



Austraplectana extranes sp. n. (Nematoda: Cosmocercoidea) from Australian amphibians *Mixophyes carbinensis* Mahony, Donnellan, Richards & McDonald (Anura: Myobatrachidae) and *Rhinella marina* (Linnaeus) (Anura: Bufonidae)

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Abstract The genus *Austraplectana* Baker, 1981 is a poorly known group of cosmocercooid nematodes. In the present study, a new species of *Austraplectana*, *A. extranes* sp. n., was described using both light and scanning electron microscopy, based on specimens collected from the carbine barred frog *Mixophyes carbinensis* Mahony, Donnellan, Richards & McDonald (Anura: Myobatrachidae) and the cane toad *Rhinella marina* (Linnaeus) (Anura: Bufonidae) in Australia. *Austraplectana extranes* sp. n. can be easily distinguished from its congener by the much longer and different morphology of tails in both sexes, the

different number and arrangement of caudal papillae, the presence of single precloacal median heart-shaped papilla and large posterior protuberance with cuticular comb-like fringe in male.

Introduction

Baker (1981) established the genus *Austraplectana*, currently including only the type species *A. kartanum* (Johnston & Mawson, 1941), which parasitizes some native frogs in Australia, including the Jervis Bay tree frog *Litoria jervisiensis* (Duméril & Bibron) (Anura: Hylidae), the striped rocket frog *L. nasuta* (Gray) (Anura: Hylidae), the motorbike frog *Ranoidea moorei* (Copland) (Anura: Hylidae) and the moaning frog *Heleioporus eyrei* (Gray) (Anura: Limnodynastidae) (Johnston & Mawson, 1941; Inglis, 1968; Baker, 1981).

During a helminthological survey in Australian amphibians, some nematode parasites collected from the carbine barred frog *Mixophyes carbinensis* Mahony, Donnellan, Richards & McDonald (Anura: Myobatrachidae) and the cane toad *Rhinella marina* (Linnaeus) (Anura: Bufonidae), were identified as a new species of *Austraplectana*. The detailed morphology of the new species was studied using light and scanning electron microscopy.

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Materials and methods

Host collection

One *M. carbinensis* and 67 *R. marina* collected from Mt Lewis (-16.580997, 145.334851), Bloomfield (-15.921538, 145.351538), Cape Tribulation (-16.088319, 145.460714) and Paluma (-19.006527, 146.189245), northern Queensland, Australia, were dissected for parasites. All hosts were collected by hand at night and placed into either a collecting bucket or moist herpetological bag and transported to the laboratory. Hosts were euthanised by an overdose of pentobarbitone sodium solution (Lethabarb Euthanasia injection, Virbac, Australia).

Light and scanning electron microscopy

Nematodes were isolated from the intestine of hosts. Specimens were fixed and stored in room temperature 70% ethanol until study. For light microscopy studies, nematodes were cleared in lactophenol consisted of phenol, lactic acid and glycerol according to the volumic ratio 1: 1: 2. For scanning electron microscopy (SEM), specimens were re-fixed in 4% formaldehyde solution, post-fixed in 1% OsO₄, dehydrated via an ethanol series and acetone, and then critical point dried. Samples were coated with gold and examined using a Hitachi S-4800 scanning electron microscope at an accelerating voltage of 20 kV. Measurements (the range, followed by the mean in parentheses) are given in micrometers (µm) unless otherwise stated. Type specimens were deposited in College of Life Sciences, Hebei Normal University, Hebei Province, China.

Results

Suborder Ascaridina Inglis, 1983

Superfamily Cosmocercoidea Railliet, 1916

Family Cosmocercidae Railliet, 1916

Subfamily Austraplectaninae Baker, 1981

Genus *Austraplectana* Baker, 1981

Austraplectana extranes sp. n. (Figs. 1–3)

