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## A new species of *Potoroxyuris* (Nematoda: Oxyuridae) from the woylie *Bettongia penicillata* (Marsupialia: Potoroidae) from southwestern Australia



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### ABSTRACT

*Potoroxyuris keninupensis* n.sp. (Nematoda: Oxyuridae) is described based on specimens recovered from the caecum and colon of two woylies, *Bettongia penicillata* (Marsupialia: Potoroidae) from Western Australia.

Only one other species of *Potoroxyuris* has been described previously, *Potoroxyuris potoroo* (Johnston and Mawson, 1939) Mawson, 1964, from *Potorous tridactylus*. The new species is most easily differentiated from *P. potoroo* by the shape of the pharyngeal lobes. The pharyngeal lobes of *P. keninupensis* n. sp. are widest at the base while those of *P. potoroo* are widest at the tip.

The genus *Potoroxyuris* most closely resembles *Macropoxyuris* based especially on structures of the caudal end of males. The other three genera of oxyurids known to infect Australian marsupials have longer caudal alae, and more caudal papillae than these two genera. The genus *Potoroxyuris* has previously been defined by the characteristic that the pharyngeal lobes protrude through the oral opening. However, the pharyngeal lobes of *P. keninupensis* n. sp. do not quite protrude, so the definition of the genus should be modified as follows. The genus *Potoroxyuris* can be easily differentiated from *Macropoxyuris* by the following differences in the morphology of the buccal cavity. The pharyngeal lobes of *Potoroxyuris* almost reach the oral opening, or protrude beyond it, whereas those of *Macropoxyuris* only reach to about the anterior third of the buccal cavity. The buccal cavity of *Potoroxyuris* is poorly cuticularized compared to *Macropoxyuris* and other genera of oxyurids known from Australian marsupials, and does not contain inter-radial lamellae.

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### 1. Introduction

The woylie or brush-tail bettong (*Bettongia penicillata*) was present across most of southern Australia until European settlement, but by the 1960s the distribution was reduced to three isolated areas in the southwest of Western Australia (Wayne et al., 2013). Woylie populations underwent a sudden decline beginning around 2001 even though there was not a concurrent increase in predators (Wayne et al., 2013), and the species is currently listed as critically endangered (IUCN, 2015). As part of an ongoing collaborative project by the Western Australian Department of Parks and Wildlife to protect the species, woylies that had died in wildlife rescue enclosures were sent to Murdoch University for post-

mortem examination. Two of those were found to be infected with a new species of *Potoroxyuris* which is described herein.

The genus *Potoroxyuris* (Nematoda: Oxyuridae) was created by Mawson (1964) for a species described from the long-nosed potoroo (*Potorous tridactylus*) from eastern Victoria by Johnston and Mawson (1939). This species has pharyngeal lobes that protrude from the oral opening. This characteristic was the only one that Petter and Quentin (1976) used to differentiate the genus from other oxyurids found in Australian marsupials, although they used the term pharyngeal tooth rather than pharyngeal lobe. The new species from *Bettongia penicillata*, while very close morphologically to the type species, has pharyngeal lobes which do not quite protrude from the oral opening, so the diagnosis of the genus *Potoroxyuris* needs to be modified as follows. The genus can be differentiated from other oxyurids by the absence of a cuticularized buccal capsule, by having pharyngeal lobes which reach almost to the oral opening or protrude through it, by the absence of inter-

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radial lamellae in the buccal cavity, and by the absence of a gubernaculum in males.

## 2. Materials and methods

### 2.1. Specimen collection

#### 2.1.1. Case 1

Adult male (ear tag number K1340/K1335) was found dead in its enclosure at Native Animal Rescue (NAR) in Malaga, a suburb of Perth WA (31.87S, 115.89E), on 20 June 2011 and taken to Murdoch University for post-mortem examination (Pathology 11/0347). The animal originally came from a captive breeding colony on a private property in Roleystone (32.12S, 116.08E), a suburb of Perth, and while at NAR was placed in an enclosure with animals sourced from Manjimup, in the southwest of WA. Faeces and gut contents were sent to the Parasitology group at Murdoch University where the adult oxyurids were collected from the caecum and colon and fixed in glycerine alcohol (5% glycerine in 70% ethanol) for identification. These specimens have been deposited in the South Australian Museum.

#### 2.1.2. Case 2

Adult male (ear tag number WC27/41) was transferred from a wild population at Keninup, WA (33.94S, 116.57E) to NAR on 11 November 2010. Keninup is a forest location close to Keninup Creek. The woylie died on 5 June 2012 and was taken to Murdoch University for post-mortem examination. Oxyurids from the caecum and colon were fixed in glycerine alcohol. The remaining caecum and contents were fixed in glycerine alcohol and nematodes were removed at a later date. These specimens have been deposited in the South Australian Museum.

