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Short Note (短　報)

Parasitic Helminths Obtained from Okinawa Rails *Gallirallus okinawae*

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Abstract. A helminthological survey was undertaken on 39 individuals of the Okinawa Rail *Gallirallus okinawae* that had either died following rescue or were collected as carcasses between 2004 and 2006 from Yanbaru region, Okinawa, Japan. A total of seven helminth species, including three nematode (*Heterakis isoloncheu, Strongyloides* sp. and *Skrjabinoclava* sp.), two trematode (*Glaphyrostomum* sp. and *Tanaisia* sp.), an unidentified cestode and an acanthocephalan species (*Plagiorhynchus* sp.) were obtained and identified morphologically. Except for *H. isoloncheu* and *Glaphyrostomum* sp., the other five helminth species were the first records for *G. okinawae*. A significantly higher occurrence of *Tanaisia* sp. was detected for male rails compared to female rails. Since *H. isoloncheu* is known to cause nodular typhlitis and/or granuloma formation in bird guts, monitoring of helminths is recommended in this endangered rail species.

Key words: *Gallirallus okinawae*, New host record, Parasitic helminth.
キーワード：ヤンバルクイナ，新宿主記録，寄生蠕虫類.

The Okinawa Rail *Gallirallus okinawae* is distributed only in the Yanbaru region, in the northern part of Okinawa Island, Japan. The rail is classified as 1A in the endangered species list (CR: Critically Endangered) and is designed as a special natural monument of Japan (Wildlife Division of the Ministry of Environment 2006). Over the last decade, as a result of the artificial development of Okinawa Rail habitat, traffic accidents, and/or predation by feral cats *Felis silvestris catus*, Java mongoose *Herpestes javanicus* and Jungle Crows *Corvus macrorhynchos*, the population of this species has declined to around 1,200 individuals (Ozaki *et al.* 2002, Kotaka & Sawashi 2004, Izawa, 2005). Rehabilitation and translocation of the rails have been implemented by the Japanese Government (Ozaki 2008, Mori *et al.* 2010).

Studies of the distribution, food habits, behavior, morphology, genetic diversity, ectoparasites and viral diseases of the Okinawa Rail have been undertaken (Brazil 1984, 1996, Harato & Ozaki 1993, Ikenaga & Gima 1993, Kuroda 1993, Ozaki *et al.* 2002, 2010, Kotaka & Sawashi 2004, Hiraoka *et al.* 2007, Yamauchi & Ozaki 2007, Neagari *et al.*

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2011). However, despite these efforts, the disease-causing agents of this species are poorly known. In conservation projects that involve the return of wild birds to free-living conditions, health risk assessments are important to avoid detrimental parasitic disease outbreaks (Friend & Franson 1999, Asakawa *et al.* 2002). In such risk assessment processes, it is essential to determine the parasitic agents. A preliminary survey on parasitic nematodes in the Okinawa Rail carried out by Yoshino *et al.* (2008) was based on only six specimens. Thus, between 2004 and 2006 we carried out a detailed survey in order to provide a more-comprehensive database.

A total of 39 Okinawa rails that died following rescue or which were collected as carcasses between 2004 and 2006 in the northern part of Okinawa Island, Japan (26° 45′ N, 128° 15′ E), were investigated. Their bodies were taken to the Laboratory of Intellectual Fundamentals for Environmental Studies, the National Institute of Environmental Studies, Japan, as a part of the Environmental Specimen Time Capsule project (www.nies.go.jp/index-j.html). The bodies were stored in a refrigerator at +4℃ until post-mortem examination. Age class (adult, subadult and juvenile) and sex was recorded following Onuma *et al.* (2011). Visceral organs of each carcass were examined under a binocular microscope. Parasitic helminths obtained were fixed in 70% ethanol. Nematodes and acanthocephalans were cleared in lacto-phenol solution, and trematodes and cestodes were stained with aceto-carmine solution for microscopic observation. Morphological and biometric data were recorded using a camera lucida (OLYMPUS Model BH-2). The occurrence (no. birds with helminths) and mean number of helminths per bird were compared between sexes and age-classes; occurrence was compared using χ^2 test and mean number of helminths per bird was compared using Student's t-test with Microsoft Excel 2000. Differences were considered to be significant at $P<0.05$. Helminth specimens have been deposited in the Wild Animal Medical Center, Rakuno Gakuen University and in the Laboratory of Intellectual Fundamentals for Environmental Studies, the National Institute for Environmental Studies, Japan.

