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***Potorostrongylus woyliei* n. sp. (Nematoda: Cloacinidae) from the
Brush-tailed Bettong *Bettongia penicillata* (Marsupialia) from Western
Australia, Australia, with Comments on Potoroid–Potorostrongylid
Associations and a Key to the Species of *Potorostrongylus***

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ABSTRACT: *Potorostrongylus woyliei* sp. n. (Nematoda: Cloacinidae), a new nematode from the stomach of the brush-tailed bettong *Bettongia penicillata* is described and a revised key to the genus provided. The new species most closely resembles *Potorostrongylus finlaysoni* Johnston and Mawson, 1939 and *Potorostrongylus temperatus* Smales, 1997 but differs from both in the shape of the submedian lips, the length of the spicules, and the proportions of the elements of the ovejector. Each potoroid host species is associated with a particular species of *Potorostrongylus*, suggesting coevolution of host and helminth. Additional potoroid–potorostrongylid associations appear to be incidental infections resulting from sympatric host distribution.

KEY WORDS: Nematoda, Cloacinidae, Labiostrongylinea, *Potorostrongylus*, marsupial, brush-tailed bettong, *Bettongia penicillata*, Western Australia, Australia.

The genus *Potorostrongylus* Johnston and Mawson, 1939 comprises species of the tribe Labiostrongylinea that parasitize potoroos and bettongs (Macropodidae: Potoroinae). The genus was revised by Smales (1997), who recognized 4 species: the type species, *Potorostrongylus finlaysoni* Johnston and Mawson, 1939; *Potorostrongylus aepyprymnus* Mawson, 1974; *Potorostrongylus temperatus* Smales, 1997; and, *Potorostrongylus tropicus* Smales, 1997. All species of *Potorostrongylus* are known from hosts in eastern Australia.

The brush-tailed bettong, *Bettongia penicillata* Gray, 1837 is reduced to remnant populations in southwestern Australia (Christensen, 1995). Recent collections of helminths from *B. penicillata* include a fifth species of *Potorostrongylus*. This new species is described in this study and a revised key to the genus provided. Additional nematode material from potoroos and bettongs held in the Commonwealth Scientific and Industrial Research Organization Wildlife Collection, Canberra, Australia (CSIRO), and the South Australian Museum, Adelaide, Australia (SAM), was also examined and the resulting host associations reported in this study.

MATERIALS AND METHODS

Specimens dissected from the stomachs of 2 brush-tailed bettongs were fixed in 10% formalin and stored in 70% ethanol. Worms were examined after clearing in lactophenol. Figures were prepared with the aid of a drawing tube and all measurements made with the aid of an ocular micrometer. Unless otherwise indicated, measurements are

presented as range values presented in micrometers followed parenthetically by the mean. Additional specimens held by CSIRO and SAM were examined and identified, providing previously unreported geographic range and host association data for *Potorostrongylus*. New and existing specimen material is deposited with CSIRO or SAM and is noted below. Host nomenclature follows Strahan (1995). Parasite nomenclature and terminology follows Chilton et al. (1997) and Beveridge (1983, 1987).

RESULTS

New determinations of existing CSIRO and SAM specimens

Specimens comprising lots CSIRO N986, N1235, N1332, and N1362 collected from the Tasmanian bettong, *Bettongia gaimardi* (Desmarest, 1822), in Blessington, Tasmania, Australia (41°31'S; 147°24'E), Epping Forest, Tasmania, Australia (41°45'S; 147°21'E), and Kempton, Tasmania, Australia (41°31'S; 147°12'E) were identified as *P. temperatus*. Specimens comprising CSIRO N3386 collected from the rufous bettong, *Aepyprymnus rufescens* (Gray, 1837), in Grafton, New South Wales, Australia (30°14'S; 150°147'E) were also identified as *P. temperatus*. This is the first record of *P. temperatus* infecting *A. rufescens*. In contrast, specimens comprising SAM AHC 32874 collected from a single specimen of *A. rufescens* in Mt. Fox, Queensland, Australia (18°49'S; 145°48'E) were identified as *P. tropicus*. Similarly, specimens comprising SAM AHC 11102, AHC 32873 collected from the northern bettong, *Bettongia tropica* (Wakefield, 1967), in Cairns, Queensland, Australia (16°55'S; 145°46'E)

