New species of *Cloacina* von Linstow, 1898 (Nematoda: Strongyloidea) parasitic in the stomachs of wallaroos, *Osphranter* spp. (Marsupialia: Macropodidae) from northern Australia

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Received: 21 January 2018 / Accepted: 30 March 2018

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This article is part of the Topical Collection Nematoda.

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Abstract Three new species of the parasitic nematode genus *Cloacina* von Linstow, 1898 (Strongyloidea: Cloacininae) are described from the stomachs of wallaroos, *Osphranter* spp. (Marsupialia: Macropodidae), from northern Australia. *Cloacina spearei* n. sp. is described from *O. robustus woodwardi* (Thomas) and *O. antilopinus* (Gould) and is distinguished from congeners by the shape of the cephalic papillae, the shallow buccal capsule, the presence of an oesophageal denticle and the convoluted but non-recurrent vagina in the female. *Cloacina longibursata* n. sp. also from *O. robustus woodwardi* and *O. antilopinus* is distinguished from congeners by the elongate dorsal lobe of the bursa, with the origin of the lateral branchlets posterior to the principal bifurcation, in the features of the spicule tip, the lack of bosses lining the oesophagus and the absence of an oesophageal denticle. *Cloacina crassicaudata* n. sp., from the same two host species was formerly identified as *C. cornuta* (Davey & Wood, 1938).

Differences in the cephalic cuticle (inflation lacking in the new species), the shape of the cephalic papillae, the dorsal oesophageal tooth and the spicule tips, as well as differences in the sequences of the internal transcribed spacers of the nuclear ribosomal DNA, indicate that this is an independent species. The geographical distribution of this species is disjunct with populations in both the Northern Territory and Queensland. Possible reasons for the disjunct distribution are discussed.

Introduction

The helminth parasites of Australian wallaroos of the genus *Osphranter* Gould (Marsupialia: Macropodidae) are relatively poorly known. The common wallaroo *Osphranter robustus* (Gould) (formerly *Macropus robustus*) is distributed across most of the continent (Van Dyck & Strahan, 2008), but the only systematic survey of its parasites is limited to Queensland (Beveridge et al., 1998). Other records (Spratt & Beveridge, 2016) are based on incidental collections. Similarly, studies of the helminth parasites of the antilopine wallaroo *Osphranter antilopinus* (Gould) (formerly *Macropus antilopinus*) which occurs across much of northern Australia (Van Dyck & Strahan, 2008) are currently based on a small sample of animals from Queensland (Beveridge et al., 1998). The black wallaroo *Osphranter bernardus* (Rothschild) is limited to rocky escarpments of Arnhem Land in the Northern Territory (Van Dyck & Strahan, 2008) and there are consequently few helminth parasite records from it (Spratt & Beveridge, 2016). In spite of these limitations, *O. robustus* is currently host to the most diverse array of species of the parasitic nematode genus *Cloacina* von Linstow, 1898, sharing most of these nematode parasites with *O. antilopinus*, with which it is broadly sympatric (Beveridge, 1998; Beveridge et al., 2002). This claim is however based on sampling primarily from the southern and eastern regions of Australia (Beveridge, 1998). At that time, there had been only limited sampling from northern Australia and consequently, Beveridge (1998, pp. 506-507) listed undescribed species from *O. robustus* and *O. antilopinus* from which there was inadequate material to formerly erect new species.

Subsequent collecting has resulted in additional material being obtained, allowing the description of two new species. In addition, morphological re-examination of material previously identified as *Cloacina cornuta* (Davey & Wood, 1938), primarily a parasite of the agile wallaby, *Notamacropus agilis* (Gould) (formerly *Macropus agilis*) (Speare et al., 1983) from both *O. robustus* and *O. antilopinus*, together with additional molecular data, have indicated that the specimens from wallaroos are a separate species which is also described here.

Materials and methods

Nematodes were collected from fresh road-killed specimens of the northern subspecies of *O. robustus*, *O. robustus woodwardi* (Thomas) and *O. antilopinus* in the Northern Territory. Nematodes were washed in saline and either fixed in Berland's fluid (Gibson, 1979) followed by storage in 70% ethanol, or were frozen at -80 C for molecular examination. A small number of specimens were obtained from animals killed by aboriginal hunters and these were fixed directly in 70% ethanol.

For morphological studies, nematodes were cleared in lactophenol and drawings were made with a drawing tube attached to an Olympus BH2 microscope. Measurements were made with an ocular micrometer and are presented in millimetres as the range followed by the mean and the number of specimens examined in parentheses. All specimens have been deposited in the South Australian Museum, Adelaide (SAM). Morphological terminology in the descriptions follows Beveridge (1998).

Host nomenclature follows Jackson & Groves (2015) in which the wallaroos are placed in the genus *Osphranter* Gould. Previously (Van Dyck & Strahan, 2008), these marsupials were included within *Macropus* Shaw. Host species included here as hosts are *O. antilopinus* (Gould) and *O. robustus woodwardi* (Thomas).

Frozen nematodes were thawed and a mid-body section removed for molecular analyses. The head and tail were cleared in lactophenol to confirm the identity of the nematodes and have been deposited in SAM as voucher specimens.

Genomic DNA was extracted from the mid-body region of each nematode using a small-scale sodium-dodecyl-sulfate/proteinase K extraction procedure (Gasser et al., 1993) and then column-purified (Wizard DNA Clean-Up kit, Promega, Madison, WI, USA). The rDNA region comprising the internal transcribed spacers (ITS) of the nuclear ribosomal DNA, including ITS-1, 5.8S rRNA gene, ITS-2, and flanking sequences (= ITS+), was amplified by polymerase chain reaction (PCR) using the primers NC5 (forward: 5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3') and NC2 (reverse: 5'-TTA GTT TCT TTT CCT CCG CT-3') (Chilton, 2004). PCRs were performed in 50 µl, with positive and no-template (negative) controls included, using a standard buffer (GoTaq® Flexi Buffer, Promega), 3.5 mM $MgCl_2$, 25 µM of each dNTP, 50 pmol of each primer and 1.5 U of *Taq* DNA polymerase (GoTaq® Flexi, Promega). The PCR cycling conditions used were: 94°C for 5 min (initial denaturation), followed by 30 cycles of 94°C for 30 s (denaturation), 55°C for 30 s (annealing) and 72°C for 90 s (extension), then 72°C

for 5 min (final extension). Amplicons were column-purified (Wizard PCR-Preps DNA Purification System, Promega) prior to automated DNA sequencing using the primers NC5 and NC2 in separate reactions.

