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## Parasitic nematodes obtained from marsupials reared at a semi-free ranging facility in a Japanese zoological park

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#### **Abstract**

Between 2006 and 2010, a total of 12 semi-free ranging marsupials comprising 10 *Petrogale xanthopus* and two *Macropus giganteus* died at the Itozu no mori Zoological Park (Kitakyushu, Fukuoka, Japan). From our postmortem examinations, 541 nematodes in total were obtained from the stomachs of the deceased marsupials. The nematodes belonged to the subfamily Cloacininae (Strongylida: Chabertiidae). The nematodes obtained from *P. xanthopus* were identified as *Rugopharynx australis*, *Cloacina pearsoni*, *Cloacina hydriformis* and *Macroponema beveridgei*, while the nematode from *M. giganteus* was identified as a Cloacina sp. member. This is the first record of *C. pearsoni* and *M. beveridgei* obtained from *P. xanthopus*. Measurements and photographs of the nematodes are provided herein to assist future continuous surveillance of them.

Keywords: Cloacininae, Japan, Macropus, Petrogale, semi-free ranging marsupials

#### Introduction

Many helminthological reports have focused on nematodes in free ranging marsupials from Australia since the 1930s (Beveridge and Chilton, 1999; Beveridge et al., 1985; Hoste and Beveridge, 1993; Johnston and Mawson, 1938, 1940; Pamment et al., 1994; Spratt et al., 1991). However, no cases of nematode infections in semi-free ranging marsupials have been reported in Japan, with the exception of our preliminary report on heavy strongylid infections, as was determined by fecal examinations (Sotohira et al.,2016). Better understanding of the helminthological background of captive marsupials is an important prerequisite for maintaining their health. Therefore, we present here the results of a direct survey of parasitic nematodes from marsupials kept in a zoological park in Kyushu, Japan.

#### **Materials and Methods**

Between 2006 and 2010, 12 semi-free ranging marsupials, 10 Petrogale xanthopus, and 2 Macropus giganteus died in the Itozu no mori Zoological Park, Postmortem Kitakyushu, Fukuoka, Japan. examinations were performed for all these cases. As a result, parasitic nematodes were observed in the stomachs of all the marsupials we examined, and a total of 541 nematode specimens were obtained (Fig. 1). The parasitic nematodes were fixed and preserved in 70 % ethanol, and the specimens were examined microscopically in lacto-phenol solution. Nematode measurements and drawings of them were done with the aid of a camera lucida, OLYMPUS Model BH2-DA. The specimens have been deposited in the Wild Animal Medical Center (WAMC), Rakuno Gakuen University, Hokkaido, Japan (WAMC-As-Nos. 9115-9121, 9565-9567, 9897 and 10363).

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Fig.1. A case of heavy parasitism by nematodes in the stomach of a *Petrogale xanthopus* kept in Itozu no mori Zoological Park.

#### **Results and Discussion**

According to the keys (Johnston and Mawson, 1940; Skryabin et al, 1992; Anderson et al., 2009), the nematodes were identified as Rugopharynx, Cloacina and Macroponema of the subfamily Cloacininae (Strongylida: Chabertiidae). Anderson et al. (2009) reported that the infection mode of these three nematode genera is a direct one via the intake of ova, but they are not able to parasitize non-marsupials. Some case reports on parasitic diseases caused by Rugopharynx spp. and Cloacina spp. appear in the scientific literature (Beveridge et al., 1985; Davis et al., 2008; Vogelnest and Woods, 2008). These reports have pointed out that veterinarians, keepers, and managers in zoos should be alert to the risk and symptoms of infection with these nematodes, even in cases that lack histopathological changes, as was noted for the present

cases (Sotohira, unpublished data). Therefore, we present here the measurements of the nematodes (in mm) and photographs of their anterior and posterior extremities to assist positive identification and future continuous surveys of them, or for monitoring animal health (Figs 2–6). Up to now, *Rugopharynx australis* and *Cloacina hydriformis* have been recorded in *P. xanthopus* (Beveridge and Presidente, 1978; Beveridge et al., 1989, 2002; Bradley et al., 2000; Mawson, 1961; 1978; Wood, 1929), but this is the first recording of *C. pearsoni* and *M. beveridgei* from *P. xanthopus*.

Rugopharynx australis (Fig. 2). Male (N=10): Body 6.2–7.6 (av. 6.76) in length, 0.3–0.4 (av. 0.37) in width, pharynx 0.05 in length, esophagus 0.8–0.9 (av. 0.82) in length, spicules, equal, 1.5–1.9 (av. 1.71). Female (N=10): Body 7.6–10.4 (av. 9.00) in length, 0.4–0.5 (av. 0.48) in width, pharynx 0.05 in length, esophagus 0.9–1.0 (av. 0.93) in length.

Cloacina pearsoni (Fig. 3). Male (N=9): Body 7.1–10.3 (av. 8.63) in length, 0.3–0.5 (av. 0.41) in width, pharynx 0.03 in length, esophagus 0.5–0.6 (av. 0.52) in length, spicules, equal, 2.0–2.2 (av. 2.10). Female (N=10): Body 8.9–14.2 (av. 11.89) in length, 0.4–0.7 (av. 0.54) in width, pharynx 0.03 in length, esophagus 0.5–0.6 (av. 0.55) in length.