were identified as *P. tropicus*. Specimens comprising CSIRO N997, N1001, and N5075 collected from the long-nosed potoroo, *Potorous tridactylus* (Kerr, 1792), in Hobart, Tasmania, Australia (41°29'S; 147°52'E) and Chudleigh, Tasmania, Australia (41°33'S; 146°28'E) were identified as *P. finlaysoni*.

***Potorostrongylus woyliei* sp. n.**
(Figs. 1–17)

Description

Cloacinidae Stossich, 1899; Cloacininae Stossich, 1899; Labiostrongylinea Beveridge, 1983. Relatively small worms, body with fine transverse cuticular striations. Cephalic extremity with 6 fleshy, well-developed lips with pulp cavities, bearing V-shaped cuticular ridges on outer surfaces; 4 submedian lips, tips wedge shaped each with single notch, bearing cephalic papilla on base (Figs. 4, 17); 2 lateral lips simple, bearing amphids; 2 interlabia, 1 dorsal, 1 ventral with V-shaped ridge but without pulp. Cervical cuticle inflated from base of lips to just anterior to nerve ring. Buccal capsule small, oval, wider than deep, does not extend beyond posterior level of lips. Esophagus long, one-ninth body length, narrow, with ovoid terminal bulb; lining of esophagus strongly cuticularized at angles of triradiate lumen, forming 3 pairs of sclerotized plates in terminal bulb. Deirids short, setiform, 20 long, posterior to excretory pore, both posterior to nerve ring which encircles esophagus at two-fifths its length.

Male (holotype and 3 paratypes): Length 8.6–10 (9.6) mm; width 340–430 (380). Buccal capsule 17 deep, 12 wide. Esophagus 1,105–1,140 (1,090) long; terminal bulb 268 long, 175–200 (185) wide. Nerve ring 395–470 (430), deirid 630, 655, excretory pore 610 from anterior end. Bursa prominent, lobes not separate, dorsal lobe longest, ventral lobes shortest. Ventroventral and ventrolateral rays fused for most of length, reaching margin of bursa; externolateral ray divergent from lateral trunk, almost reaching margin of bursa; mediolateral and posterolateral rays fused, reaching margin of bursa; externodorsal ray arising close to lateral trunk, not reaching margin of bursa; dorsal trunk stout, bifurcating at about one-third its length; lateral branches given off close to bifurcation, not reaching margin of bursa, terminal branches extending into lappets of the dorsal lobe; lateral branch of dorsal ray may have branchlet (Fig. 16). Spicules 680–985 (895) long, about one-eleventh body length, anterior extremities irregularly knobbed; dorsal tips slightly curved, blunt, striated alae extend-

ing almost to tips. Genital cone small; anterior lip larger conical, bearing pair of lateral alae, posterior lip smaller, with fringe of 6 bifid projections. Gubernaculum not present; spicule sheath with paired lateral elongate and central cordate thickenings.

Female (allotype and 2 paratypes): Length 11–12 mm; width 450–510. Buccal capsule, 12–15 deep, 17–20.5 wide. Esophagus 1,140–1,275 long; terminal bulb 290–305 long, 205–220 wide. Nerve ring 375–485, excretory pore 505–630, deirids 630–670 from anterior end. Body narrows at level of vulva, 975–1,285 from tail tip; tail 605–750 long, ending in conical tip. Vulva close to anus, leading into broad short vagina 170–200 long; ovejektor with vestibule shorter than sphincter, infundibulum shorter than vestibule. Eggs thin shelled, ellipsoidal 76–83 long, 53–56 wide.

Fourth-stage larva, female (1 specimen): Length 4.3 mm; width 165. Esophagus 830 long; terminal bulb 134 long, 37 wide, esophago-intestinal diverticula present. Nerve ring 380, excretory pore 445 from anterior end. Deirids not seen. Tail 375 long.

