Journal of Helminthology

cambridge.org/jhl

Research Paper

Cite this article: Barton DP, Smales L, Lee V, Zhu X, Shamsi S (2021). Nematodes from the Tasmanian devil (*Sarcophilus harrisii* (Boitard)), with the description of *Sarcophiloxyuris longus* n. gen. and n. sp. (Oxyuridae). *Journal of Helminthology* **95**, e38, 1–9. https://doi.org/10.1017/S0022149X21000298

Received: 4 May 2021
Revised: 20 June 2021
Accepted: 21 June 2021

Keywords:
Endangered species; parasite–host dynamics; biodiversity; conservation

Author for correspondence:
D.P. Barton, E-mail: dibarton@csu.edu.au

© The Author(s), 2021. Published by Cambridge University Press

Nematodes from the Tasmanian devil (*Sarcophilus harrisii* (Boitard)), with the description of *Sarcophiloxyuris longus* n. gen. and n. sp. (Oxyuridae)

D.P. Barton[1], L. Smales[2], V. Lee[1], X. Zhu[1,3] and S. Shamsi[1,4]

[1]School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, 2678, NSW, Australia; [2]Parasitology Section, South Australian Museum, Adelaide, South Australia, 5000, Australia; [3]Wagga Wagga Agricultural Institute, New South Wales Department of Primary Industries, Wagga Wagga, 2678, NSW, Australia and [4]Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, 2678, NSW, Australia

Abstract

The Tasmanian devil (*Sarcophilus harrisii* (Boitard)) is an endangered carnivorous marsupial, limited to the islands of Tasmania in southern Australia. The parasites of the Tasmanian devil are understudied. This study aimed to increase the knowledge of the nematode fauna of Tasmanian devils. Ten Tasmanian devils were examined for parasites from northern and southern Tasmania. Nematodes that were collected were morphologically characterized as two separate species. Molecular sequencing was undertaken to verify the identity of these species. A new genus and species of oxyurid nematode was collected from a single Tasmanian devil from the northern part of Tasmania. The nematode is differentiated from oxyurids described from other Australian amphibians, reptiles and marsupials by the characters of the male posterior end – that is, in having three pairs of caudal papillae, two pairs peri-cloacal, one large pair post-cloacal, a long tapering tail, a stout spicule and a gubernaculum and accessory piece, as well as its much larger overall size. Molecular sequencing was unsuccessful. The remaining nematodes collected from the Tasmanian devil in this study were all identified as *Baylisascaris tasmaniensis* Sprent, 1970, through morphology and molecular sequencing. This paper presents the first description of a new genus and species of oxyurid nematode from the Tasmanian devil, *Sarcophiloxyuris longus* n. gen., n. sp. The need to undertake more sampling of the parasites of endangered hosts, such as the Tasmanian devil, to assist with a better understanding of their conservation management, is discussed.

Introduction

The Tasmanian devil (*Sarcophilus harrisii* (Boitard)) is the largest living carnivorous marsupial and is endemic to the island of Tasmania, Australia (Rose *et al*., 2017). Since the late 1990s, the Tasmanian devil populations have been decimated by a soft-tissue neoplasm – Devil Facial Tumour Disease – and are now considered a threatened species (Rose *et al*., 2017; Wait *et al*., 2017).

Recent research has highlighted the importance of parasites within the 'normal' biology of their host, at the level of the host individual, population and community (Wait *et al*., 2017; Thompson *et al*., 2018; Carlson *et al*., 2020). Investigations of threatened host animals should routinely involve determination of parasite faunas (Carlson *et al*., 2020), especially as our baseline knowledge of that fauna may be extremely poor (Thompson *et al*., 2018). As threatened animals are placed in captive breeding programs, the risk of their parasites being unable to continue their life cycle increases, which could be due to a combination of increased hygiene and reduction in contact with infective stages (for parasites with an indirect life cycle especially) (Thompson *et al*., 2018). Ironically, this loss of parasites may cause more harm to the host than their presence (Thompson *et al*., 2018; Carlson *et al*., 2020).

As with many species of Australian wildlife, the parasite fauna of the Tasmanian devil is not well known, with few systematic surveys having been undertaken (Spratt & Beveridge, 2016, 2018; Wait *et al*., 2017). Currently, seven nematode species have been described from Tasmanian devils (Wait *et al*., 2017). Of these, one species *Woolleyella sarcophili* (Cameron, 1931) Mawson, 1973 (Rhabditida) is known only from the Tasmanian devil (Spratt & Beveridge, 2016; Wait *et al*., 2017). *Trichinella pseudospiralis* Garkavi, 1972 (Trichocephalida) has only been reported from Tasmanian dasyurids and birds of prey (Spratt & Beveridge, 2016; Wait *et al*., 2017). Four species – *Baylisascaris tasmaniensis* Sprent, 1970 (Ascaridida), *Physaloptera sarcophili* Johnston & Mawson, 1940, *Cercopithifilaria johnstoni* (Mackerras, 1954) Bain, Baker & Chabaud, 1983 and *Cyathospirura seurati* Gibbs,1957 (Spirurida) – have also been reported from a variety of Australian marsupials (Spratt & Beveridge, 2016; Wait *et al*., 2017). *Angiostrongylus cantonensis* Chen, 1935 (Strongylida) was introduced to

https://doi.org/10.1017/S0022149X21000298 Published online by Cambridge University Press

Table 1. Collection information and nematodes collected from the Tasmanian devil carcasses examined in this study.

Museum ID number	Geographical location	Date submitted to museum	Sex	Adult/juvenile	*Baylisascaris tasmaniensis*	*Sarcophiloxyuris longus*
TMAG A7990	Hobart, Southern Tasmania	11 May 2019	M	A	6	-
TMAG A7991	Glenlusk, Southern Tasmania	03 Apr 2019	M	A	1	-
TMAG A7992	Forestier Peninsula, Southern Tasmania	02 Apr 2019	M	A	-	-
TMAG A7993[a]	Fentonbury, Southern Tasmania	15 May 2019	M	A	3	-
TMAG A7994	Dunalley, Southern Tasmania	26 Dec 2018	F	A	-	-
TMAG A7995	Collins Cap Area, Southern Tasmania	22 May 2019	F	A	6	-
QVMAG 7492	Weymouth/Lulworth turnoff, Northern Tasmania	30 Nov 2015	F	J	2	4
QVMAG 7576[b]	Beechford, Northern Tasmania	15 May 2017	M	A	N/A	N/A
QVMAG 12239	Liffey Road, Willow Downs, Northern Tasmania	24 Jan 2019	F	J	1	-
QVMAG 14476[b]	Campania, Southern Tasmania	28 Nov 2014	M	A	N/A	N/A

[a]Devil was also infected with Facial Tumour Disease.
[b]Specimen not dissected due to degradation of carcass.

