

Helminth Parasites Found in Rhacophorid Frog, *Polypedates braueri* (Vogt, 1911), from Taiwan

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ABSTRACT.– Three species of nematodes, *Oswaldocruzia japalurae*, *Cosmocerca* sp. and *Cosmocercella iwatsukii*, and one acanthocephalan, *Pseudoacanthocephalus* cf. *bufonis*, were collected from *Polypedates braueri*, a rhacophorid frog native to Taiwan and the eastern part of subtropical continental Asia. *Cosmocercella iwatsukii* was first collected outside of the type locality on Okinawajima Island. The problem in identification of *Cosmocerca* parasitic in amphibians of Japan and Taiwan is discussed from a historical viewpoint.

KEY WORDS: Endoparasites, *Oswaldocruzia*, *Cosmocerca*, *Cosmocercella*, *Pseudoacanthocephalus*

INTRODUCTION

Only a few records of helminths have been documented in frogs of the genus *Polypedates* Tschudi, 1838 from Taiwan: Myers and Kuntz (1970) found *Cosmocercoides pulcher* Wilkie, 1930 from ‘*Polypedates leucomystax* (Gravenhorst, 1829)’, and Yang et al. (2014) reported two nematodes, *Oswaldocruzia hoepplii* Hsü, 1935 and *Cosmocerca ornata* (Dujardin, 1845), and one acanthocephalan, *Pseudoacanthocephalus bufonis* (Shipley, 1903) from *Polypedates megacephalus* Hallowell, 1861, an alien congener. The exact host species in Myers and Kuntz (1970) must be *P. braueri* (see Kuraishi et al., 2011) because the invasion of *P. megacephalus* to Taiwan was first recorded in 2006 (Yang, 2011; Lee et al., 2019). We had an opportunity to examine one individual of *P. braueri* captured in Taiwan and found four helminth species new to this host frog. They are reported herein to supplement the previous data.

MATERIALS AND METHODS

The frog (male, snout-vent length 55 mm) was caught at night in Waishuangxi, Taipei, Taiwan, 26 April 2006. It was anesthetized in 10% ethanol following the guidelines for use of live amphibians and reptiles in field research compiled by ASIH, HL, and SSAR (<http://erenweb.org/wp-content/uploads/2011/07/GUIDELINES-FOR-USE-OF-LIVE-AMPHIBIANS-AND-REPTILES-IN-FIELD-RESEARCH.pdf>). Then, a ventral incision was made on the abdomen to remove the viscera. The alimentary canal and lungs were opened in a 0.6% saline to recover living helminths. Nematodes were fixed in hot (70° C) 70% ethanol while acanthocephalans were pressed between two glass slides and fixed in 70% ethanol at room temperature. They were cleared in a glycerol-ethanol solution (glycerol 5: 70% ethanol 95) by evaporating ethanol and mounted on glass slides with a 50% glycerol aqueous

solution for observation under an Olympus BX50 microscope equipped with a differential interference contrast apparatus. Voucher helminth specimens and the host frog were deposited in the Meguro Parasitological Museum (MPM), Tokyo, and in the Zoological Collection, Kyoto University (KUZ), Kyoto, respectively. Unpublished measurements of *Oswaldocruzia japalurae* collected from saurians on Miyakojima Island, Japan, were also used for comparison.

RESULTS

Three species of nematodes, i.e., *Oswaldocruzia japalurae* Jiang & Lin, 1980, a *Cosmocerca* sp., and *Cosmocercella iwatsukii* Hasegawa, 1989, and one acanthocephalan, *Pseudoacanthocephalus* cf. *bufonis* were collected (Table 1). Short remarks are given for each of the species found:

1. *Oswaldocruzia japalurae* Jiang & Lin, 1980 (Trichostrongyloidea: Molineidae)

This nematode was originally described from a tree lizard, *Diploderma swinhonis* (Günther, 1864), from Taiwan (Jiang and