Small-sized, whitish nematodes. Body cylindrical, maximum width at about region of mid-body. Oral aperture simple, somewhat triangular, surrounded by 3 small lips, each with remarkable inner flanges (Figs. 1A, 2A). Dorsal lip with 2 double labial papillae each possessing 1 pair of small aerial-like protrusions; subventral lips each with 1 double labial papilla also possessing 1 pair of small aerial-like protrusions and large amphid (Figs. 1A, 2A). Eight large outer cephalic papillae present at base of lips (Figs. 1A, 2A). Cuticle with fine transverse striations (Fig. 1C). Somatic papillae present in both sexes (Figs. 1C, 2F). Lateral alae well developed, extending from some distance posterior to base of lips to anterior of cloaca (Figs. 1C, 2C, 3C, D). Oesophagus divided into anterior short pharynx, cylindrical corpus, slightly narrow isthmus and terminating posterior bulb with valves (Fig. 3A). Nerve ring located at about 1/2 of oesophageal length (Fig. 3A). Excretory pore situated at about middle level between nerve ring and posterior oesophageal bulb (Fig. 3A). Tail of both sexes conical, with long filamentous tip (Figs. 1D, E, 2C, 3C-E).

Male (based on 10 mature specimens): Body 1.90–3.90 (2.78) mm long; maximum width 140–440 (268). Oesophagus 386–546 (492) long in total length, representing 13.6–23.1 (18.3) % of body length; pharynx + corpus + isthmus 314–435 (398) long, size of posterior bulb 72–111 (93) × 82–121 (99). Nerve ring 163–275 (225) and excretory pore 290–386 (327) from cephalic extremity, respectively. Spicules short and robust with remarkable alae, equal in length, distal end pointed, 138–213 (178) long, representing 4.48–9.86 (6.69) % of body length (Fig. 3F). Expansion of cuticle in posterior end of body supported by 5 pairs of sub-lateral pedunculate papillae (2 pairs precloacal, 3 pairs postcloacal) (Fig. 3C); 8 pairs of additional precloacal ventral pedunculate papillae, 2 pairs of adcloacal ventral pedunculate papillae and 1 pair of postcloacal ventral pedunculate papillae present (postcloacal sessile papillae indistinguishable from somatic papillae) (Figs. 1B, D, F, 2B, D, E, 3C, D). Single median precloacal heart-shaped papilla and large posterior protuberance with cuticular comb-like fringe present (Figs. 1B, 2B, 3C). Tail 280–500 (381) long, representing 7.69–22.8 (14.5) % body length, long filamentous tip with numerous somatic papillae (Figs. 1D, 2C, 3C, D).