The quality of nucleotide sequences was appraised using the program Geneious R10 (Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012), and polymorphic sites were designated using International Union of Pure and Applied Chemistry (IUPAC) codes. Sequences were aligned using the program MEGA 7.0.26 (Kumar et al., 2016) by employing Multiple Sequence Comparison by Log-Expectation (MUSCLE) (Edgar, 2004). The alignments were then adjusted manually using the program BioEdit (Hall, 1999).

Order Strongylida (Railliet & Henry, 1913)
Family Chabertiidae (Popova, 1955)
Genus *Cloacina* von Linstow, 1898

***Cloacina spearei* Beveridge n. sp.**

Type-host: *Osphranter robustus woodwardi* (Thomas) (Marsupialia: Macropodidae).
Other host: *Osphranter antilopinus* (Gould) (Marsupialia: Macropodidae).
Type-locality: 18 km south-west of Katherine, Northern Territory, Australia.
Other localities: Northern territory: 46 km west of Victoria River; 10 km north of Katherine; Willeroo Station via Katherine; 50 km SW of Katherine.
Type-material: Holotype ♂ from stomach of *Osphranter robustus woodwardi* (Thomas), 18 km south-west of Katherine, Northern Territory, Australia (SAM 48090); allotype ♀, same data (SAM 48091); paratypes: 2 ♂♂, 7 ♀♀, same data (SAM 48092).
Material examined: types; voucher specimens: from *O. robustus woodwardi*, Northern Territory: 1 ♂, 17 ♀♀, 46 km west of Victoria River (SAM 48093); 1 ♂, 4 ♀♀, 10 km north of Katherine (SAM 27270); 8 ♀♀, Willeroo Station via Katherine (SAM 48094): from *Osphranter antilopinus* (Gould): 3 ♂♂, 4 ♀♀, 50 km south-west of Katherine (SAM 48095).
Site in host: Stomach.
Representative DNA sequences: Sequences generated from one specimen (SAM voucher number 48120) were submitted to the GenBank database under the accession numbers MH105784 (ITS1) and MH105785 (ITS2).
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new

species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Cloacina spearei* n. sp. is urn:lsid:zoobank.org:act: act:5936AE14-9146-43EB-AC6F-85E4B4DFD85E. *Etymology*: The species is named after the late Professor R. Speare, formerly of James Cook University, Queensland, who first collected this species near Katherine in 1994.

Description (Figs. 1 11)

General. Small, whitish nematodes; cervical cuticle not inflated; body covered with prominent transverse annulations *c.*0.020 apart. Cephalic collar present; 4 submedian cephalic papillae, bipartite, projecting anteriorly from cuticle of cephalic collar, 0.009 long; proximal segment bulbous, 0.005 long, distal segment sub-cylindrical, shorter, 0.004 long; 2 lateral amphids present. Buccal capsule shallow, sub-symmetrical in lateral views, sub-circular in apical view, anterior margin not lobed; walls in lateral view thicker at anterior extremities; leaf crown consisting of 8 elements, each arising from full length of buccal capsule; leaf crown elements recurved at tips, lacking lip-like inflation of perioral cuticle externally; dorsal lobe of oesophagus with low, dome-shaped, bilobed tooth projecting slightly into lumen, not clearly visible in lateral views; sub-ventral sectors of oesophagus each with small, triangular lancet projecting into buccal capsule. Oesophagus claviform, with very slight preneural swelling; lining lacking bosses; diminutive dorsal denticle anterior to nerve ring, not clearly visible in all specimens. Nerve-ring in mid-oesophageal region; deirid in anterior oesophageal region; excretory pore in posterior oesophageal region.

Male [Measurements from 5 specimens ex *M. r. woodwardi*.] Length 5.84 6.35 (6.07); maximum width 0.24 0.34 (0.29); buccal capsule 0.025 0.030 (0.027) wide, 0.005 0.010 (0.009) deep; oesophagus 0.48 0.54 (0.50) long; nerve-ring 0.21 0.25 (0.23), excretory pore 0.43 0.48 (0.46) and deirid 0.080 0.090 (0.087) from anterior extremity. Bursa with ventral, lateral and dorsal lobes poorly differentiated; cleft between ventral lobes; dorsal lobe with slight posterior indentation; dorsal lobe no longer than lateral lobes; internal surface of bursa with fine, irregular striae. Ventro-ventral and ventro-lateral rays apposed, reaching margin of bursa; externo-lateral ray divergent from lateral trunk, not reaching margin of bursa; medio-lateral and postero-lateral rays apposed, reaching margin of bursa; externo-dorsal ray arising from lateral trunk, not reaching margin of bursa; dorsal ray stout at origin, dividing at mid length; 2 pairs of terminal branchlets, external branchlets usually but not invariably shorter than internal branchlets; no terminal branchlets reach margin of bursa. Genital cone with prominent conical ventral lobe

bearing papilla 0; with paired inflations of internal surface of bursa on either side; dorsal lip of genital cone with pair of appendages bearing papillae 7. Spicules elongate, alate, 2.05 2.60 (2.22) long; anterior extremities irregulary knobbed, distal tips sharply pointed; no spicules exerted to allow observation of termination of ala; gubernaculum prominent, 0.020 0.030 (0.027) long, wider than long in dorso-ventral views; cordiform internal and elongate external thickenings of spicule sheaths present anterior to gubernaculum.