Australia with a wide range of hosts across a variety of mammals (Spratt & Beveridge, 2016; Wait *et al.*, 2017). Wait *et al.* (2017) also reported the presence of trichurid eggs of an unknown species in a number of Tasmanian devil faecal samples undertaken as part of the routine health management by the Save the Tasmanian Devil Program; this was potentially a species of the genus *Eucoleus*, species of which are known to infect other dasyurids.

Most of the parasites described from the Tasmanian devil have not been reported since their initial description (Wait *et al.*, 2017), and their overall biology and ecology has not been studied (Spratt & Beveridge, 2018). Thus, the importance of parasites in the conservation management of the Tasmanian devil remains largely unknown (Wait *et al.*, 2017; Thompson *et al.*, 2018).

This study documents the nematodes of the Tasmanian devil found during a small survey of their parasites, providing data on prevalence of infection as a baseline for future ecological studies, and reports the presence of a new species of nematode.

Materials and methods

Road-killed Tasmanian devil carcasses had been opportunistically collected and donated to the Tasmanian Museum and Art Gallery, Hobart (TMAG) and the Queen Victoria Museum and Art Gallery, Launceston (QVMAG) from November 2014 to May 2019 (table 1). Carcasses were frozen whole until they were made available for dissection in October 2019.

A longitudinal incision was made from the neck area to pubis, exposing the abdominal and thoracic cavities. All internal organs and the alimentary system were removed and separated. The lungs and liver were sliced into 5-mm-thick sections and examined for parasites under a dissecting microscope. The heart, kidneys and spleen were also dissected and placed into separate 500 ml jars of tap water and agitated for a minute; the tissue was removed and examined under a dissecting microscope for parasites. After at least 10 min, the supernatant was gently poured off from the jar and the sediment was examined in a petri dish under a dissecting microscope. The stomach and intestinal system were separated and opened. All stomach contents were removed and examined. The stomach wall and intestinal system were placed into separate 1 L jars of tap water and agitated for a minute; the tissues and liquid were examined as described above.

All parasites found were collected, individually counted and fixed in 70% ethanol. Parasite prevalence and mean intensity of infection were calculated as per Bush *et al.* (1997).

Based on morphological characters, two different types of nematodes were collected from the intestinal system of the Tasmanian devils examined in this study: *B. tasmaniensis* and an unknown oxyurid. A small piece of the mid-body of a number of the specimens was excised for molecular study, and the remaining nematodes and nematode pieces were prepared as temporary wet mounts for morphological examination. Specimens were cleared in lactophenol and studied using an Olympus BH2 microscope (Tokyo, Japan) with differential interference contrast optics. Measurements were taken using an eyepiece micrometre and are given in micrometres, unless otherwise stated, as the mean followed by the range in parentheses. Illustrations were made using a drawing tube. Photomicrographs of mounted specimens were taken using a 9-MP microscope digital camera (AmScope Model MU900, Irvine, California, USA). Specimens were identified based on the literature (Mawson, 1964, 1978; Sprent, 1970; Sprent *et al.*, 1973; Petter & Quentin, 1976).

All specimens were returned to their home institution – either TMAG or QVMAG (see table 1). The authorities for the new genus and species are attributed to the first three authors (i.e. Barton, Smales & Lee) only.

Genomic DNA was isolated from samples using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). The nuclear internal transcribed spacer (ITS) region of *B. tasmaniensis* was amplified utilizing the primer sets SS1: 5′-GTTTCCGTAGGTGAACCTG CG-3′ (forward) and NC2: 5′-TTAGTTTCTTTTCCTCCGCT-3′ (reverse) (Shamsi & Suthar, 2016). Polymerase chain reaction (PCR) reactions were performed in 25 µl master mixes containing 1X buffer, 1.5 mM Magnesium chloride, 0.1 µM of each primer, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 1.25 U of Taq DNA Polymerase and 2 µl of genomic DNA (Promega, Madison, USA). Thermocycling conditions for each run were

set as initial denaturation at 95°C for 2′, then 95°C/30″, 58°C/30″, 72°C/45″ × 40 cycles. Run completed with final extension at 72°C for 10′. The nuclear ITS1 region of the oxyurid nematode was amplified with Pinworm_18S_F: 5′-TAGGTGAACCTGCGGAA-3′ and Pinworm_5.8 R: 5′-GCAGYTHRCTGCGTTCTT-3′ and ITS2 region was amplified with Pinworm_5.8_F: 5′-CGATGAAGAACGCAGYDARCTG-3′ and Pinworm_28S_R: 5′-TGCTTAARTTCAGCGGGTA-3′. The 18S ribosomal RNA region was amplified with two primer pairs targeting a portion of 1800 bp of the gene: 18s_110: 5′-CTAGAGCTAATACATGCACCAA-3′ paired with 18S_1016_R: 5′-AGAACTAGGGCGGTATCTGA-3′ and 18S_538_F: 5′-TCTGGTGCCAGCAGC-3′ and 18S_1906_R: 5′-TGTTACGACTTTTGCCCG-3′. All attempts at amplification for the oxyurid were not successful.

Successful PCR amplicons (visualized via a clear band on gel electrophoresis) were sent to the Australian Genome Research Facility (AGRF Ltd.) in Queensland for bidirectional sequencing using the same primers. The sequences from this study were subjected to phylogenetic analysis with other *Baylisascaris* spp. from GenBank (table 2). *Ascaris suum* (Goeze, 1782) (KY964444.) was used as outgroup to root the phylogenetic tree. The Bayesian method was utilized to infer phylogenetic relationships amongst species. The HKY + G model was selected for Bayesian analysis using Jmodeltest2 (Darriba *et al.*, 2012). Bayesian analysis was undergone using the parameters: temp = 0.2, Ngen = 2,000,000 and burninfrac = 0.3. Remaining parameters were set as default. Figtree v1.4.3 (Rambaut, 2014) was used to visualize the phylogenetic trees.

