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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.

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 µl) 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) 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 µm 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

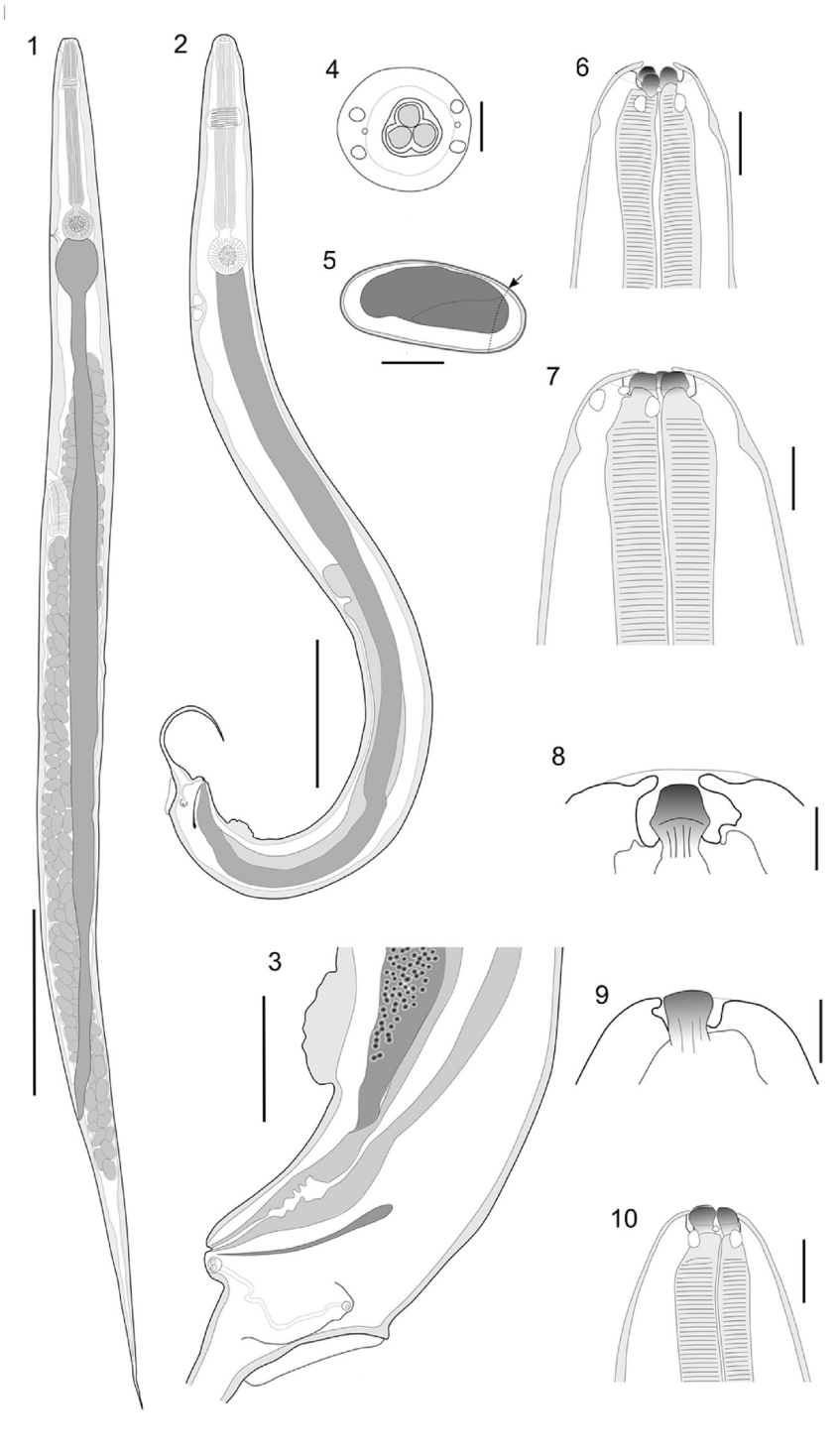

Fig. 1. 1–10. Line drawings of *Potoroxyuris keninupensis* n. sp. (1–8) and *P. potoroo* (9–10). (1) whole female, lateral view, (2) whole male, lateral view, (3) cloacal region of male, lateral view, (4) *en face* view of female, (5) egg from uterus, with the position of the operculum indicated by an arrow, (6) anterior end of male, lateral view, (7) anterior end of female, lateral view, (8) detail of pharyngeal lobe of female, (9) detail of pharyngeal lobe of female *P. potoroo* (AHC 47720), (10) anterior end of female *P. potoroo* (AHC 47720). Scale bars: (1): 500 μm, (2): 200 μm, (3): 40 μm, (4–7) and (10): 20 μm, (8–9): 10 μm.

