EUCOLEUS POTOROI SP. NOV. (NEMATODA: TRICHINELLOIDEA: CAPILLARIIDAE) FROM THE BRONCHI AND BRONCHIOLES OF CAPTIVE POTOROID MARSUPIALS

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Summary
Eucoleus potoroi sp. nov. is described from the bronchi and bronchioles of the rufous rat kangaroo, Aepyprymnus rufescens (Gray, 1837), the long-nosed potoroo, Potorous tridactylus (Kerr, 1792) and the woylie, Bettongia penicillata Gray, 1837 in a captive colony at Cowan, New South Wales. It is distinguished from all other species of Eucoleus Dujardin, 1845 from Australian native mammals by the immense length of the ejaculatory duct and the very large ratio of mean total length of oesophagus to mean total length of posterior body in males. The only known occurrence of the parasite in captive colonies and the pathology associated with infection suggest that the parasite might be a parasite of introduced or volant eutherians in abnormal hosts. However, comparative examination indicated that E. potoroi is distinct from Eucoleus aerophilus (Creplin, 1839) from the trachea of cats, from Pearsonema feliscati (Diesing, 1851) from the bladder of cats and from Calodium hepaticum (Bancroft, 1893) from the liver of rats, the only capillariids known from introduced eutherians in Australia. It is also distinct from Aonchotheca miniopterae (Thomas, 1959) comb. nov. from the stomach of the bat, Miniopterus schreibersii (Kuhl, 1817).

KEY WORDS: Nematoda, Capillariidae, Eucoleus, new species, potoroid marsupials, bronchi, captivity, Aonchotheca miniopterae comb. nov.

Introduction
Love and Reddacliff (1992) reported verminous bronchitis and bronchiolitis in captive colonies of Aepyprymnus rufescens (Gray, 1837), Potorous tridactylus (Kerr, 1792) and Bettongia penicillata Gray, 1837 at the University of New South Wales Field Station at Cowan and at Armidale, New South Wales. These conditions were associated with the presence of parasitic trichinelloid nematodes which I then recognised as Capillaria sp. nov. 7 (see also Spratt et al. 1991). The only known occurrence of the parasite in captive colonies of potoroids and the pathology associated with infection suggested that the parasite might be a known parasite of eutherian mammals in abnormal hosts. However, comparative examination indicated that this was not the case and the parasite is described herein as Eucoleus potoroi sp. nov.

Materials and Methods
Specimens were fixed in 10% neutral buffered formalin, stored in 70% ethanol and examined in lactophenol. Measurements were made using an ocular micrometer and are given in micrometres, unless otherwise stated, as the mean of n measurements followed by the range in parentheses. Drawings were made using a Zeiss Nomarski interference contrast microscope and drawing tube. Types and voucher specimens are deposited in the South Australian Museum, Adelaide (SAM), the Queensland Museum, Brisbane (QM) and in the wildlife parasite collection housed in the Australian National Wildlife Collection at CSIRO Sustainable Ecosystems, Canberra (W/L HC N----).

Some lungs of A. rufescens were transported in antibiotic saline, eggs harvested from bronchi and bronchioles, placed in 0.2% formalin in a closed McCartney bottle and aerated every second day. At 176 days some embryonated eggs were washed several times in distilled water and two 75-day-old male C57BL mice were dosed per os.
**Eucoleus potoroi**, sp. nov.

(Figs 1-13)

**Synonyms**

*Cappilaria* sp. n 7, Spratt, Beveridge & Walter, 1991: 32, 33, 70, W/L HC N1802, 2468, 2808, P108 (*Aepyprymnus rufescens, Bettongia penicillata, Potorous tridactylus*) (captive animals)

*Cappilaria* sp., Love & Reddacliff, 1992: 643-645, (same captive animals)

**Material examined:**

*Holotype* male: from epithelium of bronchi and bronchioles, *Aepyprymnus rufescens* (Gray, 1837), University of New South Wales Field Station, Cowan, New South Wales (captive colony), 22.i.1987, coll: G. Reddacliff & S. Barker, SAM AHC 34456. *Allotype* female: same data as holotype, SAM AHC 34457. *Paratypes* – all same data as holotype - 3 males: SAM AHC 34458; 3 males: QM G227649; 4 male anterior and 4 male posterior ends: SAM AHC 34459; 2 male anterior and 4 male posterior ends: QM G227650; 6 females: SAM AHC 34460; 6 females: QM G227651.