Cloacina hydriformis (Fig. 4). Male (N=1): Body 5.85 in length, 0.36 in width, pharynx 0.02 in length, esophagus 0.54 in length, spicules, equal, 1.37–1.39.

Female: Not obtained. Macroponema beveridgei (Fig. 5). Male (N=9): Body

11.0–15.0 (av. 12.35) in length, 0.23 in width, pharynx 0.04 in length, esophagus 0.7–0.9 (av. 0.79) in length, spicules, equal, 1.1–1.3 (av. 1.18). Female (N=10): Body 17.8–26.0 (av. 20.70) in length, 0.2–0.3 (av. 0.27) in width, pharynx 0.04 in length, esophagus 0.8–1.0 (av. 0.87) in length.

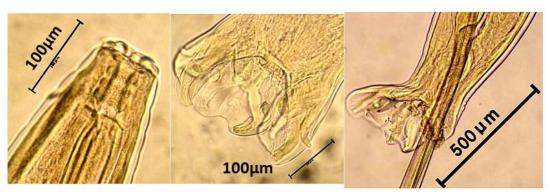


Fig. 2. Male Rugopharynx australis from Petrogale xanthopus kept in Itozu no mori Zoological Park. Head end (left), ventral view (middle) and sub-lateral view (right) of bursa.

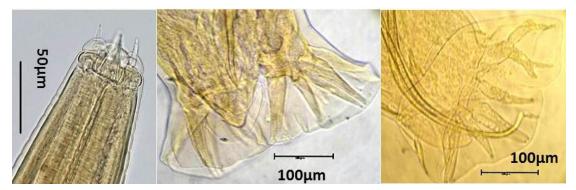


Fig. 3. Male *Cloacina pearsoni* from *Petrogale xanthopus* kept in Itozu no mori Zoological Park. Head end (left), ventral view (middle) and sub-lateral view (right) of bursa.

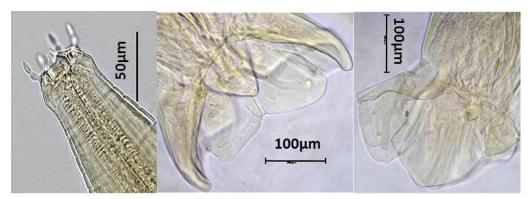


Fig. 4. Male *Cloacina hydriformis* from *Petrogale xanthopus* kept in Itozu no mori Zoological Park. Head end (left), ventral view (middle) and sub-lateral view (right) of bursa.

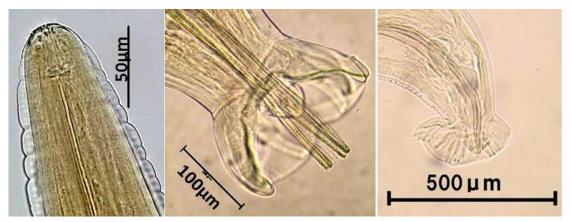


Fig. 5. Male *Macroponema beveridgei* from *Petrogale xanthopus* kept in Itozu no mori Zoological Park. Head end (left), ventral view (middle) and sub-lateral view (right) of bursa.

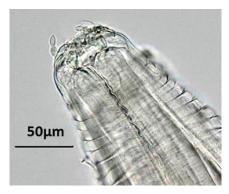


Fig. 6. Head end of female Cloacina sp. from *Macropus giganteus* kept in Itozu no mori Zoological Park.

Only one species of *Cloacina* nematode (Fig. 6) was obtained from *M. giganteus*. Species-specific naming was not possible because of the lack of a male specimen. Body 15.8–19.7 (av. 17.94) in length, 0.8–1.0 (av. 0.93) in width, pharynx 0.03 in length, esophagus 0.6–0.7 (av. 0.63) in length.

Based on their behavioral characteristics, captive large-sized marsupials are often kept in semi-free-ranging facilities, and one example of where this occurs is the Whipsnade Wild Animal Park in the UK. Itozu no mori Zoological Park in Japan also keeps

such animals in captivity (Asakawa, 2010). However, in Japan, no nematological surveys have been performed in semi-free ranging type facilities, with the exception of one survey conducted on the captive felids kept in a Japanese safari park (Hashimoto et al., 2015). Hence, the present survey is an important one for the Japanese zoological medical field.

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#### 和文要旨

国内動物園において半自然状態で飼育され斃死した有 袋類から検出された寄生線虫類

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2006年から2010年の間に北九州市到津の森公園「ひび き動物ワールド」で、半自然状態下で飼育され、斃死し た有袋類、シマオイワワラビーPetrogale xanthopus 10 個体およびオオカンガルー Macropus giganteus 2 個体 の2種12個体の剖検の結果、全検体の胃腔内から計541 個 体 の Cloacininae 亜 科 (Strongyloidea: Chabertiidae) に属する線虫類が検出された。シマオイ ワワラビーから検出された個体は、Rugopharynx australis, Cloacina pearsoni, Cloacina hydriformis および Macroponema beveridgei と同定された。また、 オオカンガルーから検出された個体は Cloacina sp. と 同定された。なお、C. pearsoni と M. beveridgei の 寄主としてシマオイワワラビーは、本研究による新たな 記録である。今後の継続的な疫学調査の一助とするため、 今回同定されたすべての線虫類の計測値と形態的な特 徴を明示した写真を掲載した。

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