Female [Measurements of 10 specimens from *M. r. woodwardi*.] Length 6.14 9.70 (7.35); maximum width 0.30 0.43 (0.35); buccal capsule 0.030 (0.030) wide, 0.010 (0.010) deep; oesophagus 0.48 0.61 (0.54) long; nerve-ring 0.21 0.26 (0.23), excretory pore 0.43 0.56 (0.48) and deirid 0.060 0.100 (0.080) from anterior extremity. Tail elongate, conical, 0.31 0.36 (0.32) long; vulva 0.47 0.61 (0.54) from posterior extremity; distal vagina straight, forming 1 or 2 coils immediately before entering ovejector; direct length from vulva to ovejector 0.45 0.67 (0.56). Eggs absent.

Measurements of specimens from *O. antilopinus*

Male [Measurements of 4 ♂♂ specimens.] Length 5.75 8.14 (7.01); maximum width 0.30 0.36 (0.28); buccal capsule 0.025 0.030 (0.027) wide, 0.010 (0.010) deep; oesophagus 0.50 0.56 (0.53) long; nerve-ring 0.20 0.26 (0.23), excretory pore 0.42 0.57 (0.47) and deirid 0.070 0.100 (0.083) from anterior extremity; spicules 2.20 2.44 (2.33) long; gubernaculum, 0.020 0.030 (0.026) long.

Female [Measurements from 3 ♀♀ specimens.] Length 7.08 9.35 (8.28); maximum width 0.34 0.49 (0.40); buccal capsule 0.030 (0.030) wide, 0.010 (0.010) deep; oesophagus 0.56 0.68 (0.60) long; nerve-ring 0.22 0.23 (0.23), excretory pore 0.43 0.51 (0.47) and deirid 0.060 0.080 (0.070) from anterior extremity. Tail 0.30 0.33 (0.31) long; vulva 0.55 0.66 (0.60) from posterior extremity; vagina 0.65 0.75 (0.70) long. Eggs absent.

Molecular data

One specimen (SAM voucher number 48120) was sequenced (ITS1, 376 bp GenBank accession numbers MH105784; ITS2, 226 bp, MH105785). The sequence for the new species was most

similar to *C. dirce* Beveridge, 1998 (95.7% for ITS1, 97.3% for ITS2; comparison with sequence MF284683 available from GenBank) and *C. dindymene* Beveridge, 1998 (93.1% for ITS1, 94.6% for ITS2; comparison with sequence MF284682 available on GenBank).

Remarks

Using the key to species of *Cloacina* in Beveridge (1998), covering 90 species, the specimens described above differ from all congeners other than *C. cornuta* and *C. dirce* in the possession of anteriorly directed cephalic papillae, a buccal capsule with a width to length ratio of less than 4:1 together with a straight anterior margin, lacking lips, and an oesophagus lacking bosses and transverse folds lining the lumen. The species differs from both *C. cornuta* and *C. dirce* in lacking a prominent inflation of the cervical cuticle and in spicule lengths which are 1.38 1.62 mm in *C. cornuta* and 3.48 3.95 mm in *C. dirce*, compared with 2.14 2.60 mm in the new species.

The same characters as well as the anterior position of the deirid, the non-recurrent vagina and the terminal origin of the branchlets of the dorsal ray distinguish *C. spearei* n. sp. from the species described subsequently from the quokka *Setonix brachyurus* (Quoy & Gaimard) and banded hare wallaby *Lagostrophus fasciatus* (Péron & Lesueur), from Western Australia by Beveridge (1999, 2014) and those from Papua New Guinea (Beveridge & Speare, 1999; Beveridge, 2002), except for *C. papuensis* Beveridge, 1992. In *C. papuensis*, the spicules are 1.84 2.26 mm long compared with 2.14 2.60 mm in the new species and the female tail is short and conical, 0.14 0.22 mm long compared with the elongate tail, 0.31 0.36 mm long in the new species. A minor difference is the pair of lancets arising from the sub-ventral sectors of the oesophagus compared with single lancets in the new species. This same array of characters differentiates *C. spearei* n. sp. from the remaining described species of *Cloacina* (see Beveridge et al., 2014a, b; Shuttleworth et al., 2016a).

The specimens from *O. antilopinus* were identical with those from *O. r. woodwardi* and all specimens were collected in the Katherine-Victoria River district of the Northern Territory. The measurements from the different host species have been presented separately in support of the claim that all the specimens described here are conspecific.

Osphranter robustus and *O. antilopinus* together are parasitised by more species of *Cloacina* than any other macropodid host species (Beveridge, 1998; Beveridge et al., 2002, 2014b; Shuttleworth et al., 2016b). Among the nematode species present in these two host species, the new species described above most closely resembles *C. macropodis* Johnston & Mawson, 1938 and *C. johnstoni* Shuttleworth, Jabbar & Beveridge, 2016 (see Beveridge, 1998;

Shuttleworth et al., 2016a). The specimens described here were compared with representatives of both of these species and the most obvious difference was the non-recurrent vagina in the present species, the vagina being prominently recurrent in the two species previously described (Beveridge, 1998; Shuttleworth et al., 2016a). In addition, both *C. macropodis* and *C. johnstoni* lack an oesophageal denticle, although it can be difficult to detect in *C. spearei* n. sp., and both *C. macropodis* and *C. johnstoni* have bosses lining the oesophagus which are again lacking in the new species. There are also differences in the shape of the cephalic papillae.

Cloacina spearei n. sp. has been found only in the northern subspecies of the common wallaroo, *O. r. woodwardi*, and has not been encountered in relatively extensive collections of *O. r. robustus* or *O. r. erubescens* from other parts of the continent (Beveridge et al., 2014a).