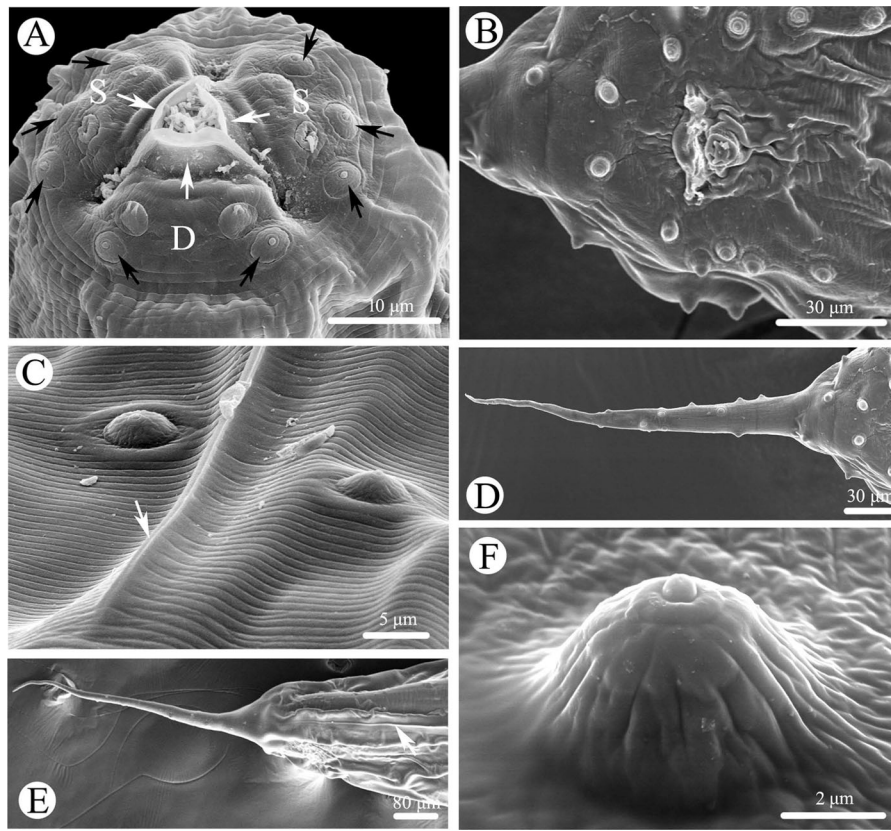


Fig. 1 Scanning electron micrographs of *Austraplectana extranes* sp. n. collected from the cane toad *Rhinella marina* (Linnaeus) (Anura: Bufonidae) in Australia. A, cephalic end of female (white arrows showing inner flanges, black arrows showing outer cephalic papillae), apical view; B, magnified image of cloacal region of male, ventral view; C, magnified image of somatic papillae and the beginning of lateral ala (white arrows showing lateral ala) of female; D, magnified image of tail tip of male, ventral view; E, posterior end of female (white arrows showing lateral ala), lateral view; F, magnified image of precloacal pedunculate papilla of male. Abbreviations: D, dorsal lip; S, subventral lip.

Female (based on 10 mature specimens): Body 2.93–4.15 (3.66) mm long; maximum width 300–520 (410). Oesophagus 507–628 (579) long in total length, representing 13.8–18.6 (16.0) % of body length; pharynx + corpus + isthmus 411–507 (465) long, size of posterior bulb 97–121 (114) × 116–135 (128). Nerve ring 213–350 (280) and excretory pore 338–449 (402) from anterior extremity, respectively. Vulva transverse slit, vulval lips not protruded, 1.10–1.80 (1.51) mm from anterior extremity, at 35.5–43.9 (41.0) % of body length (Fig. 3B). Vagina muscular, uteri didelphic, opistodelphic. Eggs oval, with smooth surface, 58–70 (65) × 40–53 (47) (n=20) (Fig. 3G). Tail 600–900 (757) long, representing 18.5–24.6 (20.8) % body length (Figs. 1E, 3E).

Taxonomic summary

Type host: Carbine barred frog *Mixophyes carbinensis* Mahony, Donnellan, Richards & McDonald (Anura: Myobatrachidae).

Type locality: Mt Lewis (-16.580997, 145.334851), northern Queensland, Australia.

Other host and localities: Cane toad *Rhinella marina* (Linnaeus) (Anura: Bufonidae), collected from Bloomfield (-15.921538, 145.351538), Cape Tribulation (-16.088319, 145.460714), Paluma (-19.006527, 146.189245), all in northern Queensland, Australia.

Site of infection. Rectum.

Prevalence and intensity of infections: Single *Mixophyes carbinensis* examined and infected with 26 nematodes; 43.3% (29 out of 67 *Rhinella marina*)