approximately 15% of body length. Vulva opens approximately one third of body length from anterior end. Tail tapers gradually to fine point. Uterus extends anteriorly beyond level of vulva but not as far as oesophageal bulb, and posteriorly into tail region. Eggs smooth to slightly mammilated, with operculum at one end.

3.1.2. Etymology

The species is named after the geographic origin of the host in which the holotype and allotype specimens were collected.

3.1.3. Specimens deposited

The holotype and allotype specimens from the Case 2 host have

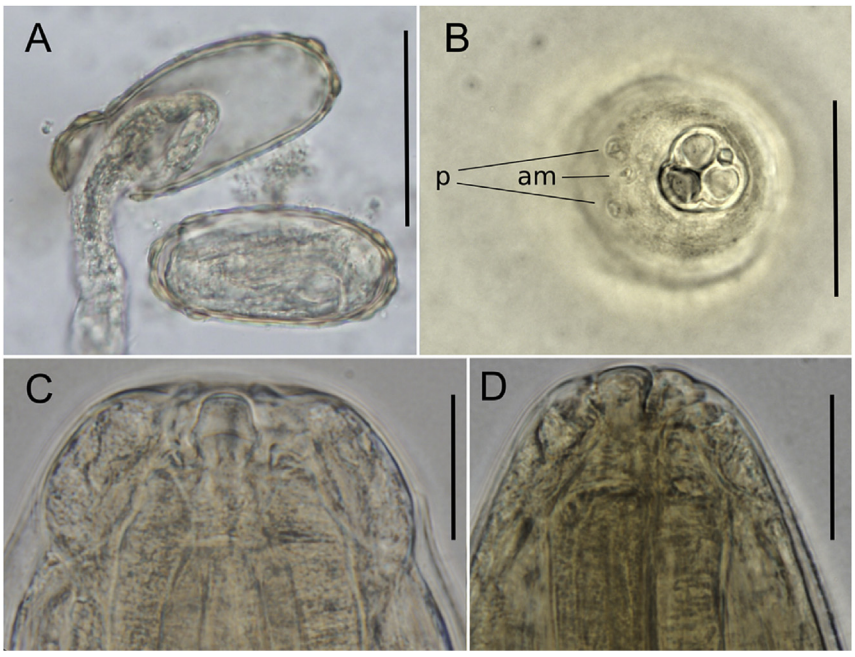

Fig. 2. Photomicrographs of *Potoroxyuris keninupensis* n. sp. and *P. potoroo*. (A) eggs of *P. keninupensis* n. sp. from faeces, one with the operculum open. (B) *En face* view of a female specimen of *P. keninupensis* n. sp. showing pharyngeal lobes. The amphid (am) and both submedian papillae (p) are in focus on the left side of the oral opening. The granule to the right of the dorsal lobe is an artefact. (C) Dorso-lateral optical section of a ventro-lateral pharyngeal lobe of allotype female *P. keninupensis* n. sp. (D) Lateral view of a ventro-lateral pharyngeal lobe of a female *P. potoroo* from AHC 47720. Scale bars: (A–B) 50 μm, (C–D) 20 μm.