***Cloacina longibursata* Beveridge n. sp.**

Type-host: *Osphranter antilopinus* (Gould) (Marsupialia: Macropodidae).
Other host: *Osphranter robustus woodwardi* (Thomas) (Marsupialia: Macropodidae).
Type-locality: 70 km south of Maningrida, Northern Territory, Australia.
Other localities: Northern Territory: 8 km north of Mataranka; 50 km south-west of Katherine ; 31 km north of Katherine; 10 km north of Katherine; Mount Smit.
Type-material: Holotype ♂ from stomach of *Osphranter antilopinus* (Gould), 70 km south of Maningrida, Northern Territory, Australia (SAM 48096); allotype ♀, same data (SAM 48097); paratypes: 3 ♂♂, 5 ♀♀, same data (SAM 48098).
Material examined: types; voucher specimens: from *O. antilopinus*, Northern Territory: 1 ♂, 8 km north of Mataranka (SAM 48099); 1 ♀, 50 km south-west of Katherine (SAM 48100). From *Osphranter robustus woodwardi*, Northern Territory: 1 ♂, 31 km north of Katherine (SAM 48101); 1 ♀, 10 km north of Katherine (SAM 48102); 1 ♂, 1 ♀, Mount Smit (SAM 48103).
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Cloacina longibursata* n. sp. is urn:lsid:zoobank.org:act:4B68CB56-FEA1-4D40-93A3-6CCB4F20110D.
Etymology: The specific name relates to the extremely elongated dorsal lobe of the bursa of this species.

Description (Figs. 12 21)

General. Small, whitish nematodes; cervical cuticle not inflated; body covered with prominent transverse annulations *c.*0.006 apart. Cephalic collar present; 4 submedian cephalic papillae present, bipartite, projecting anteriorly from cuticle of cephalic collar, 0.006 long; proximal segment slightly bulbous, 0.005 long, distal segment tiny, sub-cylindrical, 0.001 long; 2 lateral amphids present. Buccal capsule shallow, sub-symmetrical in lateral views, sub-circular in apical view, anterior margin not lobed; walls in lateral view of uniform thickness; leaf crown consisting of 8 elements, each arising from full length of buccal capsule; leaf crown elements recurved at tips, lacking lip-like inflation of perioral cuticle externally; dorsal lobe of oesophagus with low, dome-shaped, bi-lobed tooth projecting slightly into lumen, not clearly visible in lateral views; sub-ventral sectors of oesophagus each with pair of small, triangular lancets projecting into buccal capsule. Oesophagus claviform, without preneural swelling; lining lacking bosses; denticles absent. Nerve-ring in anterior oesophageal region; deirid in anterior oesophageal region, anterior to nerve-ring; excretory pore in posterior oesophageal region.

Male [Measurements from 5 specimens from *O. antilopinus*.] Length 3.40 3.82 (3.54); maximum width 0.16 0.21 (0.18); buccal capsule 0.015 0.020 (0.016) wide, 0.005 0.010 (0.008) deep; oesophagus 0.37 0.40 (0.38) long; nerve-ring 0.17 0.23 (0.19), excretory pore 0.20 0.33 (0.24) and deirid 0.050 0.055 (0.053) from anterior extremity. Bursa with ventral and lateral lobes poorly differentiated; dorsal lobe with slight posterior indentation; dorsal lobe much longer than lateral lobes; internal surface of bursa with fine, irregular striae. Ventro-ventral and ventro-lateral rays apposed, reaching margin of bursa; externo-lateral ray divergent from lateral trunk, reaching margin of bursa; medio-lateral and postero-lateral rays apposed, almost reaching margin of bursa; externo-dorsal ray arising from lateral trunk, long, slender, not reaching margin of bursa; dorsal ray elongate, slender at origin, dividing at mid length; 2 pairs of terminal branchlets; external branchlets much shorter than internal branchlets arising soon after principal bifurcation, not reaching margin of bursa; internal branchlets elongate sub-parallel, almost reaching margin of bursa. Genital cone with prominent conical ventral lobe bearing papilla 0; dorsal lip of genital cone with pair of appendages bearing papillae 7. Spicules elongate, alate, 1.35 1.47 (1.40) long; anterior extremities irregulary knobbed, distal tips sharply pointed; alae transversely striated, diminish in width towards spicule tip; gubernaculum prominent, 0.015 0.020 (0.016) long, cordiform internal and elongate external thickenings of spicule sheaths present anterior to gubernaculum.

Female [Measurements of 5 specimens from *O. antilopinus*.] Length 3.16 6.17 (4.30); maximum width 0.18 0.35 (0.23); buccal capsule 0.015 0.030 (0.018) wide, 0.005 0.010 (0.006) deep; oesophagus 0.40 0.48 (0.42) long; nerve-ring 0.17 0.23 (0.20), excretory pore 0.22 0.41 (0.29) and deirid 0.050 0.070 (0.060) from anterior extremity. Tail elongate, conical, 0.15 0.20 (0.18) long; vulva 0.32 0.41 (0.36) from posterior extremity; vagina sinuous, length 0.52 0.75 (0.60). Eggs absent.

Remarks

Immediately distinguishing features of this species are the elongate dorsal lobe of the bursa which is much longer than the lateral lobes and the elongate, sub-parallel internal branchlets of the dorsal ray. A series of known species have an elongate dorsal lobe of the bursa, but in these species, the external branchlets arise prior to the principal bifurcation rather than after it as is the case in the new species; these species are: *C. caballeroi* Mawson, 1977; *C. cretheis* Beveridge, 2002; *C. cunctabunda* Beveridge, 2002; *C. enyo* Beveridge, 1998; *C. erigone* Beveridge, 2002; *C. ips* Beveridge 1998; *C. procris* Beveridge, 2002; and *C. syphax* Beveridge & Speare, 1999. Of the species with an elongate dorsal lobe of the bursa and with the external branchlets arising posterior to the principal bifurcation, the current species is distinguishable as follows: from *C. chiron* Beveridge, 1999, in which the distal segments of the cephalic papillae are globose and much larger than the proximal lobes; from *C. cloelia* Beveridge, 1998 in lacking oesophageal bosses and in having a simple, pointed spicule tip rather than the foot-shaped spicule tip present in *C. cloelia*; from *C. leto* Beveridge, 1998 in lacking oesophageal bosses and a dorsal oesophageal denticle; from *C. mundayi* Mawson, 1972 in lacking oesophageal bosses, a very shallow buccal capsule and six rather than eight leaf crown elements; from *C. polymela* Beveridge, 2002 in lacking an undulating anterior margin of the buccal capsule. For these reasons, the species described here is considered to be new.