Lin, 1980). Subsequently, three other lizards of Taiwan, *D. polygonatum xanthostoma* (Ota, 1991), *Plestiodon elegans* (Boulenger, 1887) and *Sphenomorphus indicus* (Gray, 1853), also were reported to harbor this species (Norval et al., 2014). In the Ryukyu Archipelago, it has been found in two lizard taxa, *D. polygonatum ishigakiense* (Van Denburgh, 1912) from Ishigakijima and Miyakojima Islands and in *Scincella boettgeri* (Van Denburgh, 1912) on Miyakojima Island, and also from four frogs, *Polypedates leucomystax*, *Zhangixalus owstoni* (Stejneger, 1907), *Buergeria choui* Matsui et Tominaga, 2020 and *Kurixalus eiffingeri* (Boettger, 1895) of the Yaeyama Islands (Hasegawa and Iwatsuki, 1984; Hasegawa et al., 2018). Such a diversified host preference is not surprising because species of the genus *Oswaldocruzia* often show wide host range encompassing both amphibians and reptiles (see Baker, 1987). Although not mentioned previously, *O. japalurae* is characterized by having well developed cervical alae, being readily distinguished from *O. hoepplii* and *O. insulae* Morishita, 1926, which are also distributed in the Southern Ryukyus (Hasegawa, 1989b; Hasegawa et al., 2018).

TABLE 1. Helminths collected from *Polypedates braueri* of Waishuangxi, Taipei, Taiwan.

Helminth species	No. of worms collected	Site of infection	MPM Coll. No.
Nematoda			
<i>Oswaldocruzia japalurae</i>	8 (4 mature males; 3 gravid females; 1 larva)	Small intestine	21726
<i>Cosmocerca</i> sp.	1 (non-gravid female)	Rectum	21727
<i>Cosmocercella iwatsukii</i>	30 (10 mature males; 1 non-gravid female; 19 larvae)	Rectum	21728
Acanthocephala			
<i>Pseudoacanthocephalus</i> cf. <i>bufonis</i>	2 (2 males)	Small intestine	21729