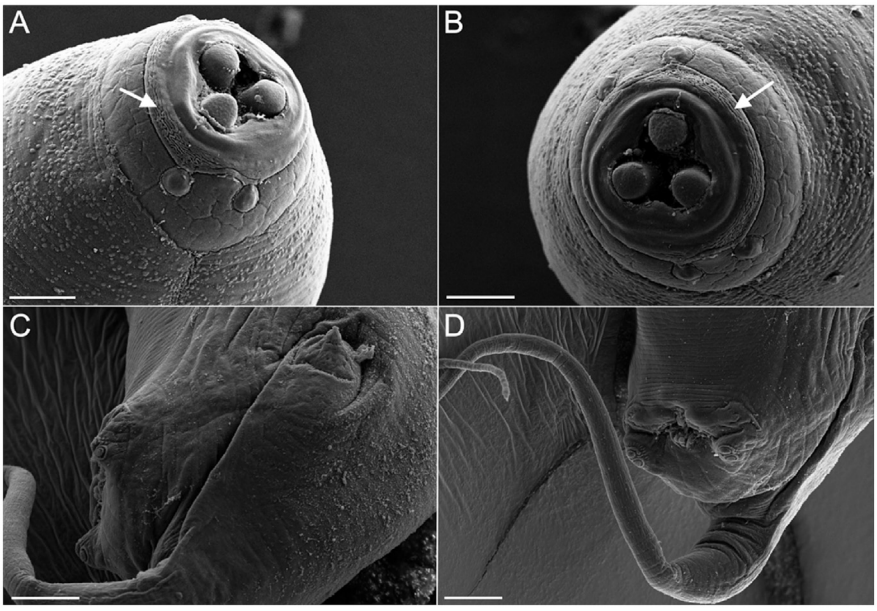

Fig. 3. Scanning electron micrographs of specimens of *Potoroxyuris keninupensis* n. sp. (A–B) Female anterior end showing submedian papillae, amphids, mouth collar and pharyngeal lobes. Arrows indicate the ventral wrinkled zone on the posterior edge of the mouth collar. (C–D) Male caudal region showing caudal papillae and cloacal opening. Scale bars all 10 μm.

been deposited at the South Australian Museum (SAM) under the collection numbers AHC 47714 and AHC 47715 respectively. Paratypes from the Case 2 host have been deposited under the collection numbers AHC 47716, AHC 47717, AHC 36234 and AHC 36235. Paratypes from the Case 1 host have been deposited under the collection numbers AHC 36236, AHC 47718 and AHC 47719.

4. Discussion

Five genera of oxyurid nematodes have been described from marsupials in Australia. *Austroxyuris* Johnston and Mawson, 1938 occurs in Pseudocheiridae, *Adelonema* Mawson, 1978 in Phalangeridae, *Paraustroxyuris* Mawson, 1964 in Pseudocheiridae and perhaps Phalangeridae, *Macropoxyuris* Mawson, 1964 in Macropodidae and *Potoroxyuris* in Potoroidae (Spratt et al., 1991).

Table 1
Measurements of male *Potoroxyuris* spp. All measurements are in micrometres (μm) unless otherwise indicated. The Australian Helminth Collection (AHC) numbers are shown in the parasite species column.

Parasite species	Host species		Total length (mm)	Maximum width	Oesophagus length	Bulb width	Nerve ring from anterior	Excretory pore from anterior	Spicule length	Tail spike length	Mouth-anus (mm)	Pharyngeal lobe width at widest
Potoroxyuris keninupensis n. sp.	*Bettongia penicillata*, WA	Holotype	1.65	92	314	47	91	365	61	162	1.46	6.5
		Mean	1.51	87.4	306.2	50.8	90.2	327.4	68.4	167.5	1.3	6.8
		Range	1.20–1.95	75–100	256–340	47–55	75–107	240–405	43–96	146–225	1.03–1.74	6.1–7.4
		n	13	12	13	12	13	10	10	13	13	11
Potoroxyuris potoroo, from Table 4 in Mawson (1964)	*Potorous tridactylus*, South Gippsland, Vic	Mean	1.4	80	316		95	294	56	185		
		Range	1.3–1.4	70–90	310–320		85–100	280–300	50–60	170–210		
		n	5	5	5		5	5	5	5		
Potoroxyuris potoroo, AHC 13954 & 47720	*Potorous tridactylus*, Hobart Tas	Mean	1.35	74.6	305.9	44.9	87.2	271.8	–	163.1	1.15	6.2
		Range	1.25–1.59	65–82	290–320	42–48	78–100	204–310	–	121–199	0.99–1.38	5.9–6.7
		n	9	11	11	11	11	10	–	9	11	7
Potoroxyuris potoroo (?), AHC 31538	*Potorous longipes*, Bendoc, Vic	Mean	1.47	78.3	346.7	43.3	88.0	–	–	125	–	6.6
		Range	1.42–1.56	72–83	325–360	42–45	85–92	–	–	–	–	6.2–6.8
		n	3	3	3	3	3	–	–	1	–	3

