal papillae forming 2 parallel rows; 1 pair adcloacal papillae, somewhat larger than precloacal papillae; 4 pairs post cloacal, 1 pair slightly behind cloaca, 1 pair at end of first third of tail, 1 pair at end of second third of tail, 1 pair at base of tail filament. Phasmids not seen.

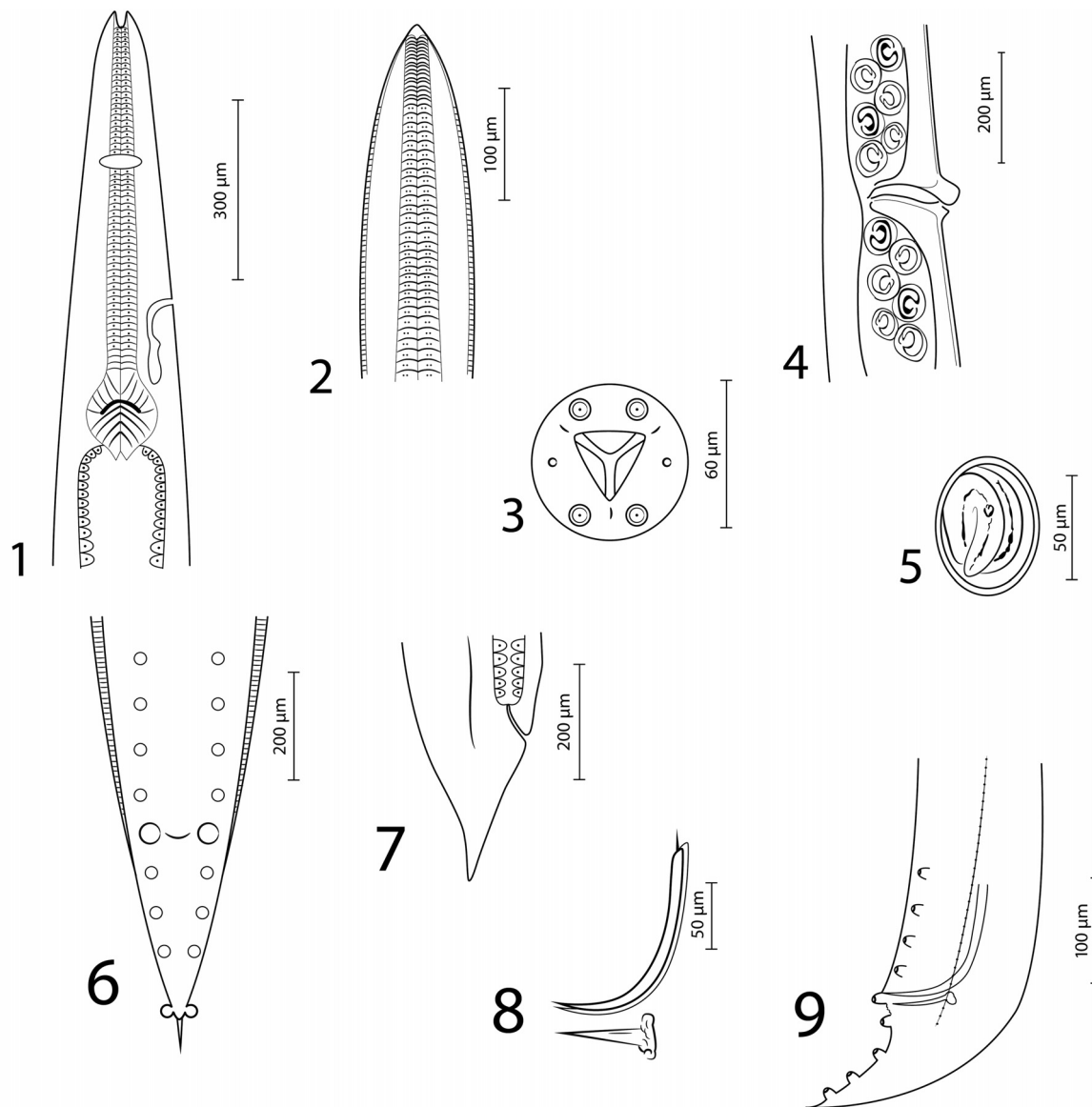
Female (*allotype and 11 paratypes*): Length 3.58 ± 0.32 mm (3.07–4.16 mm); width at vulva 218 ± 28 (191–281). Total length of esophagus 802 ± 66 (727–995); pharynx 55 ± 4 (49–61) long; corpus 585 ± 14 (561–612) long; isthmus 40 ± 4 (31–47) long; bulb 88 ± 5 (83–98) long, 80 ± 5 (73–92) wide. Nerve ring 295 ± 9 (275–305) and excretory pore 566 ± 33 (510–612) from anterior end. Vulva salient, anterior lip distinctly protruding, 1.07 ± 0.10 mm (0.84–1.17 mm) from posterior tip of tail. Muscular vagina directed dorsally joining 2 uteri near level of vulva, 1 uterus directed anteriorly, the other posteriorly. Reproductive system absent from anterior third of body; posterior uterus flexed anteriorly before reaching anal region. Ovaries directed anteriorly, both lie anterior to vulva. Eggs numerous, thin-shelled, closely packed and in various stages of development. Embryonated eggs 80 ± 6 (73–92) long, 52 ± 4 (43–59) wide. Anus 195 ± 11 (179–204) from posterior end; tail conical. Phasmids at mid-point of tail.