***Cloacina crassicaudata* Beveridge n. sp.**

Syns *Cloacina cornuta* (Davey & Wood, 1938) *sensu* Beveridge (1998), *pro parte*.

Type-host: *Osphranter robustus woodwardi* (Thomas) (Marsupialia: Macropodidae).
Other host: *Osphranter antilopinus* (Gould) (Marsupialia: Macropodidae).
Type-locality: 46 km west of Victoria River, Northern Territory.

Other localities: Northern Territory: Katherine; 8 km north of Katherine; 10 km north of Katherine; 30 km north of Katherine; 18 km south-west of Katherine; 40 km south of Katherine; 5 km north of Mataranka; 8 km north of Mataranka; Willeroo Station via Katherine; Pine Creek; 4 km south of Emerald Springs; Newry Station via Timber Creek; Mount Smit; Jabiluka; Queensland: Charters Towers; Harvest Home Station via Charters Towers; Fletcher View Station via Charter Towers; Mount Surprise; Western Australia: Napier Downs Station via Derby.

Type-material: Holotype ♂ from stomach of *Osphranter robustus woodwardi* (Thomas), 46 km west of Victoria River, Northern Territory (SAM 48104); allotype ♀, same data, (SAM 48105); paratypes, 5 ♂♂, 17 ♀♀, same data, (SAM 48106).

Material examined: types; voucher specimens: from *Osphranter r. woodwardi*: Northern Territory: 13 ♂♂, 13 ♀♀, Katherine (SAM 25223); 21 ♂♂, 24 ♀♀, 18 km south-west of Katherine (SAM 48108); 20 ♂♂, 29 ♀♀, 40 km south of Katherine (SAM 48111); 5 ♂♂, 1 ♀, 10 km north of Katherine (SAM 27267, 27278); 7 ♂♂, 9 ♀♀, 5 km north of Mataranka (SAM 48114); 19 ♂♂, 54 ♀♀, Willeroo Station via Katherine (SAM 48110); 2 ♂♂, 5 ♀♀, 30 km north of Katherine (SAM 48107); 3 ♂♂, Pine Creek (SAM (48112); 21 ♂♂, 47 ♀♀, 4 km south of Emerald Springs (SAM 48113); 5 ♂♂, 5 ♀♀, Newry Station via Timber Creek (SAM 48109); 12 ♂♂, 17 ♀♀, Mount Smit (SAM 32732); 1 ♂, 1 ♀, Jabiluka (SAM 25078); Queensland: 7 ♂, 13 ♀♀, Charters Towers (SAM 25059, 25354); 2 ♂, 33 ♀♀, Harvest Home Station via Charters Towers (SAM 13505, 25355); 2 ♀♀, Fletcher View Station via Charter Towers (SAM 25090); 7♂♂, 7 ♀♀, Mount Surprise (SAM 29085); Western Australia: 8 ♂♂, 5 ♀♀, Napier Downs via Derby (SAM 25081). From *Osphranter antilopinus*: Northern Territory: 15 ♂♂, 32 ♀♀, 8 km north of Mataranka (SAM 48115-6); 20 ♂♂, 20 ♀♀, Katherine (SAM 32703); 1 ♀, 8 km north of Katherine (SAM 32709); Queensland: 15 ♂♂ , 15 ♀♀, Mt Surprise (SAM 25907, 25908); Western Australia: 1 ♂, 12 ♀♀, Napier Downs Station via Derby (SAM 23833);

Representative DNA sequences: Sequences generated from a single specimen of the new species (SAM 48121) were submitted to the GenBank database under the accession numbers MH105786 (ITS1) and MH105787 (ITS2).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Cloacina crassicaudata* n. sp. is urn:lsid:zoobank.org:act:E054CDA8-5883-46C484DE-BBD5036C380C.

Etymology: The specific name is given on account of the broad tail in this species.

Description (Figs. 22 33)

General. Small, whitish nematodes; cervical cuticle not inflated; body covered with prominent transverse annulations *c.*0.012 apart. Cephalic collar present; 4 submedian cephalic papillae prominent, bipartite, projecting anteriorly from cuticle of cephalic collar, 0.035 long; proximal segment bulbous, slightly asymmetrical, 0.020 long, distal segment conical, 0.015 long; 2 lateral amphids present. Buccal capsule shallow, sub-symmetrical in lateral views, sub-circular in apical view, anterior margin not lobed; walls in lateral view not thicker at anterior extremities; leaf crown consisting of 8 elements, each arising from full length of buccal capsule; leaf crown elements recurved at tips, lacking lip-like inflation of perioral cuticle externally; dorsal lobe of oesophagus with low- dome-shaped tooth projecting slightly into lumen, not clearly visible in lateral views; sub-ventral sectors of oesophagus lacking lancets projecting into buccal capsule. Oesophagus claviform, with very slight preneural swelling; lining with bosses extending halfway to nerve-ring; dorsal denticle anterior to nerve ring. Nerve-ring in mid-oesophageal region; deirid in anterior oesophageal region; excretory pore in posterior oesophageal region.