**Other material examined**

From *Aepyprymnus rufescens* bronchi and bronchioles University of New South Wales Field Station, Cowan, New South Wales (captive colony) male and female fragments, eggs (W/L HC N1802). From *Potorous tridactylus* bronchi and bronchioles University of New South Wales Field Station, Cowan, New South Wales (captive colony) third- and fourth stage larvae, moulting fourth-stage larvae (W/L HC N2468) (Material too auto lysed for description or illustration). From *Bettongia penicillata* University of New England, Armidale, New South Wales (captive colony) histological section of lung containing eggs (W/L HC PA108).

**Differential Diagnosis:**

Distinguished from all other species of *Eucoleus* in Australian native mammals except *E. gastricus* by the large size of males and females, the very large ratio of mean total length of oesophagus to mean length of posterior body in males, the breadth of the spicule throughout its length (with the exception of *E. breviductus*), the immense length of the ejaculatory duct (almost double that of *E. longiductus*), the posterior position of the vulva and greater length of the rectum in females. Distinguished from *E. gastricus* by much higher ratio of total length of oesophagus to total length of posterior body in males, the breadth of the spicule throughout its length, the shorter posterior cloaca, the immense length of the ejaculatory duct, the shorter body length in females, much shorter total oesophageal length, the anterior position of the vulva, much shorter vagina and rectum, and the fine, dense punctae ornamenting the egg shell, in contrast to the reticulate ornamentation in *E. gastricus*.

**Description**

Relatively long, thin nematodes, anterior extremity attenuated. Cuticle thin, with prominent transverse annulations. Lateral bacillary bands in both sexes, commencing as single column of pore openings, widening posteriorly to maximum 5-6 pore openings width in males and 8-10 pore openings width in females, remaining wide posteriorly in tail region. Lateral alae absent. Cephalic extremity minute, tapered, details of oral opening and cephalic papillae not determined. Oesophagus commencing as narrow muscular structure, broadening short distance posteriorly. Stichosome of 38-40 stichocytes of highly variable size, generally shortest anteriorly or immediately at oesophago-intestinal junction, longest mid- to near posterior stichosome, stichocytes overlapping at proximal and distal extremities, with large oval nuclei and numerous refringent granules.
Figs 1-13  *Eucoleus potoroi* sp. nov. from *Aepyprymnus rufescens*. 1. Egg. 2. Male, oesophago-intestinal (in) junction and stichosome (st), lateral view. 3. Stichocyte, from anterior stichosome. 4. Stichocyte, from mid-stichosome. 5. Stichocyte from posterior stichosome. 6. Male, junction of seminal vesicle (sv) with ejaculatory duct (ed), lateral view; note absence of sphincter muscle around seminal vesicle at point of entry to ejaculatory duct. 7. Male, spicule ventral view. 8. Male, posterior end, ventral view. 9. Spicule, illustrating longitudinal and lateral flexibility, ventral view. 10. Male, junction of ejaculatory duct (ed) and intestine (in) with anterior cloaca (ac), lateral view. 11. Male, tail showing spiny spicule sheath (ss) partially everted, ventral view. 12. Male, tail optical section, lateral view. 13. Male, tail, lateral view. Scale bars = 20\(\mu\)m, 1, 7, 8, 11, 12, 13, 50\(\mu\)m, 2, 3, 4, 5, 6, 9, 10.
EUCOLEUS POTOROI SP. NOV. FROM POTOROIDS

Stichocytes in males 187-397 long, 25-73 wide, in females 104-187 long, 25-83 wide. Junction of oesophagus and intestine conspicuous, without two large mesenchymal cells.