Table 2. ITS sequences of *Baylisascaris* spp. used in this study.

Species name	Accession no.	Host	Localities
Baylisascaris tasmaniensis	MW063459	*Sarcophilus harrisii*	TAS, Australia*
B. tasmaniensis	MW063460	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063461	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063462	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063463	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063464	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063465	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063466	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063467	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MW063468	*S. harrisii*	TAS, Australia*
B. tasmaniensis	MH030603	*S. harrisii*	TAS, Australia
B. devosi	MH030598	*Pekania pennanti*	Ontario, Canada
B. procyonis	MH030596	*Procyon lotor*	Connecticut, USA
B. procyonis	MH030597	*P. lotor*	California, USA
B. venezuelensis	KX151726	*Tremarctos ornatus*	Venezuela
B. venezuelensis	KX151725	*Tremarctos ornatus*	Venezuela
B. venezuelensis	KX151727	*Tremarctos ornatus*	Venezuela
B. columnaris	MH030595	*Mephitis*	Illinois, USA
B. columnaris	MH030594	*M. mephitis*	Connecticut, USA
B. schroederi	JN210911	*Ailuropoda melanoleuca*	China
B. schroederi	JN210912	*Ailuropoda melanoleuca*	China
B. schroederi	MH030599	*Ailuropoda melanoleuca*	Sichuan, China
B. transfuga	MH030602	*Ursus americanus*	West Virginia, USA
B. transfuga	HM594951	*Ursus maritimus*	Tuscany, Italy
B. transfuga	AB571304	*Homo sapiens*	Japan
B. transfuga	JN617990	NA	NA
B. ailuri	MH030600	*Ailurus fulgens*	Sichuan, China
Ascaris suum	KY964444	*Sus scrofa domesticus*	Tibet, China

Ascaris suum was used as the outgroup.
*Sequences obtained in this study. NA, not available.

Results

A total of ten Tasmanian devil carcasses were available for examination in this study: six were collected from the southern part of Tasmania (dissected at TMAG) and four were collected from the northern part of Tasmania (dissected at QVMAG) (table 1). Two of the four individuals being held at QVMAG could not be examined due to the level of internal decomposition of the specimen. Of the remaining eight individuals, two specimens were not infected with any nematode parasites.

Of the nematode specimens collected, the majority were determined to be *B. tasmaniensis* based on morphological and molecular analysis. Molecular sequences obtained in this study (GenBank sequences MW063459–MW063468) were a 100% match to a specimen of *B. tasmaniensis* (GenBank sequence MH030603; Camp *et al.*, 2018) and clustered with *B. tasmaniensis* with 100% branch support (fig. 1; table 3). Overall, six of the eight devils (62.5%) were infected with *B. tasmaniensis* with a mean intensity of 3.2 (1–6). From the southern devils examined, four of the six (66.7%; mean intensity 4 (1–6)) were infected; both of the northern devils were infected (mean intensity 1.5 (1–2)).

One of the northern devils was also infected with a nematode that was determined to belong to the family Oxyuridae. A total of four male nematodes were collected. This nematode was determined to be a new genus and species and is described below.

Oxyuridae Cobbold, 1864

Sarcophiloxyuris n. gen.

Diagnosis

Oxyuridae: males relatively large, cuticle with transverse annulations; body tapering. Buccal cavity with pharyngeal lobes of interradial blades between pharyngeal teeth; cervical and lateral alae present. Three pairs caudal papillae, two pairs peri-cloacal, one pair large pedunculated posterior to cloaca; tail tapering, elongated without caudal alae. Spicule single, stout; gubernaculum with accessory piece present. Females unknown. Parasites of Dasyuridae, marsupials from Australia.

Type species. Sarcophiloxyuris longus.