Male [Measurements of 10 specimens from *O. r. woodwardi*.] Length 5.50 7.70 (6.45); maximum width 0.23 0.36 (0.30); buccal capsule 0.030 0.035 (0.032) wide, 0.010 (0.010) deep; oesophagus 0.51 0.64 (0.58) long; nerve-ring 0.20 0.29 (0.25), excretory pore 0.33 0.47 (0.43) and deirid 0.060 0.100 (0.070) from anterior extremity. Bursa with ventral, lateral and dorsal lobes poorly differentiated; cleft between ventral lobes; dorsal lobe with slight posterior indentation; dorsal lobe no longer than lateral lobes; internal surface of bursa with fine, irregular striae. Ventro-ventral and ventro-lateral rays apposed, reaching margin of bursa; externo-lateral ray divergent from lateral trunk, not reaching margin of bursa; medio-lateral and postero-lateral rays apposed, reaching margin of bursa; externo-dorsal ray arising from lateral trunk, slender, not reaching margin of bursa; dorsal ray slender at origin, dividing at mid length; 2 pairs of terminal branchlets, external branchlets similar in length to internal branchlets; no terminal branchlets reach margin of bursa. Genital cone with prominent conical ventral lobe bearing papilla 0; with paired inflations of internal surface of bursa on either side; dorsal lip of genital cone with pair of appendages bearing papillae 7. Spicules elongate, alate, 1.35 1.70 (1.48) long; anterior extremities irregulary knobbed, distal tips blunt; termination of ala relatively abrupt, anterior to spicule tip; gubernaculum prominent, 0.020 0.040 (0.030) long; cordiform internal and elongate external thickenings of spicule sheaths present anterior to gubernaculum.

Female [Measurements of 10 specimens from *O. r. woodwardi.*] Length 6.1 10.6 (7.4); maximum width 0.27 0.58 (0.36); buccal capsule 0.035 0.040 (0.037) wide, 0.010 (0.010) deep; oesophagus 0.57 0.73 (0.64) long; nerve-ring 0.25 0.32 (0.29), excretory pore 0.37 0.54 (0.45) and deirid 0.050 0.090 (0.070) from anterior extremity. Tail short, conical, 0.05 0.10 (0.08) long; vulva 0.17 0.25 (0.21) from posterior extremity; vagina short, straight, forming single coil before entering ovejector; length from vulva to ovejector 0.30 0.40 (0.36). Eggs absent.

Molecular data

A single specimen of the new species was sequenced (SAM 48121) (ITS1, 399 bp, GenBank accession number MH105786; ITS2, 227 bp, MH105787) and compared with three specimens of *C. cornuta* from *N. agilis* (SAM 48119) (GenBank registration numbers for ITS1 and ITS2: MH105788-MH105793). Excluding insertions and deletions, there were 38 bp differences between the sequences from the nematodes from the two host species in the ITS1 (Table 1) and 33 bp differences in the ITS2 (Table 2). Limited variation was noted in the three sequences of *C. cornuta*, with variation at base number 178 in the ITS1 sequences and base numbers 10 and 135 in the ITS2 sequences (Tables 1, 2).

Remarks

Specimens of this species from *O. robustus* and *O. antilopinus* in Queensland were initially attributed to *C. cornuta* by Beveridge (1998) in spite of the obvious anomalies in host distribution. Until then, *C. cornuta* had been considered to be a species specific to *N. agilis* (see Speare et al., 1983). Re-examination of the material reported by Beveridge (1998) as well as new collections from the Northern Territory listed above, suggest that an independent species is present in *O. robustus* and *O. antilopinus* and is described here as a new species.

In this species, the very short, conical female tail together with the single dorsal denticle and the convoluted but non-recurrent vagina immediately distinguish the species from most congeners. Of the species possessing these features, *C. crassicaudata* n. sp. is distinguishable from *C. australis* (Yorke & Maplestone, 1926) in lacking enlarged bosses at the anterior extremity of the oesophagus; from *C. dis* Beveridge, 1998 in lacking a spirally-coiled vagina; from *C. longispiculata* Johnston & Mawson, 1939 in the anterior position of the excretory pore (posterior to the oesophago-intestinal junction in *C. longispiculata*), and from *C. erigone*

Beveridge, 2002 in the position of the deirid (posterior-oesophageal in *C. erigone*) and in the morphology of the spicule tip (lobed in *C. erigone*) (Beveridge, 1998, 2002).

The new species is therefore most closely related to *C. cornuta* as indicated by Beveridge (1998). However, it differs from *C. cornuta* in having oesophageal bosses extending half way to the nerve-ring (absent in *C. cornuta*), in lacking a prominent inflation of the cervical cuticle (present in *C. cornuta*), in having a bulbous proximal segment of the cephalic papillae (cylindrical in *C. cornuta*), a less prominent dorsal oesophageal tooth, a circular buccal capsule in transverse section (sub-triangular in *C. cornuta*), the excretory pore anterior to the oesophago-intestinal junction rather than posterior to it in *C. cornuta* and blunt spicule tips with the ala diminishing in size abruptly, compared with sharp tips and an ala gradually diminishing in size towards the tip in *C. cornuta* (see figure 194 in Beveridge, 1998). These morphological differences are relatively subtle but are supported by substantial genetic differences (Tables 1, 2).

As this species can be differentiated from it congeners based on both morphological and genetic characters, it is considered to be new.

Discussion

The antilopine wallaroo, *O. antilopinus*, and common wallaroo, *O. robustus*, are sympatric across much of northern Australia (Eldridge et al., 2014) and are parasitised by a wide range of species of *Cloacina* (Table 3) which exhibit several distinct patterns of geographical distribution. *Cloacina spearei* n. sp. and *C. longibursaris* n. sp., both of which appear to be restricted the northern region of the Northern Territory, exhibit a similar geographical range to *C. tyro* Beveridge, 1998. Two additional species, *C. eleithyia* Beveridge, 1998 and *C. polyxena* Beveridge, 1998 also occur in both species of wallaroo in the Northern Territory, but their distribution extends westward into the Kimberley Region of Western Australia (Table 3). By contrast, *C. crassicaudata* n. sp. is distributed in Western Australia, the Northern Territory and Queensland, resembling the patterns seen with *C. dindymene* Beveridge, 1998, *C. dirce*, and *C. ixion* Beveridge, 1998. *Cloacina frequens* Johnston & Mawson, 1938 is found in the Northern Territory and Queensland. Finally, several of the species from northern Australia have restricted distributions, *C. dis* in the Charters Towers region of northern Queensland (Beveridge, 1998), *C. epona* Beveridge, 1998 in the Mount Isa region of northern Queensland (Shuttleworth et al., 2016b) and *C. atthis* Beveridge, 2014 in the Pilbara region of Western Australia (Beveridge et al., 2014b).