A total of seven helminth species were identified by their measurements and morphological characters (Table 1); three nematodes (*Heterakis isolonche, Strongyloides* sp. and *Skrjabinoclava* sp.), two trematodes (*Glaphyrostomum* sp. and *Tanaisia* sp.), one cestode (unidentified) and one acanthocephalan (*Plagiorhynchus* sp.). The cestode segments were heavily degenerated and we were unable to identify accurately to species. Almost all individuals (97.4% of rails; 38 of 39 examined) were infected by at least one helminth species. *Heterakis isolonche* was the most common (87.2% occurrence). *H. isolonche, Glaphyrostomum* sp. and *Tanaisia* sp. were found in 34, 12 and 12 birds, respectively (Table 1). Among these three common helminth species, *H. isolonche* and *Glaphyrostomum* sp. were found in all age classes and sexes, with no significant differences in their occurrence and mean number of parasites per bird (Table 2). *Tanaisia* sp. was more common in male birds than in females ($\chi^2=4.25$, $P=0.039$, $df=1$, Table 2), but no significant difference was found between adults and non adults (a combination of subadults and juveniles) ($\chi^2=2.01$, $P=0.16$, $df=1$).

Previous studies of parasite taxa of the Okinawa Rail have recorded one nematode (*H. isolonche*), one trematode (*Glaphyrostomum* sp.) and one louse fly (*Ornithoica exilis*)

Table 1. The occurrence (percentage of birds infected) and the number of parasitic helminth species per bird found in the Okinawa Rail.

Species	Infected site*	No. birds infected	Occurrence (%)**	No. of parasites per bird		
				Mean	SD***	Range
Nematoda						
Rhabditida						
Rhabditoidea						
Strongyloididae						
Strongyloides sp.	si	1	2.6	2	—	2
Ascaridida						
Heterakoidea						
Heterakidae						
Heterakis isolonche	si, li, ce, ki	34	87.2	6.5	8.9	1–27
Spirurida						
Acuarioidea						
Acuariidae						
Skrjabinoclav sp.	gi	1	2.6	1	—	1
Trematoda						
Plagiorchida						
Eucotylidae						
Tanaisia sp.	ki	12	30.8	1.2	0.6	1–3
Brachylaimida						
Brachylaimidae						
Glaphyrostomum sp.	si, ce	12	30.8	13.8	21.2	1–70
Cestoda						
unidentified	si	1	2.6	1	—	1
Acanthocephala						
Polymorphida						
Plagiorhynchidae						
Plagiorhynchus sp.	si	2	5.1	1	0	1

* Abbreviations of site; gi: gizzard, si: small intestine, li: large intestine, ce: caeca, ki: kidney.
** A total of 39 birds were examined.
*** (−) means no value.

(Yamauchi & Ozaki 2007, Yoshino *et al.* 2008, Zhao *et al.* 2009). Thus, all the present helminth species, except for *H. isolonche* and *Glaphyrostomum* sp., were the first records for Okinawa Rails.