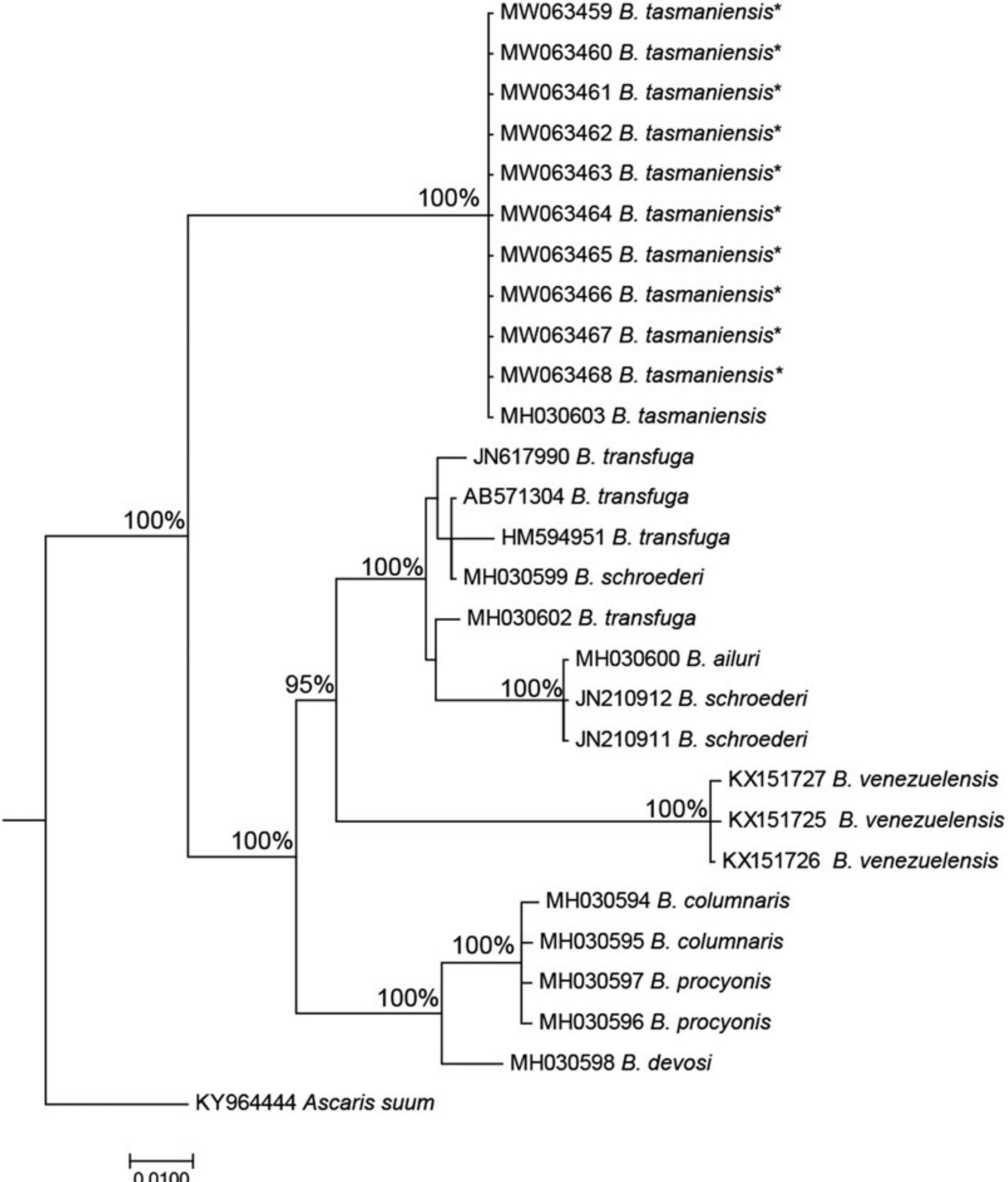

Fig. 1. Bayesian phylogenetic tree of *Baylisascaris tasmaniensis* specimens collected in this study (marked with *) in comparison to sequences available from GenBank based on ITS sequences. Scale bar shows the number of substitutions per site.

Sarcophiloxyuris longus n. sp.

Description

General (figs 2 and 3). Relatively large nematodes, cuticle with transverse cuticular striations. Cephalic end with four sub-median papillae, two lateral amphids; cervical alae present, lateral alae double crested, begin at posterior level of cervical alae and extend to level just posterior to cloaca. Buccal capsule wall lightly sclerotized, with inter-labial lamellae. Pharyngeal part of oesophagus lobed. Oesophagus with distinct isthmus, terminating in a subspherical bulb.

Male (measurements from three worms). Body length 6 mm; maximum width 300–305. Cervical alae extend from 50 from anterior end to 180, 250 from anterior end. Oesophagus 589 (470–690) long; bulb 141 (125–167) long, 129 (99–167) wide. Nerve ring, excretory pore not seen. Tail elongated, tapering 487 (480–500) long. Spicule single, robust 147 (130–170) long, maximum width 20, spicule tip curved ventrally; gubernaculum 50 long, accessory piece 45 long. Caudal papillae three pairs; two pairs peri-cloacal; one pair, large pedunculated caudal, placed 140, 160 post-cloacal.

Female. Unknown.

Taxonomic summary

Type host. Sarcophilus harrisii (Boltard, 1841).

Site in host. Caecum.

Type locality. Weymouth/Lulworth turnoff (41.017°S, 147.103°E), northern Tasmania.

Type specimens. Holotype male (QVMAG registration number QVM:2019:18:0036), three paratype males (QVM:2019:18:0037-0039) collected by Vanessa Lee, 29 viii 2019.

https://doi.org/10.1017/S0022149X21000298 Published online by Cambridge University Press

https://doi.org/10.1017/S0022149X21000298 Published online by Cambridge University Press

Table 3. Pairwise genetic distance matrix of the ITS regions of sequences obtained from this study compared to closely related species in GenBank, shown as *p*-difference (below the diagonal) and number of differences (above the diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. MW063459		0	76	78	77	96	97	97	76	78	81	81	65	68	70	65	68	81	73
2. MH030603	0.0%		76	78	77	96	97	97	76	78	81	81	65	68	70	65	68	81	73
3. MH030598	9.3%	9.3%		22	22	80	81	81	22	23	63	63	45	48	48	45	47	63	72
4. MH030596	9.5%	9.5%	2.7%		2	80	81	81	2	3	66	66	48	51	51	48	50	66	74
5. MH030597	9.4%	9.4%	2.7%	0.2%		78	79	79	2	3	66	66	48	51	51	48	50	66	74
6. KX151726	11.7%	11.7%	9.8%	9.8%	9.5%		1	1	80	81	78	78	66	67	69	66	69	78	98
7. KX151725	11.8%	11.8%	9.9%	9.9%	9.6%	0.1%		2	81	82	79	79	67	68	70	67	70	79	99
8. KX151727	11.8%	11.8%	9.9%	9.9%	9.6%	0.1%	0.2%		81	82	79	79	67	68	70	67	70	79	99
9. MH030595	9.3%	9.3%	2.7%	0.2%	0.2%	9.8%	9.9%	9.9%		3	66	66	48	51	51	48	50	66	74
10. MH030594	9.5%	9.5%	2.8%	0.4%	0.4%	9.9%	10.0%	10.0%	0.4%		67	67	49	52	52	49	51	67	75
11. JN210911	9.9%	9.9%	7.7%	8.1%	8.1%	9.5%	9.6%	9.6%	8.1%	8.2%		0	20	21	25	20	23	0	80
12. JN210912	9.9%	9.9%	7.7%	8.1%	8.1%	9.5%	9.6%	9.6%	8.1%	8.2%	0.0%		20	21	25	20	23	0	80
13. MH030599	7.9%	7.9%	5.5%	5.9%	5.9%	8.1%	8.2%	8.2%	5.9%	6.0%	2.4%	2.4%		5	5	0	4	20	65
14. MH030602	8.3%	8.3%	5.9%	6.2%	6.2%	8.2%	8.3%	8.3%	6.2%	6.3%	2.6%	2.6%	0.6%		10	5	8	21	68
15. HM594951	8.5%	8.5%	5.9%	6.2%	6.2%	8.4%	8.5%	8.5%	6.2%	6.3%	3.1%	3.1%	0.6%	1.2%		5	9	25	69
16. AB571304	7.9%	7.9%	5.5%	5.9%	5.9%	8.1%	8.2%	8.2%	5.9%	6.0%	2.4%	2.4%	0.0%	0.6%	0.6%		4	20	65
17. JN617990	8.3%	8.3%	5.7%	6.1%	6.1%	8.4%	8.5%	8.5%	6.1%	6.2%	2.8%	2.8%	0.5%	1.0%	1.1%	0.5%		23	68
18. MH030600	9.9%	9.9%	7.7%	8.1%	8.1%	9.5%	9.6%	9.6%	8.1%	8.2%	0.0%	0.0%	2.4%	2.6%	3.1%	2.4%	2.8%		80
19. KY964444	8.9%	8.9%	8.8%	9.0%	9.0%	12.0%	12.1%	12.1%	9.0%	9.2%	9.8%	9.8%	7.9%	8.3%	8.4%	7.9%	8.3%	9.8%	

All sequences obtained from this study were identical and shown as one haplotype in the table (1. MW063459). Please refer to GenBank accession number in table 2 for details of sequences.