Taxonomic summary

Type host: Brush-tailed bettong, *B. penicillata* Gray, 1937.

Type locality: Batalling, Western Australia, Australia (33°20'S; 116°34'E).

Infection site: Stomach.

Collection date: 11 December 2001.

Specimens deposited: Holotype male (SAM AHC 32862), allotype female (SAM AHC 32863), paratypes (SAM AHC 32864).

Specimens examined: (CSIRO N3203) 2 males, 10 females from *B. penicillata*, from Perup, Western Australia, Australia, (34°19'S, 116°27'E) collected 12 December 1990.

Etymology: The species is named from the indigenous name for the host, the woylie.

Key to the species of *Potorostrongylus*

- 1a. Buccal capsule within lip region 2
- 1b. Buccal capsule extends posterior to region of lips 4

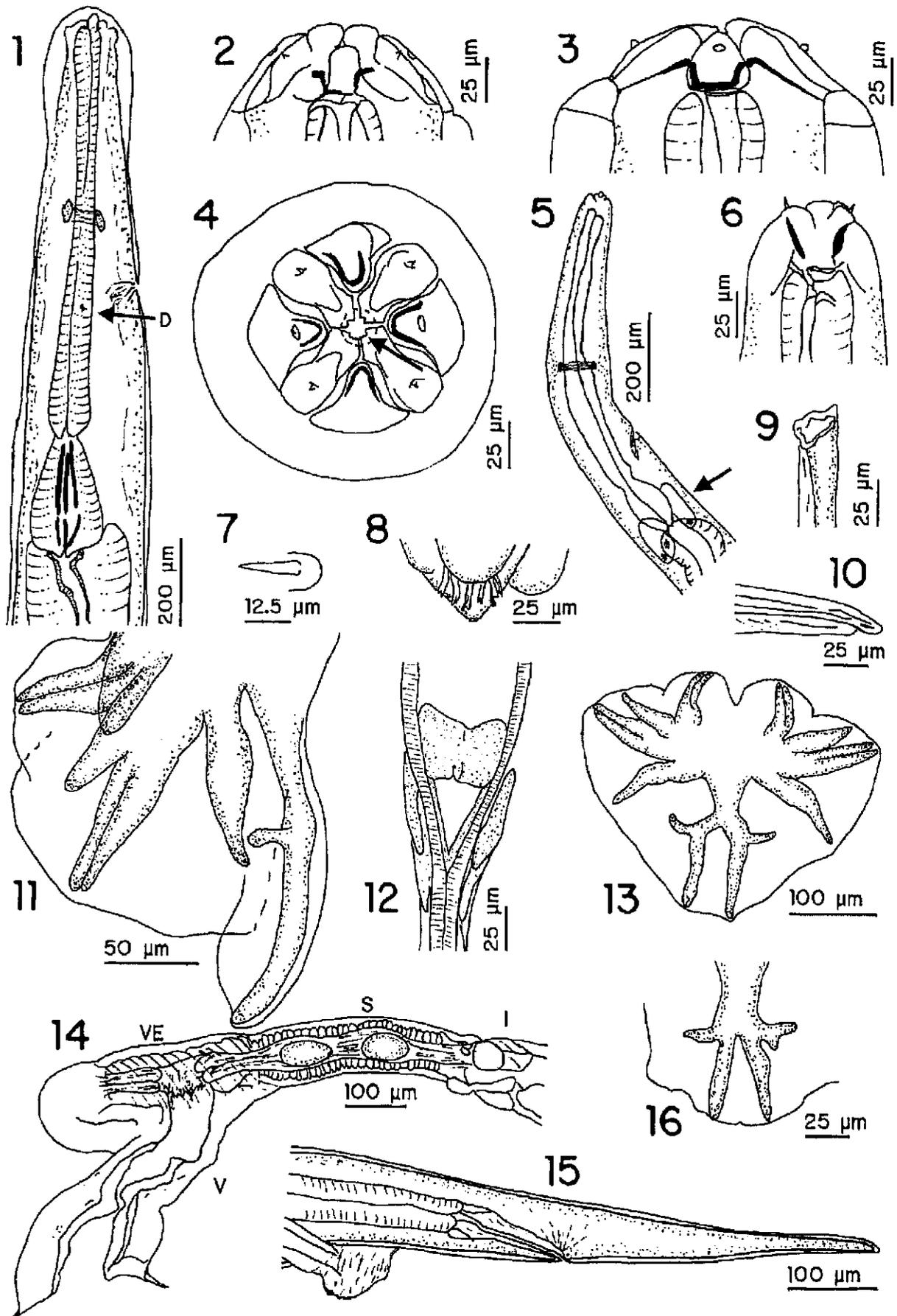




Figure 17. *Potoroststrongylus woyliei* n. sp. from *Bettongia penicillata* in Western Australia. Cephalic end, en face view showing notched tips of submedian lips (arrow).

- 2a. Submedian lips with notched tips; spicules <1,000, one-eleventh body length, vagina <230 μm *P. woyliei* n. sp.
- 2b. Submedian lips with wedge-shaped tips, spicules >1,000, one-fifth to one-eighth body length, vagina >230 μm long 3
- 3a. Dorsal lobe of bursa longer than laterals; spicule tips straight; 3 pairs of bifid projections on posterior lip of genital cone; deirids short, stout; vulva >650 μm anterior to anus *P. finlaysoni*
- 3b. Dorsal lobe of bursa same length as laterals; spicule tips curved; 4 pairs of projections on posterior lip of genital cone; deirids setiform; vulva <600 μm anterior to anus *P. temperatus*
- 4a. Dorsal lobe of bursa longer than laterals; spicule tips slightly curved; up to 10 projections on posterior lip; large lateral alae on anterior lip of genital cone; deirids short; female tail >600 μm in length *P. tropicus*
- 4b. Dorsal ray same length as laterals; spicule tips very curved; 2 pairs of projections on posterior lip; small lateral alae on anterior lip of genital cone; deirids long; female tail <300 μm in length *P. aepyprymnus*