Table 2
Measurements of female *Potoroxyuris* spp. All measurements are in micrometres (μm) unless otherwise indicated. The Australian Helminth Collection (AHC) numbers are shown in the parasite species column.

Parasite species	Host species		Total length (mm)	Maximum width	Oesophagus length	Bulb width	Nerve ring from anterior	Excretory pore from anterior	Vulva from anterior	Tail length	Pharyngeal lobe width at widest	Egg length	Egg width
Potoroxyuris keninupensis n. sp.	*Bettongia penicillata*, WA	Allotype	3.74	185	530	85	120	520	1275	800	9.6	65	32
		Mean	3.36	179.6	482.9	77.5	121.5	492.1	1086.5	712.9	9.8	62.6	30.1
		Range	2.72 –3.82	160–195	440–530	65–87	103–145	375–580	870 –1275	580 –800	8.7–10.5	55 –68	28 –33
		n	11	11	11	11	11	11	10	11	9	33	33
Potoroxyuris potoroo, from Table 4 in Mawson (1964)	*Potorous tridactylus*, South Gippsland Vic	Mean	2.3	113	446		110	404	889	530			
		Range	2.2–2.5	90–130	420–450		100–120	370–460	810 –1000	480 –600		45 –52	20 –28
		n	10	10	10		10	10	10	10		?	?
Potoroxyuris potoroo, Syntypes AHC 41375	*Potorous tridactylus*, South Gippsland Vic	Mean	2.02	102.5	407.5	53.5	93.5	365	790	400	6.3	49.8	26.0
		Range	1.85 –2.18	100–105	400–415	50–57	92–95			360 –440	6.1–6.5	48 –53	24 –28
		n	2	2	2	2	2	1	1	2	2	6	6
Potoroxyuris potoroo, AHC 47720 & 13954	*Potorous tridactylus*, Hobart Tas	Mean	2.50	133.5	458.6	66.9	99.6	356.1	827.5	679.5	7.7	48.1	23.9
		Range	2.21 –2.78	117–150	415–511	60–73	85–120	290–435	706 –1050	400 –878	7.1–8.7	45 –52	21 –26
		n	15	15	15	15	14	14	14	13	14	45	45
Potoroxyuris potoroo (?), AHC 31538	*Potorous longipes*, Bendoc, Vic	Mean	2.70	137.7	483.3	62.7	108.3	590	980	680	7.5	46.4	24.3
		Range	2.66 –2.72	130–143	480–485	60–65	88–125				7.0–7.7	44 –48	22 –26
		n	3	3	3	3	3	1	1	1	3	9	9

In our opinion, based on descriptions of the structure of male posterior ends (Johnston and Mawson, 1938; Mawson, 1964, 1978), *Potoroxyuris* most closely resembles *Macropoxyuris*. The other three genera have longer caudal alae and a greater number of caudal papillae. Male *Macropoxyuris* specimens seen in this study closely resembled the original description of *M. brevigularis* Mawson, 1964. *Potoroxyuris* is easily differentiated from *Macropoxyuris*, by the morphology of the buccal cavity and pharyngeal lobes. The buccal cavity in species of *Potoroxyuris* is shallower, very weakly sclerotized and does not have inter-radial lamellae as species of *Macropoxyuris* do. The pharyngeal lobes of species of *Potoroxyuris* are smooth, without protuberances, reaching almost to or extending through the oral opening, whereas the pharyngeal lobes of species of *Macropoxyuris* do not project into the anterior third of the buccal capsule, are mammilated and possess anterior processes.