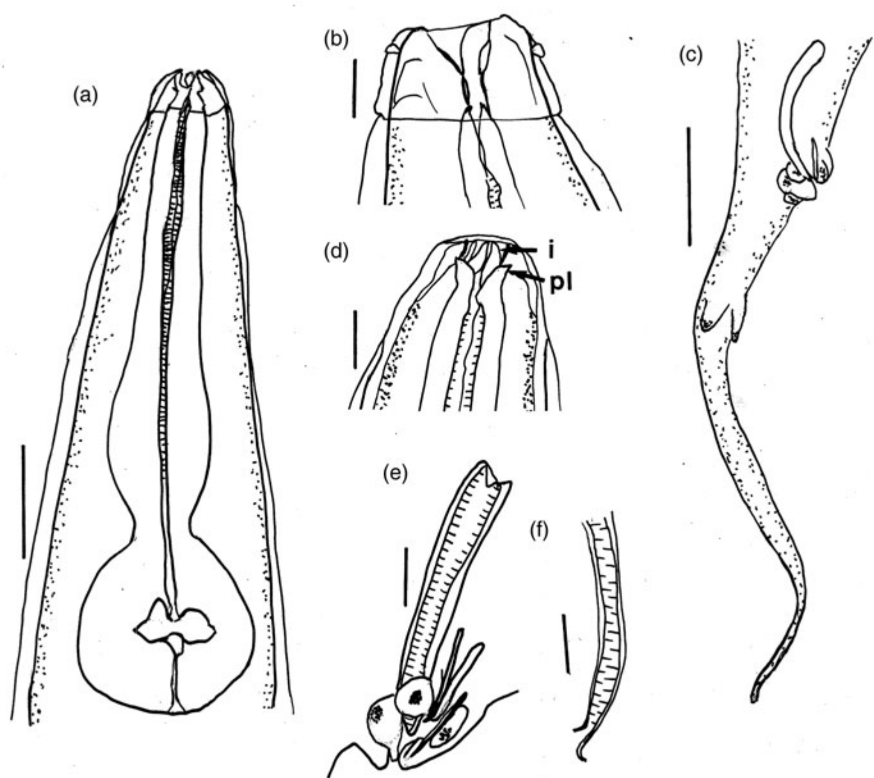

Fig. 2. *Sarcophiloxyuris longus* n. gen., n. sp. male: (a) anterior-end lateral view; (b) cephalic-end dorso-ventral view, slightly flattened; (c) tail ventro-lateral view; (d) cephalic-end lateral view; (e) cloacal region lateral view, showing gubernaculum complex; (f) spicule tip. Abbreviations: i, inter-labial lamella; pl, pharyngeal lobe. Scale bars: (a, c) 100 µm; (b, d) 50 µm; (e, f) 25 µm.

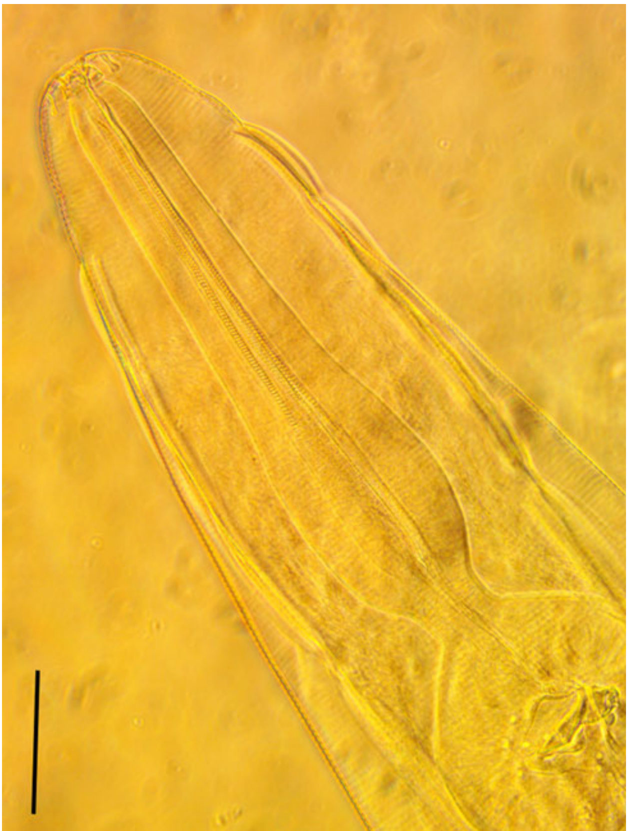

Fig. 3. Photomicrograph of anterior-end lateral view of *Sarcophiloxyuris longus* n. gen., n. sp. male. Scale bar: 50 µm.

Intensity of infection. Four.

Prevalence. One of eight Tasmanian devils examined.

Etymology. The genus is named after the host genus from which it was collected. The species is named after the much larger overall body size in comparison to the other oxyurids collected from Australian marsupials.