DISCUSSION

Of the 4 valid species of *Potoroststrongylus*, *P. woyliei* most closely resembles *P. temperatus* and *P.*

finlaysoni in which the buccal capsule is also placed within the lip region. *Potoroststrongylus woyliei* is most similar to *P. finlaysoni*: in both species the dorsal lobe of the bursa is longer than the lateral lobes, the dorsal ray gives off lateral branches close to the bifurcation of the trunk, and there is a fringe of 6 bifid elements on the posterior lip of the genital cone. A notch in the wedge-shaped tip of the submedian lips distinguishes *P. woyliei* from *P. finlaysoni* and all other *Potoroststrongylus* species. *Potoroststrongylus woyliei* is also differentiated from *P. finlaysoni* by shorter spicules (895, one-eleventh body length vs. 1,303, one-fifth to one-seventh body length) and longer deirids (20 vs. 10). Female *P. woyliei* differ from *P. finlaysoni* in having the vulva closer to the anus (370–536 vs. 665–790), an ovejector with the sphincter the longest element, and a shorter vagina (170–200 vs. 375–440). *Potoroststrongylus woyliei* can be distinguished from *P. temperatus* in having 6 bifid rather than 8 setate elements on the posterior lip of the genital cone, shorter spicules (895, one-eleventh body length vs. 1,175, one-eighth body length) and the dorsal lobe of the bursa longer than the lateral lobes. In female *P. woyliei*, the infundibulum is the shortest element of the ovejector, but in *P. temperatus* the infundibulum and vestibule are about the same length.

The fourth-stage larva was found together with fifth-stage adult specimens in the stomach lumen of the host. The shape of the posterior end allowed the identification of the worm as female. The cephalic extremity and the presence of esophago-intestinal diverticula indicated the fourth stage of development.