Specimens of *Potoroxyuris* seen in this study from *P. tridactylus* in Tasmania do not appear to differ in size or morphology from the redescription of *P. potoroo* in Mawson (1964) or from the 2 remaining female type specimens of *P. potoroo* seen in this study.

Specimens of *P. keninupensis* n. sp., particularly the females, are much larger than those of *P. potoroo*, as are the eggs. Most of the male specimens of *P. potoroo* available to us had tightly curved posterior ends, making details of the papillae arrangement and structure very difficult to see. No consistent differences in male caudal structure were seen between the two species. Mawson (1964) described the presence of a small papilla at the base of the tail spike for *P. potoroo*, but we were unable to locate this structure in any of the specimens available to us.

The critical character distinguishing *P. keninupensis* n. sp. from *P. potoroo*, is the shape of the pharyngeal lobes in both males and females. The widest point of the lobe (as seen in radial view) of *P. potoroo* is close to the tip (Fig 1.9), whereas that of *P. keninupensis* n. sp. is near the base (Fig. 1.8). The lobes of *P. potoroo* usually protrude from the oral opening, but those of *P. keninupensis* n. sp.

reach the opening but do not protrude.

A less consistent character differentiating the two species, is that the excretory pore of *P. potoroo* is usually close to the junction of the oesophagus and the intestine, or slightly anterior to the junction, whereas that of *P. keninupensis* n. sp. is usually slightly posterior to the junction.

Although most of the eggs in the uterus of *P. keninupensis* n. sp. appear to be predominately smooth-shelled in the specimens available, eggs passed in faeces presumed to belong to this species (Fig. 2A), have a mammilated outer covering. It is unclear whether this layer is deposited just prior to the egg passing out the vulva, or that the layer has dissolved in the fixation or clearing medium. The operculum is difficult to see in eggs inside the uterus, but is seen more easily in eggs in host faeces.

Mawson (1964) notes that the amphids of *Potoroxyuris potoroo* are prominent. It is interesting that although the amphids of *P. potoroo* and *P. keninupensis* n. sp. can be seen clearly under the surface using light microscopy, on SEMs it is difficult to see the amphids of the latter species on the surface.

The ventral wrinkled zone along the posterior or aboral border of the mouth collar which can be seen clearly in the scanning electron micrographs (Fig 3A–B) is of unknown function.

Due to the poor sclerotization and almost hair-like fineness of the spicule tip, some measurements taken of the spicule lengths may be underestimates. Note that spicule lengths were even more difficult to measure in the *P. potoroo* specimens than the *P. keninupensis* n. sp. specimens, so these were not recorded.

The specimens from *P. longipes* collected from a geographical location close to that of the *P. potoroo* type specimens are larger. However, no consistent morphological characteristics were seen in these specimens that could differentiate them from *P. potoroo*, and the sample size was too small to be able to use size as a factor, so for the present we have designated them as probably *P. potoroo*. Further study of oxyurids from *P. longipes* may well show that it is actually a new species. Many studies of helminths of Australian marsupials have shown that closely related host species usually harbour separate but closely related parasite species (Beveridge and Spratt, 2015). In some parasite groups, the different species are difficult to separate morphologically, but can be shown to differ using molecular techniques (Beveridge and Gasser, 2014).

Unfortunately specimens of helminths from potoroids are generally difficult to obtain because many potoroids are endangered or rare. The fact that woylies are critically endangered and rare (Wayne et al., 2013) meant that few specimens of *P. keninupensis* n. sp. were available for this study. It is likely that the woylie is the only host of this parasite so the latter should be regarded as endangered as well. Since endangered wildlife are regularly treated with anti-parasitic drugs prior to translocation (Northover et al., 2015), there is the danger that non-pathogenic parasites could be affected. Although there are no data on the pathogenicity of *Potoroxyuris*, oxyurid nematodes are seldom regarded as being pathogenic (Soulsby, 1982; Taffs, 1976), so anti-parasitic treatment of woylies could result in placing *P. keninupensis* n. sp. at greater risk than the host, which would be an unfortunate consequence for this newly recognised species.

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