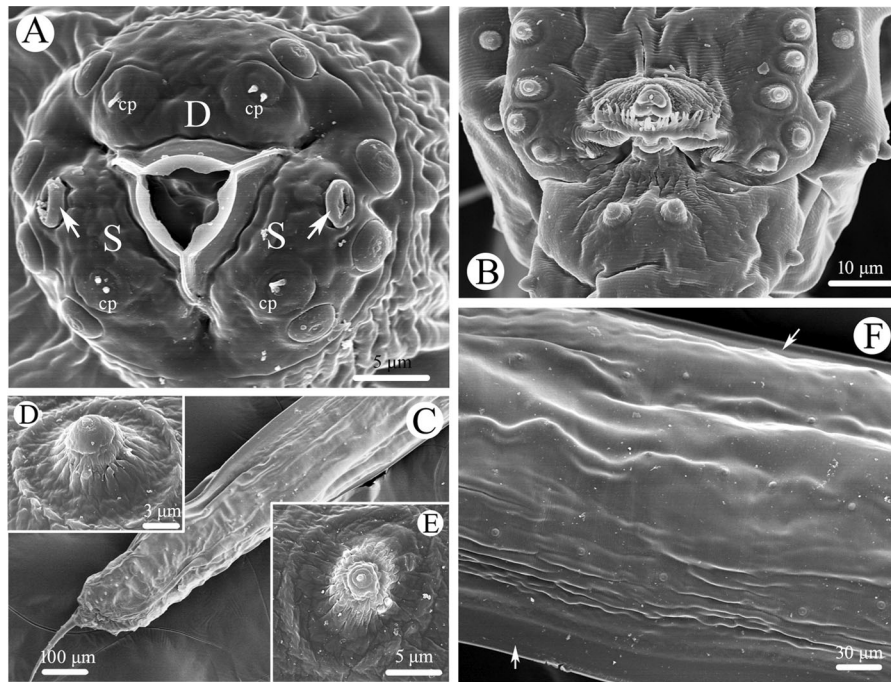


Fig. 2 Scanning electron micrographs of *Austraplectana extranes* sp. n. collected from the cane toad *Rhinella marina* (Linnaeus) (Anura: Bufonidae) in Australia. A, cephalic end of male (white arrows showing amphids), apical view; B, magnified image of cloacal region of male, ventral view; C, posterior end of male, ventral view; D, magnified image of preloacal pedunculate papilla; E, magnified image of preloacal pedunculate papilla; F, somatic papillae and lateral alae (arrowed) of male. *Abbreviations*: cp, double labial papillae; D, dorsal lip; S, subventral lip.

infected with intensity of 3–100 (mean 27.1) nematodes.

Type specimens. Holotype, male (HBNU–N-2022A0830-1L-N); allotype, female (HBNU–N-2022A0830-2L-N); paratypes: 30 males, 50 females (HBNU–N-2022A0830-3L-N).

Etymology: The specific epithet is derived from the Latin word *extrane-*, referring to the strange characteristics of the new species.

Discussion

With remarkable lateral alae and somatic papillae in both sexes, well developed pedunculate papillae in the male and the didelphic uteri containing eggs of normal size (less than 100 µm in diameter) in the female, the morphological characters of our present specimens agreed well with the monotypic genus *Austraplectana* Baker, 1981. *Austraplectana extranes* sp. n. can be easily distinguished from *A. kartanum* by the size and morphology of tails in both sexes (male tail 0.28–0.50 mm, female tail 0.60–0.90 mm, tail with long

filamentous tip in the new species vs male tail 0.13–0.17 mm, female tail 0.27–0.33 mm, tail without long filamentous tip in the latter) and the presence of single median preloacal heart-shaped papilla and large posterior protuberance with cuticular comb-like fringe in male (vs preloacal papilla and large posterior protuberance absent in *A. kartanum*).

The genus *Austraplectana* is a poorly known group of cosmoceroid nematodes, which has received little attention since its inception. The cephalic structures are very important characters for species identification and delimitation of genera in zooparasitic nematodes. However, the detailed morphology of cephalic structures of most of cosmoceroid species have not been adequately described (Ni et al., 2022). The present study clearly showed the particular cephalic structures of *A. extranes* sp. n. using SEM, including each double cephalic papillae with 1 pair of aerial-like protrusions and the presence of large outer papillae at base of each lip. Moreover, the new species shared some characters (*i.e.*, tail with long filamentous tip, cloacal region of male with single median preloacal papilla and large posterior protuberance with cuticular comblike fringe)