### 2.2. Other specimens

All of the other *Potoroxyuris* spp. specimens examined were from the South Australian Museum, listed under Australian Helminth Collection (AHC) numbers as follows.

AHC 41375. *Potoroxyuris potoroo* (Johnston and Mawson, 1939) Mawson, 1964 type specimens, from *Potorous tridactylus*, South Gippsland, Victoria. The original description did not include males (Johnston and Mawson, 1939), but subsequently, males were found and included in the re-description (Mawson, 1964). The only vial of type specimens in the collection included just two female specimens, only one of which was gravid, and no males were found.

AHC 13954. One male and two female specimens of *P. potoroo* from *P. tridactylus*, Hobart, Tasmania. Neither the name of the collector nor the date of collection were available.

AHC 5027. 10 male and 13 female specimens of *P. potoroo* from *P. tridactylus*, near Hobart, collected by (V.V.?) Hickman. The collection date was unavailable. These specimens were extracted by the authors from fixed caecal contents, and are now stored under the accession numbers AHC 47720 and AHC 36248.

AHC 31538. Three male and three female specimens of *Potoroxyuris* sp., from *Potorous longipes*, Bendoc, Victoria, collected by M. Mitchell, 20 Jul 2001, were examined.

In addition, specimens of *Macropoxyuris* spp. donated to the Murdoch University Parasitology collection (X01/62) by Ian Beveridge, from a western grey kangaroo (*Macropus fuliginosus*) from Waroona WA, 10 December 2001, were examined for comparison. Two of these specimens were cleared and identified by one of the authors (RPH) as *Macropoxyuris brevigularis* Mawson, 1964.

### 2.3. Specimen preparation and processing

Specimens were placed in a small quantity (approximately 200  $\mu$ l) of fresh glycerine alcohol in glass embryo blocks. Additional glycerine was added to clear the specimens, and gently mixed, one drop at a time, ensuring that the specimens did not collapse due to rapid osmotic pressure change. The ethanol was allowed to evaporate off over the course of a day to a week, then the specimens were mounted on glass slides in the remaining glycerine, making sure that paper or glass spacers were included under the cover slip to prevent squashing.

The head ends of some specimens were cut off, mounted on slides in Hoyer's medium, and arranged to sit mouth up as *en face* mounts.

Photographs were taken using an Olympus BX50 microscope with an Olympus DP71 camera. Measurements were made either directly using an Olympus BH microscope equipped with an ocular micrometer, or from photographs, using ImageJ software (<http://imagej.nih.gov/ij/>). Drawings were made using open-source Inkscape software ([www.inkscape.org](http://www.inkscape.org)) over layered photographs as a guide.

Three specimens from *B. penicillata* Case 1 were dehydrated to absolute ethanol, critical-point dried via carbon dioxide, and sputter-coated in gold for visualization at 5 kV by a Zeiss Supra field emission scanning electron microscope at the Centre for Microscopy, Characterisation and Analysis at the University of Western Australia.

## 3. Results

### 3.1. Description

#### 3.1.1. Morphology

*Potoroxyuris keninupensis* n. sp.  
(Figs. 1.1–1.8, 2A–C and 3).

Worms small and slender, with faint transverse cuticular striations (see Fig. 3A). Lateral alae reduced to very small ridges along most of body. Oral opening triangular with 3 cuticular lappets. Mouth collar present between oral opening and papillae, with wrinkled zone along posterior edge of ventral half (see Fig 3A–B). Two pairs of cephalic submedian papillae present, quite close to lateral amphids. Buccal cavity not well defined, almost filled by 3 dome-shaped pharyngeal lobes. Pharyngeal lobes reach oral opening but do not project beyond it. Each lobe widest near its base, with smoothly pointed projection on each side. Nerve ring one quarter the length of oesophagus from anterior end. Oesophagus with constriction anterior to spherical bulb. Excretory pore usually slightly posterior to junction of oesophageal bulb and intestine.

3.1.1.1. *Males* ( $n = 13$ ). Measurements are shown in Table 1. The holotype male was from the Case 2 host. Oesophagus length approximately 20% of body length. Mid-ventral pre-cloacal cuticular ridge present, cuticle expanded in region of cloaca. Tail tapers to fine point posterior to cuticular expansion. One pair of large pedunculate papillae present on expanded lateral edge of the body, near level of cloaca. Two pairs of lateral mounds present within 10  $\mu$ m of cloacal opening. Anterior mound without papillae, posterior mound with 2 sessile papillae very close to each other. Internal duct present between posterior mound and large lateral pedunculate papilla. Spicule poorly sclerotized, with rounded head and very finely pointed tip. Gubernaculum absent.

3.1.1.2. *Females* ( $n = 11$ ). Measurements are shown in Table 2. The allotype female was from the Case 2 host. Oesophagus length

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