The presence or absence of esophago-intestinal diverticula has not been used in phylogenetic analyses of the Cloacinidae. Smales (2002) noted that such diverticula are present in the adults of all genera comprising the tribe Labiostrongylinea except *Potoroststrongylus*. The presence of esophago-intestinal diverticula in a juvenile *P. woyliei* (Fig. 5) suggests that this is the plesiomorphic state for the tribe and that their absence in adults is a derived state. This is consistent with the hypothesis that *Potoroststrongylus* are derived from *Labiomultiplex* Smales, 2002, a related genus common in the stomachs of macropodid marsupials (kangaroos and wallabies) (Smales, 2002).

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Figures 1–16. *Potoroststrongylus woyliei* n. sp. from the brush-tailed bettong, *Bettongia penicillata*, in Western Australia. 1. Anterior end, male lateral view deirid (D). 2. Cephalic end, dorsoventral view. 3. Cephalic end, lateral view. 4. En face view showing notched tips of submedian lips (arrow). 5. Juvenile female, lateral view showing esophago-intestinal diverticula (arrow). 6. Juvenile female, cephalic end, lateral view. 7. Deirid. 8. Genital cone, dorsal view. 9. Proximal end spicule. 10. Spicule tip. 11. Bursa, lateral view. 12. Spicule sheaths, dorsal view. 13. Bursa, end on. 14. Ovejector, lateral view showing vagina (V) vestibule (VE) 1 branch of sphincter (S) and infundibulum (I). 15. Female posterior end, lateral view. 16. Dorsal ray showing branchlet.

There are 7 extant species of potoroos and bettongs all of which have been surveyed for helminths (Spratt et al., 1991) except the long footed potoroo, *Potorous longipes* Seebeck and Johnston, 1980, a rare species restricted to northeastern Victoria, Australia (Strahan, 1995). Of the 6 host species surveyed for helminths, no species of *Potorostrongylus* is reported from the burrowing bettong, *Bettongia lesueur* (Quoy and Gaimard, 1824), which is extinct on the mainland and found only on islands off the coast of Western Australia. The remaining 5 host species are each associated with 1 species of *Potorostrongylus*: *P. tridactylus* with *P. finlaysoni*, *B. tropica* with *P. tropicus*, *B. penicillata* with *P. woyliei*, *B. gaimardi* with *P. temperatus*, and *A. rufescens* with *P. aepyprymnus* (Smales, 1997). In addition, incidental associations are reported for *A. rufescens* and *B. gaimardi*. *Potorostrongylus finlaysoni* was reported from the type host, *P. tridactylus*, and *B. gaimardi* on Maria Island, off the coast of Tasmania, Australia (Smales, 1997). Infections in *B. gaimardi* are probably incidental associations resulting from host sympatry on the island. *Potorostrongylus temperatus* occurs in *A. rufescens* from New South Wales, Australia as well as *B. gaimardi*, the type host, from Tasmania, Australia. The host distribution of *P. temperatus* may be relictual: *B. gaimardi* was distributed on mainland Australia as recently as the early twentieth century, occurring sympatrically with *A. rufescens* in New South Wales (Dennis and Johnson, 1995; Rose and Johnson, 1995). Additional survey is required to determine the host range and geographic distribution of *P. temperatus*, particularly on mainland Australia. Finally, an infection of *P. tropicus* is reported from an individual *A. rufescens* in Queensland, Australia. Again, this is probably an incidental infection resulting from sympatric overlap of *A. rufescens* with *B. tropica*, the normal host of *P. tropicus*.

The potoroids and their labiostrongyline parasites appear to have coevolved with 2 exceptions: the relationship between *P. temperatus* and its hosts, *A. rufescens* and *B. gaimardi*; and the relationship between *P. tropicus* and its hosts, *B. tropica* and *A. rufescens*. These associations may reflect incidental infections among sympatric host species, true host switching, or some combination of the 2. Whatever the mechanisms involved, *A. rufescens* has developed the most complex relationship with its

community of *Potorostrongylus* species: no mixed infections are known, but host-helminth association varies geographically.

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