Remarks

Owing to the condition of the material (the specimens had been kept under a coverslip in lactophenol for an extended period of time) and the lack of female specimens, a complete characterization of the species was not possible. Features such as the buccal capsule and gubernaculum had been flattened and could only be visualized laterally and in two dimensions. Focussing through the specimens, however, provided sufficient information to prepare a limited delineation of the morphology of the specimens. For example, from the lateral view the gubernaculum appeared to be a simple rod shape with an unornamented accessory piece, similar to a less developed gubernaculum as described by Hugot (1988), although the dorso-ventral shape could not be mapped. Therefore, it was possible to prepare a generic diagnosis and species description informed by the combination of those features of the cephalic and posterior ends that could be described. From the key to the Oxyurida of Petter & Quentin (1976) these specimens can be placed in the Oxyuridae because they have non-pedunculate amphids. Further, the specimens can be placed with parasites of rodents, ruminants and hyracoids in having four or less pairs of well-separated genital papillae, of which only one pair is pedunculate, a gubernaculum and simple pharyngeal teeth. However, the specimens cannot be placed in any of the known genera comprising the family because of the differences

https://doi.org/10.1017/S0022149X21000298 Published online by Cambridge University Press

Table 4. Measurements of *Sarcophilus longus* n. gen., n. sp. males compared to several oxyurids from Australian hosts as reported by Mawson (1964), Hugot & Quentin (1985) and Weaver & Smales (2010).

Characters	*Sarcophiloxyuris longus*	*Paraustroxyuris parvus*	*Syphacia boodjamullaensis*	*Syphacia darwini*	*Syphacia helidonensis*	*Syphacia muris*
Body length (mm)	6.0	1.12	1.25	1.865	0.9–1.2	1.14
Maximum width	300–305	--	130	230	170–230	75
Oesophagus length	589 (470–690)	520	175	320	103–216	160
Tail length	487 (400–500)	--	113	247	84–144	115
Spicule length	147 (130–170)	35–60	77)	80	65–74	48
Gubernaculum length	50	--	41	40	26–38	28
Caudal papillae	Two pairs peri-cloacal, one pair pedunculate post-cloacal	Two pairs pre-cloacal, two pairs ad-cloacal pedunculate, one pair peri-cloacal sessile	Two pairs cloacal, one pair postanal	One pair pre-cloacal, one pair peri-cloacal, one pair post-cloacal pedunculate	Two pairs peri-cloacal, one pair pedunculate post-cloacal	Two pairs peri-cloacal, one pair pedunculate post-cloacal
Host	*Sarcophilus harrisii*	*Petauroides volans*	*Zyzomys argurus*	*Melomys cervinipes*	*Pseudomys gracilicaudatus*	*Rattus fuscipes*, *Rattus tunneyi*
Geographical location	Northern Tasmania	Queensland	Queensland	Northern Territory	Queensland	New South Wales

in those characters that can be determined. In particular mamelons, typical of the genera, *Syphacia* (found in Australian murids) and *Sypharista* (found in Asian flying squirrels) are not present. Some characters of the posterior end of the new genus, including the three pairs of caudal papillae (two pairs peri-cloacal, one large pair post-cloacal) and a long tapering tail, are similar to those usually found in genera parasitizing rodents. Having a gubernaculum with accessory piece places *Sarcophiloxyuris* n. gen. closest to the genera *Sypharista* and *Syphacia*. In contrast to the filiform spicule of *Sypharista* and *Syphacia*, the spicule of *S. longus* is stout. The gubernaculum is neither hook-shaped nor ornamented, nor is the accessory piece ornamented. The only oxyurids that have been reported from Australian murids (*Melomys*, *Pseudomys*, *Rattus* and *Zyzomys*) are all representatives of the genus *Syphacia*. They comprise the cosmopolitan species, *Sy. muris* and seven endemic *Syphacia* spp., all of which have mamelons (Weaver & Smales, 2010). The available characters of the cephalic end of *S. longus*, a buccal cavity with inter-radial blades between three pharyngeal teeth without tubercules not protruding from the oral opening, show no similarities with the characters of the buccal capsules of the genera *Syphacia* and *Sypharista*, but, interestingly, some similarities with the buccal cavity of the genus *Paraustroxyuris*, a parasite of the Australian marsupial, *Petauroides volans* (see (Mawson, 1964). *Sarcophiloxyuris longus* are relatively large, males 6 mm long, compared with 1–2 mm for males of *Syphacia* spp. Representative comparative measurements for males are given in table 4. Studies of populations of species of *Syphacia* have shown that males are usually difficult to find because they disappear or die after copulation (Levine, 1968). That only large males were found in the Tasmanian devil in this study suggests that *S. longus* may follow a different lifecycle strategy.

Alternatively, *S. longus* may be aligned with the family Pharyngodonidae, which are oxyurid parasites of reptiles and amphibians, although they do not appear to have the key character of pedunculate amphids. The pharyngodondid genera that have been reported from Australia (*Parathelandros*, *Pharyngodon*, *Skryabinodon*, *Thelandros* (syn. *Parapharyngodon*) and *Veversia*) have not been reported from Tasmanian reptiles (Pichelon *et al.*, 1999), although *Pharyngodon* sp. and *Thelandros* sp. (reported also as *Parapharyngodon* sp.) have been reported from Tasmanian amphibians (Munday & Green, 1972). The genus *Sarcophiloxyuris* differs from these genera as follows: from *Parathelandros* in having a robust, not poorly developed, spicule, narrow lateral alae that do not constrict sharply anterior to the cloaca and without a raised genital cone, post-cloacal spherical swelling or posterior directed process; from *Pharyngodon* in not having caudal alae supported by pedunculated papillae and in having a robust spicule; from *Thelandros* in having a gubernaculum and not having the posterior end truncate with a mid-dorsal process; from *Skryabinodon* in having a robust spicule, not having the cloacal region raised as a narrow elongate cone; from *Veversia* in not having thick lateral alae, a thick cuticle covered in dense hairs and an oesophageal bulb containing a masticatory apparatus (Baylis, 1930; Yorke & Maplestone, 1936; Inglis, 1968; Skryabin *et al.*, 1991).