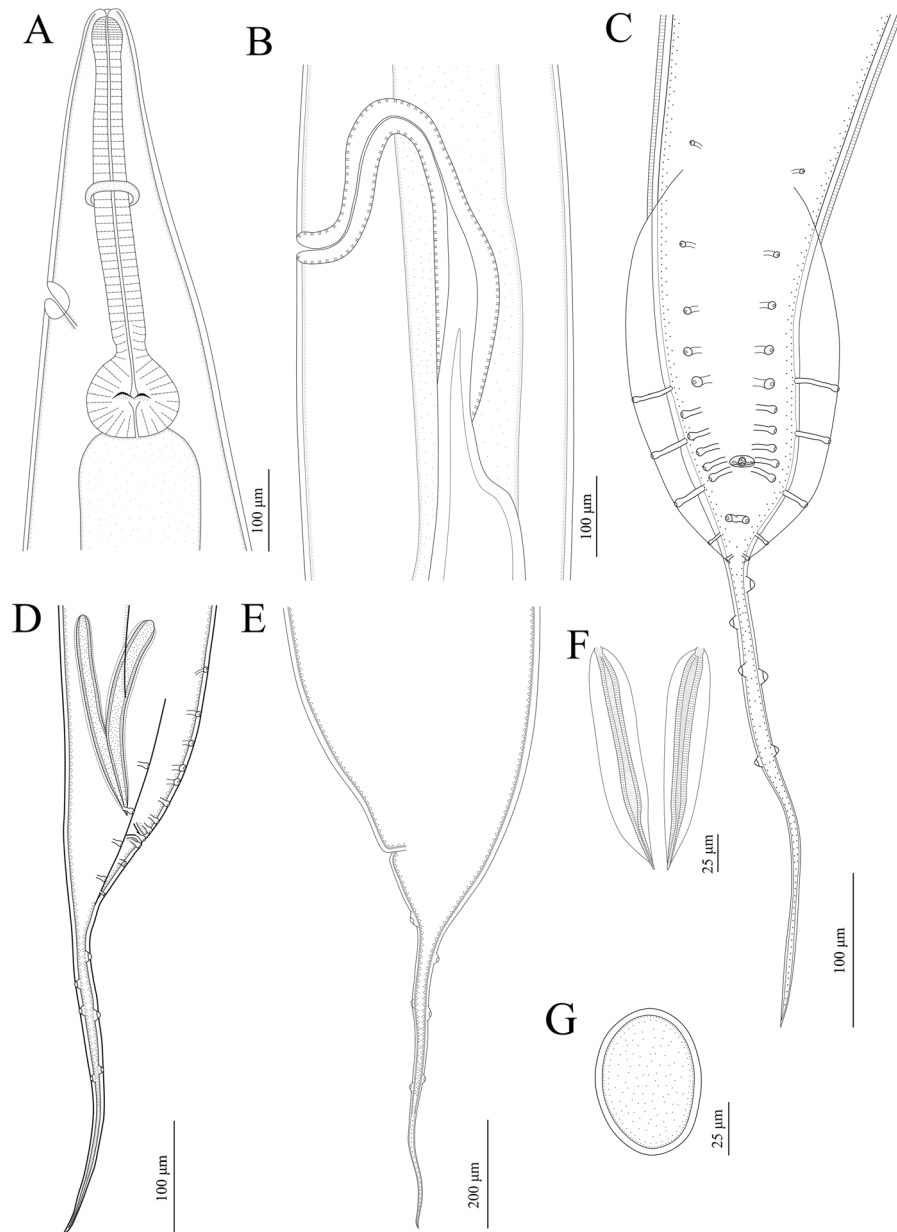


Fig. 3 *Austraplectana extranes* sp. n. collected from the cane toad *Rhinella marina* (Linnaeus) (Anura: Bufonidae) in Australia. A, anterior part of male, lateral view; B, region of vulva, lateral view; C, posterior end of male, ventral view; D, posterior end of male, lateral view; E, posterior end of female, lateral view; F, spicules, ventral view; G, egg.

with some species of the genera *Cosmocera*, *Oxysomatium* and *Raillietnema* in the family Cosmocercidae, for example, the tail of *Cosmocera* spp. commonly possessing long filamentous tip (Sou & Nandi, 2015; Chen et al., 2020), the cloacal region of male of *Oxysomatium* spp. and *Raillietnema* spp. usually with single median preloacal papilla

(ornament) and large posterior protuberance with cuticular comb-like fringe (Baker, 1980, 1982; Felix-Nascimento et al., 2020). However, the morphology of caudal papillae is the very important character for differentiating different genera in the Cosmocercidae, in which only the genus *Austraplectana* has well developed pedunculate papillae supported the

expansion of cuticle in posterior end of male. Consequently, we considered that it is more reasonable to assign the present species in *Austraplectana*. A molecular phylogenetic analysis is needed to further clarify the systematic position of *A. extranes* sp. n. in the future.

According to the previous studies (Johnston & Mawson, 1941; Inglis, 1968; Baker, 1981), the genus *Austraplectana* seems to be endemic to Australia. In the present study, the new species was found in both Australian native frog *M. carbinensis* and the exotic cane toad *R. marina* in northern Queensland, Australia. The life cycle of *Austraplectana* species has not been studied. Anderson (1992) indicated that the amphibian host becomes infected the third-stage larvae of cosmoceroid nematodes by either orally ingestion or by skin penetration. We speculated that the cane toad became infected with *A. extranes* sp. n. since *R. marina* has been introduced to Australia by sharing the same habitat or similarity in diet with the native frog *M. carbinensis*.

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Author contribution XFN and LL identified the nematode specimens, prepared figures 1–3 and wrote the main manuscript text. DPB collected specimens. XFN, DPB and LL reviewed and approved the final manuscript.

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Declarations

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

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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