**Male** \( (n=7 \text{ plus 6 anterior and 8 posterior ends}; \text{number of measurements in square parentheses}) \)

Body length [7] 23.7 (21.5-25.7) mm. Maximum width [7] 99 (94-104) in posterior third of body. Nerve ring not observed. Muscular oesophagus [12] 426 (419-432) long; glandular oesophagus [9] 2412 (2388-2423) long. Ratio mean total length oesophagus to mean length posterior body (oesophago-intestinal junction to tail tip) [7] 1:7.4. Spicule [13] 585 (572-592) long, with narrow, striated lateral margins, broad. 10.2 (10.0-10.4) wide at head, broadening to 16.7 (14.6-18.7) maximum width just anterior to rounded distal tip, flexible longitudinally and laterally. Anterior cloaca [7] 1925 (1832-2017) long, with thick muscular wall, thick cuticular lining. Posterior cloaca [7] 325 (220-426) long, depending on degree of protraction of spicule sheath, with robust, sharply-pointed spines, thick cuticular lining, plicate in retracted state. Junction of seminal vesicle with ejaculatory duct conspicuous, valvular, without large muscular sphincter. Seminal vesicle [7] 213 (180-230) long. Ejaculatory duct [7] 1789 (1624-1892) long, intestine and ejaculatory duct join anterior cloaca at same level. Ratio of ejaculatory duct to total cloaca [7] 1:1.3. Caudal lateral alae absent. Caudal end of male with rudimentary pseudobursa formed by posteriorly-directed latero-ventral lobes connected by reduced dorsal cuticular membrane. Each lobe with two small papillae. Ventral cuticular flap originating anteriorly and forming conspicuous anterior lip of cloacal aperture.

**Female:** \( (n=13) \)

Body length 30.6 (21.5-42.2) mm. Maximum width 166 (112-202) in posterior half of body. Nerve ring not observed. Muscular oesophagus 225 (102-343) long; glandular oesophagus 5804 (4522-7080) long. Ratio mean total length oesophagus to mean length posterior body 1:4.1. Vulva at or slightly anterior to oesophago-intestinal junction, 5758 (4522-7074) from anterior extremity, cuticular flap absent. Vagina 539 (457-582) long, muscular, with thick cuticular lining. Tail terminating in two small ventro-lateral swellings. Anus sub-terminal, 2 (1-3) from caudal extremity. Distal intestine relatively narrow, moderately thick-walled, narrowing posteriorly. Rectum 212 (185-244) long, relatively narrow, weakly muscular, with thick cuticular lining. Eggs 70 (67-72) long, 33 (28-36) wide, tanned, unembryonated, with conspicuous polar plugs. Egg shell moderately thick-walled, with minute, densely distributed punctuations.

Etymology: The species is named for the family Potoroidae, to which all three known hosts belong.

Hosts: *Aepyprymnus rufescens* (type host), *Potorous tridactylus*, *Bettongia penicillata* (see Love and Reddacliff, 1992).

On post mortem examination of mice 28 days after dosing with embryonated eggs of *E. potoroi*, no worms were found in any of the tissues of one animal. The other animal contained a single lesion in the liver. Dissection of the lesion revealed a live female nematode surrounded by host cells. The female contained numerous unfertilised eggs without egg shells, some just commencing formation of polar plugs. No nematodes were found in lungs, stomach, intestine or bladder, and no eggs were found in the faeces.

**Discussion**

The new species is placed in the genus *Eucoleus* Dujardin, 1845 as re-defined by Moravec (1982) due to the absence of caudal lateral alae in the male, the presence of a rudimentary pseudobursa formed by two minute, rounded, posteriorly-directed lateral lobes connected by a reduced dorsal cuticular membrane and the long spicular sheath densely covered with cuticular spines in males, and the non-protuberant vulva in females. The new species is distinguished from *Pearsonema feliscati* (Diesing, 1851) Freitas and Mendonça, 1960 from the bladder of cats, *Calodium hepaticum* (Bancroft, 1893) Moravec, 1982 from the liver of rats and *Capillaria miniopterae*...
Thomas, 1959 from the stomach of bats, by the presence of numerous spines on the posterior cloaca (= spicule sheath when protracted), a feature distinguishing the genus *Eucoleus* from the genera *Pearsonema* Freitas and Mendonça, 1960, *Calodium* Dujardin, 1845 and *Aonchotheca* López-Neyra, 1947. The latter genus is the one in which the species from the common bent-wing bat, *Miniopterus schreibersii* (Kuhl, 1817), *A. miniopterae* (Thomas, 1959) comb. nov., belongs under Moravec’s (1982) re-classification, on the basis of the pre-anal alae and the absence of spines on the posterior cloaca in males.