The distribution of *C. eilethyia*, *C. polyxena*, *C. tyro*, *C. spearei* and *C. longibursaris* being restricted to the Northern Territory and Western Australia is potentially explicable by the Carpentaria Barrier at the base of the Gulf of Carpentaria, separating genetically distinct populations of both *O. antilopinus* and *O. robustus* from those in Queensland (Eldridge et al., 2014; Wadley et al., 2016). However, the remaining species, *C. dindymene*, *C. dirce*, *C. ixion* and *C. crassicaudata* occur in north-eastern Queensland as well as in the Northern Territory and Western Australia. All records of these species from Queensland are from *O. robustus robustus*, based on molecular identifications of the host from a small number of representative localities (Richardson & Sharman, 1976; Eldridge et al., 2014). By contrast, none of these species have been found in *O. r. erubescens*, including 12 representatives of this sub-species examined in north-western Queensland between Hughenden and Mount Isa (Fig. 34) (unpublished observations), that is in the region of the Carpentaria Barrier. Here, other species of *Cloacina* are present. This apparent disjunction in their distribution, which could be resolved by additional collecting in the intervening regions, may be due to environmental influences as well as to host genetic relationships but clearly requires further exploration, in particular since the distributions of *O. r. robustus* and *O. r. erubescens* in north-eastern Queensland are not well defined (Van Dyck & Strahan, 2008).

In each of the three new species described here, although female nematodes were mature, none was gravid. This feature was reported by Beveridge (1998) for *C. crassicaudata* (as *C. cornuta*) and is in contrast to the related species, *C. cornuta* from *N. agilis*, in which females are commonly gravid. The reasons for this are unknown. In southern Australia, the related cloacinine nematodes *Labiosimplex longispicularis* (Wood, 1929), found in the stomach of red kangaroos, *Osphranter rufus* (Desmarest) and *Labiomultiplex eugenii* (Johnston & Mawson, 1940) in the stomach of the tammar wallaby, *Notamacropus eugenii* (Desmarest), exhibit seasonal patterns of fecundity, with gravid females present only in the cooler months of the year when survival of eggs and larvae in the external environment is likely to be favourable (Mykytowycz & Dudzinski, 1965; Smales & Mawson, 1978). Comparable studies have not been conducted in northern Australia, but a similar phenomenon may be involved. All collecting reported here was carried out in the dry season; collections during the wet season, the season likely to be better suited to nematode transmission, may reveal the presence of gravid nematodes.

The present study suggests that more extensive collecting of parasites of *O. robustus* and *O. antilopinus* in various areas of Australia will reveal the presence of even more species of *Cloacina* based on the relatively localised distributions of several species to date such as *C. atthis* from the Pilbara region of Western Australia and *C. epona* from north-western Queensland. A study of the helminth parasites of the wallaby *Wallabia bicolor* (Desmarest) over

its entire geographical range (Beveridge, 2016) highlighted the significance of regional variation in its parasite fauna. The same is likely to be true for the wallaroos.

Acknowledgements The collections reported here were made with financial assistance from the Australian Research Council and the Australian Biological Resources Study. We would like to thank Ben Murphy for collection of material from Maningrida.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Material was collected under permits from the Queensland National Parks and Wildlife Service (T00436, T1131), the Northern Territory Department of Primary Industry (15747) and the Western Australian Department of Environment and Conservation (SF007407). All applicable institutional, national and international guidelines for the care and use of animals were followed.

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Captions to figures

Figs. 1–11 *Cloacina spearei* n. sp. 1, Anterior region, right lateral view; 2, Anterior extremity, right lateral view; 3, Anterior extremity, ventral view; 4, Anterior extremity, apical view; 5, Cephalic papilla; 6, Optical transverse section through buccal capsule; 7, Oesophageal denticle, right lateral view; 8, Oesophageal denticle, ventral view; 9, Bursa, apical view; 10, Vagina and ovejector; 11, Female tail, right lateral view. *Scale-bars*: 1, 9, 10, 11, 0.1 mm; 2–4, 6, 7, 8, 0.01 mm; 5, 0.005 mm

Figs. 12–21 *Cloacina longibursaris* n. sp. 12, Anterior region, left lateral view; 13, Anterior extremity, right lateral view; 14, Anterior extremity, ventral view; 15, Anterior extremity, apical view; 16, Optical transverse section through buccal capsule; 17, Bursa, right lateral view; 18, Bursa, ventral view; 19, Spicule tip, lateral view; 20, Female tail, left lateral view; 21, Vagina and ovejector, left lateral view. *Scale-bars*: 12, 17, 18, 20, 21, 0.1 mm; 13–16, 19, 0.01 mm

Figs. 22–33 *Cloacina crassicaudata* n. sp. 22, Anterior region, right lateral view; 23, Anterior extremity, right lateral view; 24, Anterior extremity, ventral view; 25, Anterior extremity, apical view; 26, Optical transverse section through buccal capsule; 27, Oesophageal denticle, right lateral view; 28, Bursa, right lateral view; 29, Bursa, apical view; 30, Spicule tips, ventral view; 31, Gubernaculum, ventral view; 32, Female tail, right lateral view; 33, Vagina and ovejector, right lateral view. *Scale-bars*: 22, 28, 29, 31–33, 0.1 mm; 23–27, 30, 0.01 mm

Fig. 34 Distribution of *Cloacina crassicaudata* n. sp. in *Osphranter robustus* (closed circles) and *O. antilopinus* (triangles) together with localities at which *O. robustus* has been collected but at which no *C. crassicaudata* was found (open circles). Dotted lines show the distributions of the three mainland subspecies of *O. robustus*, *M. r. robustus*, *M. r. erubescens* and *M. r. woodwardi* based on Van Dyck & Strahan (2008)

Table 1 Base pair differences in the ITS1 sequences of a specimen of *Cloacina crassicaudata* n. sp. and three specimens of *C. cornuta* (Davey & Wood, 1938)