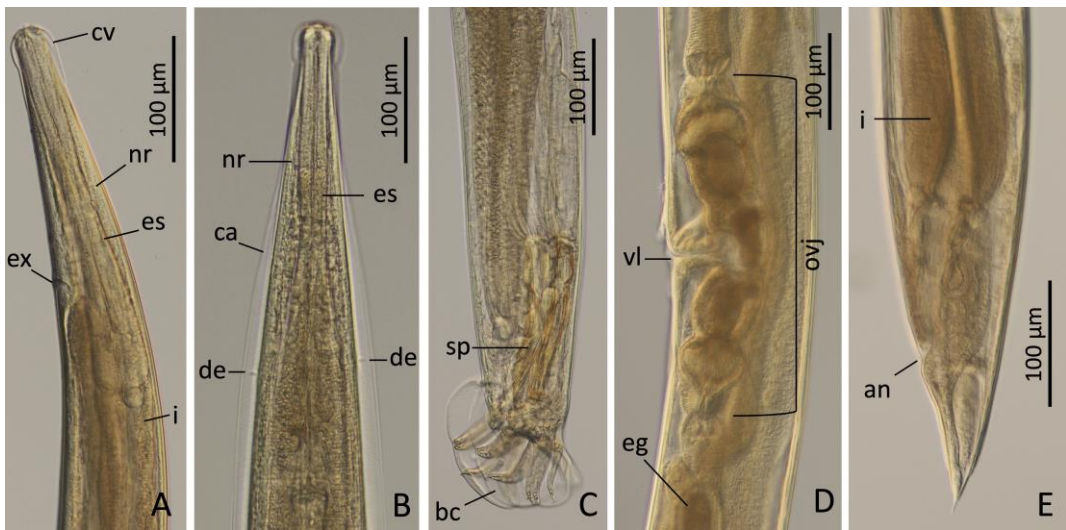


FIGURE 1. *Oswaldocruzia japonica* collected from *Polypedates braueri* of Taiwan. **A, B.** Anterior extremity of male, left lateral (A) and ventral (B) views. **C.** Posterior extremity of male, left lateral view. **D.** Vulval region of female showing ovejector, left lateral view. **E.** Posterior extremity of female, left lateral view. Abbreviations: an, anus; bc, bursa copulatrix; ca, cervical ala; cv, cervical vesicle; de, deirid; eg, egg; es, esophagus; ex, excretory pore; i, intestine; nr, nerve ring; ovj, ovejector; sp, spicule; vl, vulva.

The present worms from *P. braueri* are morphologically identical with the original description and hitherto-known specimens (Figs. 1A – E). Meanwhile, the measurements differed greatly among the worms from the different hosts (Table 2). The worms from tree lizards were larger while those from the skinks were smaller, possibly related to the difference of host body size.

2. *Cosmocerca* sp. (Cosmocercoidea: Cosmocercidae)

Only one female was found. The body was 6.70 mm long and 0.48 mm wide in midbody. The pharynx was 65 µm long × 40 µm wide; the esophageal corpus was 0.55 mm long × 55 µm wide; the isthmus was 55 µm long × 50 µm wide; the esophageal bulb was 0.13 mm long × 0.15 mm wide. Distances from the cephalic apex to the nerve ring, excretory pore and vulva were 0.35 mm, 0.56 mm and 3.60 mm, respectively. The lateral alae were 13 µm

wide, commencing at a distance of 0.17 mm from the cephalic apex and terminating 0.19 mm from the caudal apex. From the Taiwanese frogs and toads, *Cosmocerca ornata* (Dujardin, 1845) and *C. japonica* Yamaguti, 1938 have been recorded (Yamaguti and Mitunaga, 1943; Myers and Kuntz, 1970; Norval et al., 2013a, b; Yang et al., 2014). Species identification of the present material is withheld because no male worms were collected and DNA sequence data were not available (see Discussion below).

3. *Cosmocercella iwatsukii* Hasegawa, 1989 (Cosmocercoidea: Cosmocercidae)

This species was first found in one of 14 individuals of *Zhangixalus viridis* (Hallowell, 1861) of Kunigami, Okinawajima Island (Hasegawa, 1989a), but thereafter no additional worms have been recorded. Hence, this is the second report of *C. iwatsukii*, with *P. braueri* and Taiwan being its new host and locality records, respectively. The present

TABLE 2. Morphometric comparison between Okinawan and Taiwanese specimens of *Oswaldocruzia japalurae* (Mean followed by range in parenthesis in μm unless otherwise stated)