The combination of biological and morphological characters, including the larger size and those of the cephalic and posterior ends, of *S. longus* support the erection of the new genus *Sarcophiloxyuris*. This is the first record of an oxyurid from a Tasmanian devil (Spratt & Beveridge, 2016). It is possible that these nematodes are actually parasites of prey items of the Tasmanian devil; however, their condition in an intestine that did not contain obvious digested prey suggests not. Further surveys are needed to either reveal alternative hosts or confirm *S. harrisii* as the definitive host and to complete the female component of the species description.

https://doi.org/10.1017/S0022149X21000298 Published online by Cambridge University Press

Discussion

This paper presents the first description of a species of oxyurid nematode from the Tasmanian devil. Oxyurids are considered rare in carnivorous dasyurid marsupials, as they are more commonly parasites of herbivores and invertebrates (Oakwood & Spratt, 2000). Oakwood & Spratt (2000) reported a single specimen of an unknown species of oxyurid from the stomach of the northern quoll, *Dasyurus hallucatus* Gould, but suggested that it was more likely a parasite of a prey item, rather than a true parasite of the quoll. The four oxyurid specimens collected from the Tasmanian devil in this study were all in excellent condition at the time of collection, and did not appear to be degraded by digestion. Although it is possible that *S. longus* might be a parasite of a prey item, the condition of the worms, in an intestinal system also holding fully digested prey items, would suggest otherwise. Tasmanian devils are effective predators and specialized scavengers of medium to large prey such as macropods, possums and wombats (Jones, 2008; Rose *et al.*, 2017) and are unlikely to consume small mammals, such as rodents, reptiles or amphibians. However, it is possible that this parasite is not a true parasite of the Tasmanian devil, but of a prey item. Similar to the genus *Sarcophiloxyuris*, the other oxyurid genera reported from marsupials in Australia (see table 4), have also only been reported sporadically from hosts (Spratt & Beveridge, 2018). Thus, it is possible that this is a rare example of an oxyurid parasitizing a carnivorous marsupial. Given the sporadic nature of parasitological research on wildlife species in Australia (Spratt & Beveridge, 2018); however, much more research needs to be undertaken to assess their true relationship with this host group.

Baylisascaris tasmaniensis is one of the few parasites of the Tasmanian devil that has received attention from researchers (Sprent, 1970; Sprent *et al.*, 1973; Munday & Gregory, 1974; Camp *et al.*, 2018). There are currently 11 recognized species of *Baylisascaris* (Camp *et al.*, 2018). *Baylisascaris tasmaniensis*, however, is a phylogenetic anomaly being the only species within the genus to utilize a marsupial carnivore as its definitive host. All other species utilize eutherian (placental) carnivores which do not naturally exist in Australia. Sprent (1970) suggested the possibility of convergent evolution whereby parasites have infected hosts which occupy similar, though geographically separate, niches. The result is the development of morphologically similar, phylogenetically different species of parasites. Camp *et al.* (2018) reported on the molecular phylogenetics and species-level systematics of the genus *Baylisascaris*. The topology of the trees produced by Camp *et al.* (2018) and this study showed that *B. tasmaniensis* was distantly grouped from the other *Baylisascaris* species, as indicated by the long branches. If Camp *et al.* (2018) had been able to include more samples of *B. tasmaniensis*, they may well have also created an independent clade. The other two clades that were present – a clade of species collected from bears and pandas from China and the Americas, and a clade of species collected from skunks, racoons and a mustelid from the USA and Canada – were all highly supported by Camp *et al.* (2018). The placement of *B. tasmaniensis* differed between the studies in that *B. tasmaniensis* was basal to both clades in this study, but was only basal to the clade of species collected from bears and pandas in Camp *et al.* (2018). This difference could be due to the number of genes sequenced (one in this study vs. eight in Camp *et al.* (2018)), the species included (*Baylisascaris venezuelensis* from the spectacled bear in Venezuela was included in this study), the primers used and/or the type of tree presented (concatenated in Camp *et al.* (2018) vs. ITS only in this study). Importantly, however, the placement of *B. tasmaniensis* is always separate to these other clades, suggesting that *B. tasmaniensis* is not closely related to these species and that the origin of *Baylisascaris* in Tasmanian devils, as postulated by Sprent, still remains unresolved.

All species of *Baylisascaris* – excluding *Baylisascaris laevis* (Leidy, 1851) – are trophically transmitted, utilizing carnivores as their definitive hosts, and a vast variety of small mammals and herbivores as intermediate and paratenic hosts (Sapp *et al.*, 2017). Via the experimental inoculation of laboratory mice and the Tasmanian devil, Sprent *et al.* (1973) were able to shed light on the full life cycle of *B. tasmaniensis*. Within the small intestine of marsupial carnivores, adult nematodes undergo maturation, with females releasing eggs which are subsequently shed in faeces. Provided conditions are optimal, zygotes undergo development into infective-stage larvae (Sapp *et al.*, 2017). After ingestion by intermediate hosts, larvae hatch and migrate to somatic tissues and undergo a second moult within one to two weeks post infection. Third-stage larvae then survive within the intermediate host for an indefinite period until ingestion by a definitive host (Sapp *et al.*, 2017). Though visceral, ocular and neural larva migrans syndrome has been extensively described in literature in relation to *Baylisascaris procyonis*, there has only been one study so far investigating the potential pathogenic effects of larval migration in *B. tasmaniensis* within wildlife. Munday & Gregory (1974) found visceral granulomata within the mesentery, intestinal wall, liver, spleen, kidneys, heart and lungs of wombats from the north-eastern region of Tasmania; feeding of these larvae to captive Tasmanian devils resulted in infestation by *B. tasmaniensis*.

Wait *et al.* (2017) reviewed the parasites of the Tasmanian devil and highlighted the importance of parasites for the biodiversity and conservation of their host species. Also, various researchers (Wait *et al.*, 2017; Thompson *et al.*, 2018; Carlson *et al.*, 2020) have highlighted the importance of understanding the impact that conservation management measures can have on parasite prevalence and diversity. Although a number of Tasmanian devil parasites are not host-specific, at least six species (including *S. longus*) are, so there is a risk of extinction of these species with declining Tasmanian devil populations (Wait *et al.*, 2017; Thompson *et al.*, 2018). Indeed, Beveridge & Spratt (2015) noted that no parasites had yet been included in the recovery program for the Tasmanian devil, even though the cestode *Dasyurotaenia robusta* Beddard, 1912 is an officially recognized endangered species.

Acknowledgments. The authors are indebted to Belinda Bauer (TMAG), Sarah Peck (Department of Primary Industries, Parks, Water and Environment, Tasmania), and David Maynard and Judy Rainbird (QVMAG) for access to Tasmanian devil specimens and facilities for dissection of specimens. The authors would also like to thank Ian Beveridge (University of Melbourne) for provision of literature and access to his knowledge of parasites of Tasmanian devils. This project was undertaken as part of an Honours project (V.L.).