Taxonomic Summary

Type host: *Limnonectes magnus* (Stejneger, 1910), Mindanao Fanged Frog, symbiotype, OMNH 45073, collected 26 June 2016 (by CDS), male, SVL 71 mm.

Table I. Site of infection, number of helminths, prevalence, mean intensity and range of infection for 4 endoparasitic species in *Limnonectes magnus* from the Philippine Islands

Helminth	Site of Infection	Number	Prevalence	Mean Intensity \pm SD	Range	HWML Accession #
<i>Aplectana samarensis</i> sp. nov.	Large intestine	155	8/8 (100%)	19.4 ± 25.5	3–80	110376–110379
<i>Falcaustra dubia</i>	Small intestine	6	3/8 (38%)	2.3 ± 0.5	2–3	110381
<i>Pseudoacanthocephalus bufonis</i>	Large intestine	4	2/8 (25%)	2	–	110382
Cestoda (plerocercoids)	Small intestine cyst	13	1/8 (13%)	13	–	110380



Figs 1-9. *Aplectana samarensis* sp. nov. **1.** – Female, anterior end, lateral view. **2.** – Female, anterior end, dorsal view. **3.** – Female, en face view. **4.** – Female, vulvar region, lateral view. **5.** – Larvated egg. **6.** – Male, posterior end, ventral view. **7.** – Female, posterior end, ventral view. **8.** – Spicules (in lateral view) and gubernaculum (in dorsal view). **9.** – Male, posterior end, lateral view

Type locality: Philippines, Samar Island, Western Samar Province, Municipality of San Jose de Buan, Barangay, Uno, Mt. Huraw, 12.05262°N, 125.03429°E.

Site of infection: large intestine.

Type specimens: Holotype male, HWML 110376; allotype female HWML 110377; paratypes HWML 110378; voucher specimens, HWML 110379.

Etymology: The new species is named in reference to the locality of collection of its host.

Remarks

Based upon the taxonomical hierarchy of Baker (1987), three of the 9 genera assigned to the Cosmocercinae are superfi-

cially similar, i.e., *Aplectana*, *Oxyascaris*, and *Oxysomatium*. Males and females of *Oxyascaris* spp. exhibit significant sexual dimorphism (females are twice the length of males), while *Aplectana* and *Oxysomatium* exhibit little sexual dimorphism. However, the 3 genera are distinguished on the basis of uterus and ovary positions. In *Aplectana* spp., although 1 uterus extends anterior of the vulva, both ovaries are anterior to the vulva. In *Oxysomatium* spp., the ovary connected to the anteriorly directed uterus folds back to come to lie posterior to the vulva. In *Oxyascaris* spp., both uteri lie posterior to the vulva. We have assigned our specimen to *Aplectana* because both ovaries are anterior to the vulva.

Six species of *Aplectana* have now been reported from the Oriental region (Table II), i. e., *A. akhrami* (Islam, Farooq et

Table II. Nominal species of *Aplectana* from the Oriental region and selected characteristics of males and females