Host	<i>Polypedates braueri</i>	<i>Diploderma swinhonis</i>	<i>Diploderma polygonatum ishigakiense</i>	<i>Scincella boettgeri</i>
Locality	Waishuangxi, Taipei, Taiwan	Taichung, Taiwan	Miyakojima Is., Okinawa	Miyakojima Is., Okinawa
Source	Present study	Jiang & Lin (1980)	Present study	Present study
Male [No. measured]	[4]	[8]	[3]	[3]
Body length, mm	5.13 (4.30 – 6.04)	7.35 – 8.17	6.85 (6.50 – 7.18)	2.59 (2.00 – 3.17)
Body width	126 (107 – 155)	175 – 210	179 (173 – 185)	70 (58 – 88)
Cephalic vesicle length	69 (65 – 73)		69 (58 – 78)	58 (55 – 63)
Cephalic vesicle width	35 (33 – 36)		43 (40 – 45)	36 (35 – 38)
Esophageal length	306 (295 – 325)	350 – 433	407 (400 – 420)	277 (265 – 288) [n = 2]
Esophageal width	47 (45 – 50)		64 (63 – 65)	31 (28 – 33) [n = 2]
Nerve ring*	150 (140 – 163)	154 – 208	175 (168 – 185)	129 (108 – 143)
Deirids*	249 (235 – 270)		273 (255 – 285)	198 (175 – 225)
Excretory pore*	212 (205 – 223)		215 (203 – 223)	171 (143 – 210)
Spicule length	185 (172 – 198)	183 – 252	189 (185 – 193)	139 (129 – 145)
Female [No. measured]	[3]	[6]	[9]	[4]
Body length, mm	7.52 (6.81 – 8.66)	9.52 – 10.74	11.46 (8.68 – 13.60)	6.20 (5.00 – 7.06)
Body width	139 (128 – 145)	168 – 211	174 (145 – 203)	106 (90 – 125)
Cephalic vesicle length	70 (68 – 73)		80 (75 – 85)	65 (63 – 68)
Cephalic vesicle width	36 (33 – 38)		46 (43 – 48)	40 (38 – 41)
Esophageal length	348 (335 – 360)	317 – 400	431 (410 – 450)	346 (325 – 365)
Esophageal width	51 (49 – 53)		67 (60 – 73)	45 (39 – 48)
Nerve ring*	160 (160 – 183)	100 – 167	176 (143 – 198)	167 (155 – 183)
Deirids*	260 (260) [n = 3]		280 (235 – 313)	245 (225 – 265)
Excretory pore*	214 (205 – 220)		234 (213 – 255)	204 (180 – 223)
Vulva**, mm	2.59 (2.27 – 3.12)	3.33 – 3.81	3.84 (2.80 – 4.82)	1.94 (1.50 – 2.35)
Ovejector length	393 (375 – 420)		547 (480 – 640)	365 (350 – 380)
Tail length	112 (96 – 122)		125 (120 – 138)	95 (88 – 100)
Egg	80 (73 – 88)	46 – 62	85 (80 – 90)	84 (80 – 88)
	x 47 (43 – 50)	x 33 – 42	x 47 (45 – 50)	x 46 (43 – 48)

* Distance from cephalic apex ** Distance from caudal apex

worms from *P. braueri* are morphologically much similar to the holotype in original description (Figs. 2A – D), though there are some differences in measurements (Table 3). The body lengths of the male worms from *P. braueri* and *Z. viridis* overlapped though the former tended to be a little smaller. Meanwhile, the body width and the tail length were larger in the males parasitic in *P. braueri* than those in *Z. viridis*. Likewise, the unique female from *P. braueri* had a longer tail (Table 3).

4. *Pseudoacanthocephalus cf. bufonis* (Echinorhynchidae)

The aspinose trunk was spindle-shaped lacking a narrower region in the middle, 9.5–10.1 mm long \times 1.6–1.8 mm wide. The proboscis was cylindrical, 0.55–0.63 mm long \times 0.29–0.31 mm wide, ornamented with 16 hook rows, each composed of 5–6 hooks of which anterior ones the longest. The lemnesci were 1.04–1.18 mm in length, much longer than the proboscis receptacle, which was 0.55–0.63 mm long. The cement



FIGURE 2. *Cosmocercella iwatsukii* collected from *Polypedates braueri* of Taiwan. **A.** Anterior extremity of male, left lateral view. **B.** Posterior extremity of male, left lateral view. **C.** Vulval region of female, left lateral view. **D.** Posterior extremity of female, left lateral view. Abbreviations: an, anus; eb, esophageal bulb; ec, esophageal corpus; ex, excretory pore; i, intestine; ph, pharynx; rp, rosette papillae; sp, spicule; vl, vulva.

glands were 6 in number, forming a cluster. According to the keys proposed by Amin et al. (2008) and Tkach et al. (2013), the present males could be assigned to *Pseudoacanthocephalus bufonis* (Shiple, 1903). This acanthocephalan is distributed widely in Central Asia, Southeast Asia, East Asia and on Pacific islands (Amin et al., 2008; Bush et al., 2009; Tkach et al., 2013). Its presence in the Ryukyus and Taiwan has been already reported (Hasegawa, 1984; Norval et al., 2013a, 2014; Hasegawa and Ota, 2017, Uchida and Ooi, 2018). However, recent DNA sequence analysis suggested presence of cryptic species in so-

called *P. bufonis* (M. Nakao, personal commun. see Hasegawa et al., 2018). In the recent reports, species identification was withheld (Hasegawa et al., 2018; Nagasawa and Nakamura, 2018).