Financial support. This project was partly funded by Charles Sturt University (S.S., grant number A512-828-xxx-66770).

Conflicts of interest. None.

Ethical standards. No approval of research ethics committees was required for this study as all specimens had been collected previously.

References

Baylis HA (1930) Some Heterakidae and Oxyuridae (Nematoda) from Queensland. *Annals and Magazine of Natural History Ser.10* **5**, 354–366.

Beveridge I and Spratt DM (2015) Biodiversity and parasites of wildlife: helminths of Australasian marsupials. *Trends in Parasitology* **31**, 142–148.

Bush AO, Lafferty KD, Lotz JM and Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* **83**, 575–583.

Camp LE, Radke MR, Shihabi DM, Pagan C, Yang G and Nadler SA (2018) Molecular phylogenetics and species-level systematics of *Baylisascaris*. *International Journal for Parasitology: Parasites and Wildlife* **7**, 450–462.

Carlson CJ, Hopkins S, Bell KC, *et al.* (2020) A global parasite conservation plan. *Biodiversity Conservation* **250**, 108596.

Darriba D, Taboada GL, Doallo R and Posada D (2012) Jmodeltest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

Hugot JP (1988) Les nématodes syphaciinae parasites de rongeurs at de lagomorphes. Taxonomie. Zoogéographie. Evolution. *Mémoires du Museum National d'Histoire Naturelle, Zoologie* **141**, 13–149.

Hugot J-P and Quentin J-C (1985) Étude morphologie de six espèces nouvelles ou peu connues appartenant au genre *Syphacia* (oxyuridae, Nematoda), parasites de rongeurs cricétidés et muridés. *Bulletin de Museum National d'Histoire Naturelle, Serie 4* **7**, 383–400.

Inglis WG (1968) Nematodes parasitic in western Australian frogs. *Bulletin of the British Museum (Natural History) Zoology* **16**, 163–183.

Jones M (2008) Tasmanian devil. pp. 78–80 *in* Van Dyck S and Strahan R (*Eds*) *The mammals of Australia*. 3rd edn. Sydney, Reed New Holland.

Levine ND (1968) *Nematode parasites of domestic animals and of man*. 599 pp. Minneapolis, Burgess Publishing Company.

Mawson PM (1964) Some Nematoda (Strongylina and Oxyurina) from kangaroos (*Macropus* spp.) from eastern Australia. *Parasitology* **54**, 237–262.

Mawson PM (1978) A new genus *Adelonema* (Nematoda: Oxyuridae) from Australian phalangerid marsupials. *Transactions of the Royal Society of South Australia* **102**, 223–226.

Munday BL and Green RH (1972) Parasites of Tasmanian native and feral fauna part II, helminthes. *Records of the Queen Victoria Museum* **44**, 1–15.

Munday BL and Gregory GG (1974) Demonstration of larval forms of *Baylisascaris tasmaniensis* in the wombat (*Vombatus ursinus*). *Journal of Wildlife Diseases* **10**, 241–242.

Oakwood M and Spratt DM (2000) Parasites of the northern quoll, *Dasyurus hallucatus* (Marsupialia: Dasyuridae) in tropical savanna, northern territory. *Australian Journal of Zoology* **48**, 79–90.

Petter AJ and Quentin JC (1976) Keys to genera of the Oxyuroidea. *in* Anderson R, Chabaud A and Willmott S (*Eds*) *CIH keys to the nematode parasites of vertebrates No. 4*. Farnham Royal, England, Commonwealth Agricultural Bureaux.

Pichelin S, Thomas PM and Hutchinson MN (1999) A checklist of helminth parasites of Australian reptiles. *South Australian Museum Monograph Series* **5**, 1–61.

Rambaut A (2014) FigTree v1.4.2, a graphical viewer of phylogenetic trees. http://tree.bio.ed.ac.uk/software/figtree.

Rose RK, Pemberton DA, Mooney NJ and Jones ME (2017) *Sarcophilus harrissii* (Dasyuromorphia: Dasyuridae). *Mammalian Species* **49**, 1–17.

Sapp SGH, Gupta P, Martin MK, Murray MH, Niedringhaus KD, Pfaff MA and Yabsley MJ (2017) Beyond the raccoon roundworm: the natural history of non-raccoon *Baylisascaris* species in the New World. *International Journal for Parasitology: Parasites and Wildlife* **6**, 85–99.

Shamsi S and Suthar J (2016) Occurrence of *Terranova* larval types (Nematoda: Anisakidae) in Australian marine fish with comments on their specific identities. *PeerJ* **4**, e1722.

Skryabin KI, Shikhobalova NP and Mozgovoi AA (1991) *Key to parasitic nematodes. Volume 2 Oxyurata and Acscaridata*. English translation. Leiden, E.J. Brill.

Spratt DM and Beveridge I (2016) Helminth parasites of Australasian monotremes and marsupials. *Zootaxa* **4123**, 1–198.

Spratt DM and Beveridge I (2018) Wildlife parasitology in Australia: past, present and future. *Australian Journal of Zoology* **66**, 286–305.

Sprent JFA (1970) *Baylisascaris tasmaniensis* sp. nov. in marsupial carnivores: heirloom or souvenir? *Parasitology* **61**, 75–86.

Sprent JFA, Lamina J and McKeown A (1973) Observations on migratory behaviour and development of *Baylisascaris tasmaniensis*. *Parasitology* **67**, 67–83.

Thompson RCA, Lymbery AJ and Godfrey SS (2018) Parasites at risk – insights from an endangered marsupial. *Trends in Parasitology* **34**, 12–22.

Wait LF, Peck S, Fox S and Power ML (2017) A review of the parasites in the Tasmanian devil (*Sarcophilus harrisii*). *Biodiversity Conservation* **26**, 509–526.

Weaver HJ and Smales LR (2010) Three new species of *Syphacia* (*Syphacia*) (Oxyurida: Oxyuridae) from Queensland, Australia, and a key to the species in the Australian bioregion. *Comparative Parasitology* **77**, 9–19.

Yorke W and Maplestone PA (1936) *The nematode parasites of vertebrates*. London, J. & A. Churchill.

https://doi.org/10.1017/S0022149X21000298 Published online by Cambridge University Press