<i>Aplectana</i> spp.	Type host	Male characters				Female characters				Reference
		Length (mm)	Spicule (µm)	Gubernaculum (µm)	Caudal papillae pre:ad: post anal	Length (mm)	Vulva from anterior (mm)	Tail (µm)		
<i>A. akhrami</i> (Islam, Farooq et Khanum, 1979) Baker, 1987	<i>Duttaphrynus stomaticus</i>	1.8–3.4	150–180	absent	16:0:12	3.0–3.6	0.9–1.1	160	Islam <i>et al.</i> 1979	
<i>A. dubrajपुरi</i> Sou et Nandi, 2015	<i>Hoplobatrachus tigerinus</i>	1.1–1.2	147–167	absent	6:2:28	1.4–1.5	0.7–0.9	250–280	Sou and Nandi, 2015	
<i>A. duttaphryni</i> Sou, Sow et Nandi, 2014	<i>Duttaphrynus melanostictus</i>	2.3–2.5	222–240	15–17	6:2:24	3.5–3.6	1.7–1.8	43–46	Sou <i>et al.</i> 2014	
<i>A. macintoshii</i> (Stewart, 1914) Travassos, 1931	<i>Duttaphrynus stomaticus</i>	1.2–2.6	138–320	22–90	Variable	2.4–6.7	1.2–4.1	337–494	Baker 1980	
<i>A. ranae</i> (Walton, 1931) Baker, 1980	<i>Limnonectes magnus</i>	2.5	160	25	14:0:8	3.8	1.8	230	Walton 1931	
<i>A. samarensis</i> sp. nov.	<i>Limnonectes magnus</i>	2.6–3.4	158–220	49–67	8:2:8	3.1–4.2	2.3–3.0	179–204	This paper	

Khanum, 1979) Baker, 1987; *A. dubrajपुरi* Sou et Nandi, 2015; *A. duttaphryni* Sou, Sow et Nandi, 2014, *A. macintoshii* (Stewart, 1914) Travassos, 1931, *A. ranae* Wang, 1980 (Walton, 1931) Baker, 1980; and the new species *A. samarensis*. *Aplectana samarensis* sp. nov. is easily separated from the other five species by the presence/absence of a gubernaculum and the distribution pattern of male caudal papillae: *A. akhrami* and *A. dubrajपुरi* lack a gubernaculum; *A. duttaphryni* has only 3 pairs of preloacal papillae arranged in rows; *A. ranae* has 6 pairs of preloacal papillae arranged in rows; *A. macintoshii* has an irregular pattern of caudal papillae, none in rows. *Aplectana samarensis* sp. nov. has 4 pairs of preloacal papillae arranged in rows. Only 2 other species have been reported to have 4 pairs of preloacal papillae, *A. micropenis* Travassos, 1925 and *A. vellardi* Travassos, 1926 both from the Neotropical region (Burse *et al.*, 2018). *A. micropenis* and *A. vellardi* have very short gubernacula, 10 and 12 respectively, compared to *A. samarensis* sp. nov. at 49–67.

Discussion

Falcaustra dubia was originally described from *Limnonectes macrodon* (as *Rana macrodon*) collected in Selangor State, Malaysia by Yuen (1963). To our knowledge, *L. magnus* is the second host to harbor *F. dubia*. *Limnonectes magnus* represents a new host record and the Philippines is a new locality record for *F. dubia*.

Pseudoacanthocephalus bufonis was originally described as *Echinorhynchus bufonis* from *Duttaphrynus melanostictus* (as *Bufo melanostictus*) collected at Patani, Malaysia (Shiple 1903). It was reassigned to *Acanthocephalus* by Southwell and Macfie (1925); however, Petrochenko (1956) erected a new genus, *Pseudoacanthocephalus*, for it. *Pseudoacanthocephalus bufonis* is widely distributed throughout the Oceanian, Oriental, and Sino-Japanese biogeographical regions. A list of Chinese hosts can be found in Bush *et al.* (2009); additional hosts will be found in the list by Goldberg *et al.* (2013a) to which should be added reports of *Sphenomorphus indicus* from Taiwan (Norval *et al.* 2014); *Litoria angiana* and *L. arfakiana* and *L. modica* from Papua New Guinea (Goldberg *et al.* 2013b). *Limnonectes magnus* represents a new host record and the Philippines is a new locality record for *P. bufonis*.

Plerocercoid larvae (Cestoda) constitute the second larval stage of the Pseudophyllidea, Tetraphyllidea and Trypanorhyncha. They typically exhibit an adult scolex but lack embryonic hooks (Smyth 1976). *L. magnus* represents a new host record for plerocercoid larvae.

Currently, the helminth list for *L. magnus* is as follows: Cestoda, *Diphyllobothrium latum* and plerocercoids; Nematoda, *Aplectana samarensis* sp. nov., *Falcaustra dubia*, unidentified spp., and Acanthocephala, *Pseudoacanthocephalus bufonis*.

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