DISCUSSION

The identification of *Cosmocerca* species in the Far East has been confused. Yamaguti (1938) established *C. japonica* but compared it only with *C. parva* (Travassos, 1925), a South American congener. Moreover, he gave only a diagrammatic figure of female

TABLE 3. Morphometric comparison between Okinawan and Taiwanese specimens of *Cosmocerca iwatsukii* (Mean followed by range in parenthesis in μm unless otherwise stated)

Host	<i>Polypedates braueri</i>	<i>Zhangixalus viridis</i>	<i>Polypedates braueri</i>	<i>Zhangixalus viridis</i>
Locality	Waishuangxi, Taipei, Taiwan	Hentona, Kunigami, Okinawajima Is., Japan	Waishuangxi, Taipei, Taiwan	Hentona, Kunigami, Okinawajima Is., Japan
Source	Present study	Hasegawa (1989)	Present study	Hasegawa (1989)
Sex [No. worms measured]	Male [10]	Male [15]	Female [1]	Female [16]
Body length, mm	1.66 (1.58 – 1.75)	1.71 (1.66 – 1.80)	1.92	2.04 (1.96 – 2.15)
Body width	79 (73 – 93)	68 (65 – 70)	90	119 (108 – 130)
Pharynx length	14 (13 – 16)	19 (15 – 21)	15	21 (18 – 25)
Esophageal corpus length	270 (255 – 290)	295 (280 – 330)	322	327 (310 – 340)
Esophageal corpus width	22 (20 – 25)	24 (23 – 25)	28	28 (25 – 30)
Esophageal bulb length	59 (55 – 68)	62 (58 – 63)	70	70 (65 – 73)
Esophageal bulb width	45 (41 – 48)	51 (48 – 55)	53	60 (58 – 63)
Nerve ring*	169 (160 – 178)	154 (143 – 168)	180	167 (153 – 180)
Excretory pore*	259 (248 – 265)	282 (270 – 310)	295	323 (310 – 340)
Spicule length	141 (128 – 153)	135 (128 – 145)	–	–
Vulva*	–	–	1.03	1.16 (1.09 – 1.24)
Tail length	161 (150 – 170)	135 (123 – 143)	220	187 (168 – 203)

* Distance from cephalic apex

reproductive organs along with photomicrographs of female general view and perianal region of a male in lateral view. Apparently, he mistook the gubernaculum as a single spicule, though Travassos (1925) recognized two spicules and a gubernaculum in *C. parva*. Thereafter, *Cosmocerca* nematodes from Japanese amphibians have been identified as *C. japonica* (see Uchida et al., 2019). Some records of this species also have been made from Taiwan, the Philippines and Vietnam (see Baker, 1987). In continental China, Kung and Wu (1945) erected a new genus *Paracosmocerca* with *P. mucronata* Kung & Wu, 1945 as the type species. They also mistook the gubernaculum as a spicule, and differentiated their new genus from *Cosmocerca* by this feature. Apparently, Kung and Wu (1945) did not know the paper by Yamaguti (1938), possibly due to difficulties in scientific exchange between Japan and China during the wartime.