According to taxonomic reviews and/or keys to the species of the genus *Heterakis* (Skrjabin *et al.* 1961), the present heterakid nematode was identified as *H. isolonche*, based on measurements, in particular for spicule length (nearly equal spicules = 1.3–2.3 mm) and the arrangement of the caudal papillae of the male individuals (Skrjabin *et al.* 1961, Yoshino *et al.* 2008). Several species belonging to the genus *Heterakis*, including *H. isolonche*, have been previously reported from wild and captive birds in Japan (Uchida *et al.* 1991, Sato *et al.* 2005, Ushigome *et al.* 2010, Yoshino *et al.* 2008). *H. isolonche* has been found to inhabit the caecum of pheasants and gallinaceous birds and often causes fatal nodular typhlitis and/or granuloma formation by invading the intestinal wall of

Table 2. Age and sex differences in the occurrence (percentage of birds infected) and the mean number of common helminth parasites per bird for the Okinawa Rail.

Age-classes or sex	No. birds examined	*Heterakis isolonche*			*Glaphyrostomum* sp.			*Tanaisia* sp.		
		No. birds infected	Occurrence (%)	Mean number of parasites per bird	No. birds infected	Occurrence (%)	Mean number of parasites per bird	No. birds infected	Occurrence (%)	Mean number of parasites per bird
Adult	25	21	84.0	4.6	8	32.0	13.6	10	40.0	1.2
Subadult and juvenile	12	11	91.7	11.1	4	33.3	14.7	2	16.7	1
Age-class unidentified	2	2	100.0	1	0	0.0	0	0	0.0	0
Males	23	21	91.3	6.4	7	30.4	18.9	10	43.5	1
Females	16	13	81.3	6.5	5	31.3	6.8	2	12.5	2

infected birds (Balaguer *et al.* 1992, Menezes *et al.* 2003).

Members of the genus *Skrjabinoclava* are acuariid nematodes and include over 20 species which parasitize the Charadriidae, Scolopacidae, Alcedinidae, Rallidae, Cinclidae, and mammals around the world (Wong & Anderson 1987). *Skrjabinoclava cincli, S. halcyoni* and *S. horrida* have been previously recorded in the Brown dipper *Cinclus pallasii*, Ruddy Kingfisher *Halcyon coromanda bangsi* and Whimbrel *Numenius phaeopus* from Japan (Uchida *et al.* 1991, Yoshino *et al.* 2009). *Skrjabinoclava amaurornae* and *S. rallae* have been found in the White-breasted Waterhen *Amaurornis phoenicurus* and the Slaty-breasted Rail *G. striatus*, respectively, in the Philippines (Schmidt & Kuntz 1972). We could not identify the present specimen in this paper, because only one immature female specimen was found. Although the genus *Skrjabinoclava* is known to often cause severe nematodiasis in the digestive tract between the esophagus and the gizzard (Friend & Franson 1999), we found no evidence of any diagnostic glossy region in the digestive tract of Okinawa Rails in this study.

Seven species belonging to the genus *Strongyloides* have been recorded from wild and captive birds (Sakamoto & Sarashina 1968, Speare 1989). In Japan, two species belonging to this genus have been recorded: *S. avium* has been found in the Water Rail *Rallus aquaticus indicus*, and *S. pavonis* in the Green Peafowl *Pavo muticus*, Indian Peafowl *P. cristatus* and Red Jungle-fowl *Gallus gallus domesticus* (Sakamoto & Sarashina 1968, Sakamoto & Yamashita 1970). In this study, *Strongyloides* specimens could not be identified to species level as they were heavily degenerated. No apparent lesions caused by the nematodes were evident, but the pathogenicity of the genus *Strongyloides* is known to result in a thickening of the caecal wall and filling of the caecal lumen with desquamated debris of degenerated tissue, mucous exudates and blood (Sakamoto & Yamashita 1970, Speare 1989).