***Cloacina* spp. (Genbank ID)**	**3**	**10**	**19**	**22**	**23**	**56**	**59**	**60**	**84**	**98**	**112**	**133**	**134**	**136**	**142**	**178**	**179**	**215**	**216**	**235**
C. crassicaudata (MH105786)	A	T	G	A	A	T	G	A	G	T	G	C	G	T	A	T	G	T	G	T
C. cornuta (MH105788)	C	A	A	G	G	C	C	G	A	A	A	T	A	A	T	T	A	G	A	C
C. cornuta (MH105789)	C	A	A	G	G	C	C	G	A	A	A	T	A	A	T	T	A	G	A	C
C. cornuta (MH105790)	C	A	A	G	G	C	C	G	A	A	A	T	A	A	T	C	A	G	A	C

	236	**238**	**242**	**245**	**246**	**247**	**255**	**275**	**286**	**290**	**291**	**295**	**314**	**317**	**321**	**323**	**346**	**348**
C. crassicaudata (MH105786)	T	C	T	C	G	G	T	T	G	G	A	C	A	T	A	C	A	T
C. cornuta (MH105788)	G	T	C	T	A	A	C	C	C	A	G	A	T	A	G	A	G	A
C. cornuta (MH105789)	G	T	C	T	A	A	C	C	C	A	G	A	T	A	G	A	G	A
C. cornuta (MH105790)	G	T	C	T	A	A	C	C	C	A	G	A	T	A	G	A	G	A

Table 2 Base pair differences in the ITS2 DNA sequences of a specimen of *Cloacina crassicaudata* n. sp. and three specimens of *C. cornuta* (Davey & Wood, 1938)

***Cloacina* spp. (GenBank ID)**	**10**	**28**	**59**	**62**	**65**	**68**	**91**	**105**	**107**	**109**	**114**	**120**	**121**	**122**	**123**	**124**	**125**	**126**	**127**
C. crassicaudata (MH105787)	C	A	A	G	C	A	A	A	C	T	T	C	A	T	A	G	T	C	C
C. cornuta (MH1057	C	G	C	A	G	G	C	G	T	C	C	T	T	C	G	T	C	T	A

91)																			
C. cornuta (MH105792)	A	G	C	A	G	G	C	G	T	C	C	T	T	C	G	T	C	T	A
C. cornuta (MH105793)	C	G	C	A	G	G	C	G	T	C	C	T	T	C	G	T	C	T	A

	128	**129**	**132**	**134**	**135**	**138**	**144**	**152**	**153**	**173**	**180**	**185**	**195**	**223**
C. crassicaudata (MH105787)	A	T	T	C	A	A	A	G	C	C	A	G	G	T
C. cornuta (MH105791)	G	C	C	T	-	T	T	A	T	T	G	A	A	G
C. cornuta (MH105792)	G	C	C	T	-	T	T	A	T	T	G	A	A	G
C. cornuta (MH105793)	G	C	C	T	G	T	T	A	T	T	G	A	A	G

Table 3 Valid species of *Cloacina* von Linstow, 1898 parasitic in common and antilopine wallaroos, *Osphranter* spp., in Australia

Nematode species	**Host species**	**Distribution**
C. atthis Beveridge, 2014	*O. robustus*	WA
C. crassicaudata n. sp.	*O. robustus*, *O. antilopinus*	NT, QLD, WA
C. clymene Beveridge, 1998	*O. robustus*	NT, SA
C. communis Johnston & Mawson, 1938	*O. robustus*, *O. antilopinus*	NSW, NT, QLD, SA
C. curta Johnston & Mawson, 1938	*O. robustus*	NT, SA, WA
C. daveyi Mawson, 1977	*O. robustus*	NT, SA
C. dindymene Beveridge, 1998	*O. robustus*, *O. antilopinus*	NT, QLD, WA
C. dirce Beveridge, 1998	*O. robustus*, *O. antilopinus*	NT, QLD, WA
C. dis Beveridge, 1998	*O. robustus*	QLD
C. echidne Beveridge, 1998	*O. robustus*, *O. antilopinus*	NSW, QLD, SA
C. eileithyia Beveridge, 1998	*O. robustus*, *O. antilopinus*	NT, WA
C. epona Beveridge, 1998	*O. robustus*	QLD
C. feronia Beveridge, 1998	*O. robustus*	NSW, QLD, SA
C. frequens Johnston & Mawson, 1938	*O. robustus*, *O. antilopinus*	NT, QLD
C. herceus Beveridge, 1998	*O. robustus* (usual host *Macropus giganteus* Shaw)	NSW, QLD
C. hydriformis Johnston & Mawson, 1938	*O. robustus* (usual host *Osphranter rufus* (Desmarest))	NSW
C. ixion Beveridge, 1998	*O. robustus*, *O. antilopinus*	NT, QLD, WA
C. johnstoni Shuttleworth, Jabbar & Beveridge, 2016	*O. robustus*	NT, QLD, WA
C. longelabiata Johnston & Mawson, 1938	*O. robustus*	NT, SA
C. longibursata n. sp.	*O. robustus*, *O. antilopinus*	NT
C. macropodis Johnston & Mawson, 1938	*O. robustus*, *O. antilopinus*	NSW, NT, QLD, SA, WA
C. nike Beveridge, 1998	*O. robustus*, *O. antilopinus*	QLD
C. parva Johnston & Mawson, 1938	*O. robustus*, *O. antilopinus*	NSW, NT, QLD, SA, WA
C. phaethon Beveridge, 1998	*O. robustus*, *O. antilopinus*	NSW, NT, QLD, SA, WA
C. polyxena Beveridge, 1998	*O. robustus*, *O. antilopinus*	NT, WA

C. spearei n. sp.	*O. robustus*, *O. antilopinus*	NT
C. typhon Beveridge, 1998	*O. robustus*, *O. antilopinus*	QLD, WA
C. tyro Beveridge, 1998	*O. robustus*	NT

Abbreviations: NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; WA, Western Australia.