The establishment of *P. mucronata* caused further confusion. In Taiwan, Myers

and Kuntz (1970) recorded both *C. japonica* and *P. mucronata* from *Duttaphrynus melanostictus* (Schneider, 1799) and *Bufo bankorensis* (Barbour, 1908), while they also recorded the latter nematode from *Rana* spp. and *Microhyla fissipes* Boulenger, 1884 but without giving any annotation to justify their identification. Later, Chabaud (1978) pointed out the mistake of the gubernaculum as a spicule by Kung and Wu (1945), and synonymized *Paracosmocerca* with *Cosmocerca*. Subsequently, Baker and Vaucher (1984) synonymized *P. mucronata* with *C. ornata*. They considered that *C. ornata* not only was distributed widely in the Old World, but also in South America. Although they argued the distinguishing characteristics of *C. ornata* are mostly shared with *C. japonica*, they did not refer to Yamaguti's (1938) work at all. Later, Baker (1987) listed *C. japonica* as a valid taxon in his synopsis of parasitic nematodes of amphibians and reptiles. In recent years, Taiwanese specimens of *Cosmocerca* have

been identified as *C. ornata* only (Norval et al., 2013a, b; Yang et al., 2014). Nevertheless, such wide geographical distribution of an amphibian helminth may accompany genetic diversification and isolation. Indeed, DNA sequence analysis of the samples of *C. japonica* from Japanese mainland suggested the presence of cryptic species (Sato et al., 2015). Hence, the systematic reassignment of the Taiwanese and Japanese populations of *Cosmocerca* should be made carefully.

It is of special interest that *Cosmocercella iwatsukii* is distributed both in Taiwan and Okinawajima Island, whereas it has not been recorded from Miyakojima and the Yaeyama Islands, which are situated between those two localities even by recent helminthological surveys on *P. leucomystax* and other frogs from these islands (Hasegawa and Ota, 2017; Hasegawa et al., 2018). Similar distribution also is known for *Batrachonema synaptospicula* Yuen, 1965 and *Pseudabbreviata yambarensis* Hasegawa et Otsuru, 1984 (syn. *Pseudabbreviata nudamphida* sensu Jiang & Lin, 1980 nec Lichtenfels & Quigley, 1968). The former nematode was first described from the peninsular Malaysian frogs (Yuen, 1965) and subsequently from *Fejervarya* cf. *limnocharis* of Southern Taiwan and *Odorrana narina* (Stejneger, 1901) and *Z. viridis* of the northern area of Okinawajima Island (Hasegawa, 1987, 1989a). The latter nematode species has been known only from the tree lizards *D. swinhonis* in Taiwan and *D. p. polygonatum* Hallowell, 1861 of the northern region of Okinawajima Island (Jiang and Lin, 1980; Hasegawa and Otsuru, 1984; Norval et al., 2014). Meanwhile, some helminths, such as *Oswaldocruzia japalurae* and *O. hoepplii*, are common between Taiwan and the Southern Ryukyus, but absent in the Central Ryukyus. It will be important to determine whether these records reflect their actual segregated distributions deriving from

vicariant events, such as the complicated geological history of the Ryukyu Archipelago in relation to Taiwan (e.g., Kizaki and Oshiro, 1980; Ota, 1998), or are mere consequences of insufficiency in appropriate surveys.

Most of the herpetofauna of the Ryukyu Archipelago have their closest phylogenetic affinity with those in southern China and/or Taiwan (Ota, 2000; Yokoyama et al., 2018). Hence, it is not surprising that their helminths also show close affinity with helminths from Taiwanese herps. Nonetheless, it is a bit curious that *C. iwatsukii*, *B. synaptospicula* and *P. yambarensis* have never been reported in hosts from continental China. One congener of *C. iwatsukii* (i.e., *Cosmocercella neveri* Hsü & Hoepplii, 1933) was recorded from a frog, *Quasipaa spinosa* (David, 1875), collected at Amoi of continental China on the opposite side of the strait to Taiwan (Hsü and Hoepplii, 1933), but since this species is morphologically distinct from *C. iwatsukii* by having numerous somatic papillae, distally alate spicules in males, and much smaller eggs in females (Hasegawa, 1989), its conspecificity with *C. iwatsukii* is unlikely. Further surveys in continental China are desirable to confirm phylogenetically closest counterparts of *C. iwatsukii* or other Ryukyu-Taiwan species herein discussed.

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