The trematode genus *Tanaisia* has been reported from the kidneys of the Galliformes, Columbiformes and Passeriformes. *Tanaisia zarudnyi* has been reported from the Eurasian Jay *Garrulus glandarius* and Grey Bunting *Emberiza variabilis* in Japan (Uchida *et al.* 1991). The pathogenicity of this genus is not well known, but the closely related

genus *Paratanaisia* often causes granulomatous nephritis (Luppi *et al.* 2007). The genus *Glaphyrostomum* has been reported from mammals and birds from China, Taiwan, the Philippines and Japan. Among birds, *G. garrulum, G. hunanensis, G. minor* and *G. taiwanensis* have been reported from the Melodious Laughing-thrush *Garrulax canorus; G. hunanensis* and *G. sanguinolentum* from the Red-billed Leiothrix *Leiothrix lutea; G. capellae* from the Needle-tailed Snipe *Gallinago megala; G. rallinarum* from the Philippine Banded-crake *Rallina eurizonoides eurizonoides;* and *Glaphyrostomum* sp. from the Okinawa Rail (Fischthal & Kuntz 1976, Wang 1982, Qiu *et al.* 1999, Eduardo & Gaddi 2004, Zhao *et al.* 2009).

The genus *Plagiorhynchus* has been recorded from several bird species. In Japan, *Plagiorhynchus* (*Prothorhynchus*) *charadrii* and *P.* (*P.*) *malayensis* have been found in the Little Ringed Plover *Charadrius dubius* and White-breasted Waterhen, respectively (Uchida *et al.* 1991, Yoshino *et al.* 2009). Acanthocephalans, including the genus *Plagiorhynchus*, are regarded as highly pathogenic agents responsible for severe, and sometimes fatal, enteritis in various wild and captive birds (Friend & Franson 1999, Asakawa *et al.* 2002).

Although no evidence of pathogenicity or mortality directly attributable to helminth infection was found in the present survey, it is known that birds subjected to stress (e.g. capture, deterioration of habitat) are more susceptible to infectious agents, including parasites, than areunstressed wild individuals (Friend & Franson 1999, Asakawa *et al.* 2002). Thus, ongoing and future efforts toward conserving the Okinawa Rail must include more detailed surveys and monitoring of infectious agents, including parasites, notably helminth infections, and pathogenicity and epidemiological aspects. As we could not identify some specimens morphologically, genetic identification is required.

Among the three helminth species recorded, the occurrence and the mean number of each helminth species did not differ significantly with the age classes and sex of the hosts. Interestingly, *Tanaisia* sp. occurred more frequently in male birds. Trematodes belonging to the genus *Tanaisia* require terrestrial snails as an intermediate host (Luppi *et al.* 2007). Hiraoka *et al.* (2007) and Ozaki (2008) reported bradybaenid snails, such as *Bradybaena circulus,* from the stomach of Okinawa Rails. Sex difference in the occurrence of *Tanaisia* might indicate a sex difference in the intake of snails, though more detailed studies of food habits of *G. okinawae* that conside age, seasonal changes and location are required to confirm this.

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ヤンバルクイナ *Gallirallus okinawae* から得られた寄生蠕虫類

2004年から2006年にかけて，沖縄島北部各地にて保護収容後に死亡，あるいは死体として回収されたヤンバルクイナ *Gallirallus okinawae* 計39個体について寄生虫学的調査を実施した。その結果，線虫3種 (*Heterakis isolonche*, *Strongyloides* sp., *Skrjabinoclava* sp.)，吸虫2種 (*Glaphyrostomum* sp., *Tanaisia* sp.)，条虫1種（属種不明）および鉤頭虫1種 (*Plagiorhynchus* sp.) の計7種の寄生蠕虫類を得た。*H. isolonche* および *Glaphyrostomum* sp. を除く5種は新宿主記録であった。*Tanaisia* sp. の寄生率はオスの方がメスに比べて高かった。*H. isolonche* は結節性，肉芽腫性腸炎の原因となることが知られており，絶滅危惧種であるヤンバルクイナの保護管理

上，今後寄生虫のモニタリングが必要であると考えられた。

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