*Eucoleus potoroi* sp. nov. is distinguished from all other species of *Eucoleus* described from Australian marsupial and murid hosts by the immense length of the ejaculatory duct and the very large ratio of mean total length of oesophagus to mean total length of posterior body in males. The latter is not a feature in females. Female *E. potoroi* are distinguished from all other species of *Eucoleus* from Australian marsupial and murid hosts except *E. gastricus* by the following combination of features: greater body length, greater ratio of mean total length of oesophagus to mean length of posterior body, more posterior position of the vulva and the greater length of the vagina and rectum. These same features distinguish *E. gastricus* from *E. potoroi* in addition to the reticulate pattern on the egg shell of the former species. *Eucoleus longiductus* Spratt, 2006 was named originally on account of the great length of the ejaculatory duct in males however, that in *E. potoroi* is almost twice as long.

Love and Reddacliff (1992) reported verminous bronchitis and bronchiolitis in captive colonies of *A. rufescens*, *P. tridactylus* and *B. penicillata* at the University of New South Wales Field Station at Cowan and at Armidale, New South Wales. These conditions were associated with the presence of what I then recognised as *Capillaria* sp. nov. 7 (see Spratt et al. 1991) and herein describe as *Eucoleus potoroi* sp. nov. Oral treatment of infected animals with fenbendazole (Panacur 10®, Hoechst Australia Limited, Melbourne, Australia) at a dose rate of 25 mg/kg bodyweight once daily for 5 days resulted in remission of clinical signs over a period of a few weeks and the rapid disappearance of trichinelloid eggs from the faeces. However, re-infection did occur.

The finding of an unfertilised female in the liver of one mouse infected with embryonated eggs of *E. potoroi*, the occurrence of *E. potoroi* in captive animals at field stations and the lesions associated with infection suggested that the parasite might be a known species in abnormal hosts. Potential candidates were *C. hepaticum* from the liver of introduced rats (Singleton et al., 1991), *Eucoleus aerophilus* (Creplin, 1839) Dujardin, 1845 previously recorded from the trachea of domestic cats in Sydney (Holmes and Kelly, 1973; Kelly and Ng, 1975) and feral cats in Tasmania (Gregory and Munday, 1976), *P. feliscati* from the bladder and urinary tract of domestic cats in Brisbane (Waddell, 1967, 1968; Kelly and Ng, 1975; Wilson-Hanson and Prescott, 1982a,b) or *A. miniopterae* from the stomach of *M. schreibersii*. Since *E. potoroi* clearly belongs to the genus *Eucoleus*, *C. hepaticum*, *P. feliscati* and *A. miniopterae* could be excluded.

Male *E. potoroi* are distinguished from male *E. aerophilus* by the much shorter glandular oesophagus, much greater ratio of oesophagus to posterior body length (oesophagus represents approximately one-third the body length in *E. aerophilus* and approximately one-sixth the body length in *E. potoroi*), absence of two large mesenchymal cells at the oesophago-intestinal junction, much shorter and broader spicule, much longer (approx. 3 times) ejaculatory duct and the absence of a prominent sphincter muscle at the junction of the seminal vesicle with the ejaculatory duct. Female *E. potoroi* are distinguished from female *E. aerophilus* by the absence of two large mesenchymal cells at the oesophago-intestinal junction, much shorter vagina, longer rectum and the punctate pattern of ornamentation on the egg shell in contrast to the fine, reticulate pattern of ridges in *E. aerophilus*.

*Eucoleus aerophilus* is a cosmopolitan parasite of canids, felids and mustelids which has not been reported in canids in Australia and which uses earthworms as intermediate host (Borovkova, 1947 cited in Anderson, 1992). Given the result of the experimental infection of mice in this study, it seems likely that *E. potoroi* has a direct life cycle. *Eucoleus potoroi* sp. nov. appears to be a
“native” species and its known occurrence only in captive animals probably reflects the lack of examination of the lungs of wild Potoroidae. I have not found *E. potoroi* in the lungs of one *A. rufescens* from south of Marlborough, Queensland and one from Grafton, New South Wales, three *B. penicillata* from Battalling, Western Australia, two *Betongia gaimardi* (Desmarest, 1822) from Kingston and one from Epping Forest, Tasmania and two *Potorous tridactylus* from Margate, Tasmania.

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