

## **BREINLIA (BREINLIA) VENTRICOLA SP. NOV., A NEMATODE PARASITE FROM THE HEART OF THE RED KANGAROO, *MACROPUS RUFUS*, IN WESTERN AUSTRALIA.**

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### Summary

SPRATT, D. M. & HOBBS, R. P. (2004) *Breinlia (Breinlia) ventricola* sp. nov. (Nematoda:Filarioidea) from the heart of the red kangaroo, *Macropus rufus*, in Western Australia. *Trans. R. Soc. S. Aust.* 128(1), 67-71, 31 May, 2004.

*Breinlia (Breinlia) ventricola* sp. nov. is described from the right ventricles and pulmonary arteries of the red kangaroo, *Macropus rufus* (Desmarest, 1822), from the Pilbara region of Western Australia. It is the largest and most robust species of *Breinlia (Breinlia)* known to date and is distinguished from all other species by the close similarity in size between males and females, the presence of a filamentous membrane surrounding the distal tip of the left spicule and the presence of a pair of caudal glands secreting a material which adheres to the caudal end of some fixed specimens, masking external cuticular features. The new species is most similar to *B. (B.) trichosuri*, *B. (B.) boltoni* and *B. (B.) mundayi* but distinguished from all 3 by its much greater size and the absence of internolateral cephalic papillae.

KEY WORDS: Nematoda, marsupials, kangaroo, *Breinlia*, new species, *Macropus*.

### Introduction

The genus *Breinlia* Yorke and Maplestone, 1926, subgenus *Breinlia* represents a group of parasitic filarioid nematodes occurring in the body cavities, subcutaneously and in the vascular system of murid and sciurid rodents and primates in Asia (Chabaud and Bain, 1976; Bain *et al.*, 1983), in dasyurid, peramelid, petaurid, pseudocheirid, phalangerid, phascolaretid, potoroid and macropodid marsupials in Australia and Papua New Guinea (Spratt and Varughese, 1975; Spratt *et al.* 1991), and in native murid rodents in northwestern Western Australia (Beveridge and Spratt, 1996). Microfilariae occur in the peripheral circulation and in the four life cycles that have been investigated (*B. booliati* Singh and Ho, 1973, *B. manningi* Bain, Petit, Ratanaworabhan, Yenbutra and Chabaud, 1981, and *B. sergenti* Mathis and Léger, 1909 in Southeast Asia and *B. macropi* Spratt and Varughese, 1975 in Australia) mosquitoes act as intermediate hosts (Ho *et al.*, 1973; Bain *et al.*, 1981; Ramachandran and Dunn, 1968; Zaman and Chellappah, 1968; Beveridge and Spratt, 1996). There are many species of *Breinlia* but only one, *B. woerlei* (Spratt and Varughese, 1975) Bain, Baker and Chabaud, 1983 occurs in the heart. However, in the filarioid genus, *Dirofilaria* Railliet and Henry, 1911 a number of species occur in the heart, the best known example in Australia being the dog

heartworm, *D. immitis* (Leidy, 1856). An inspector in a wild meat export abattoir in Perth encountered large filarioid nematodes in the right ventricles and pulmonary arteries (Fig. 1) of red kangaroos, *Macropus rufus*, from the Pilbara region of Western Australia, which represents a new species of *Breinlia (Breinlia)* described below.

### Materials and Methods

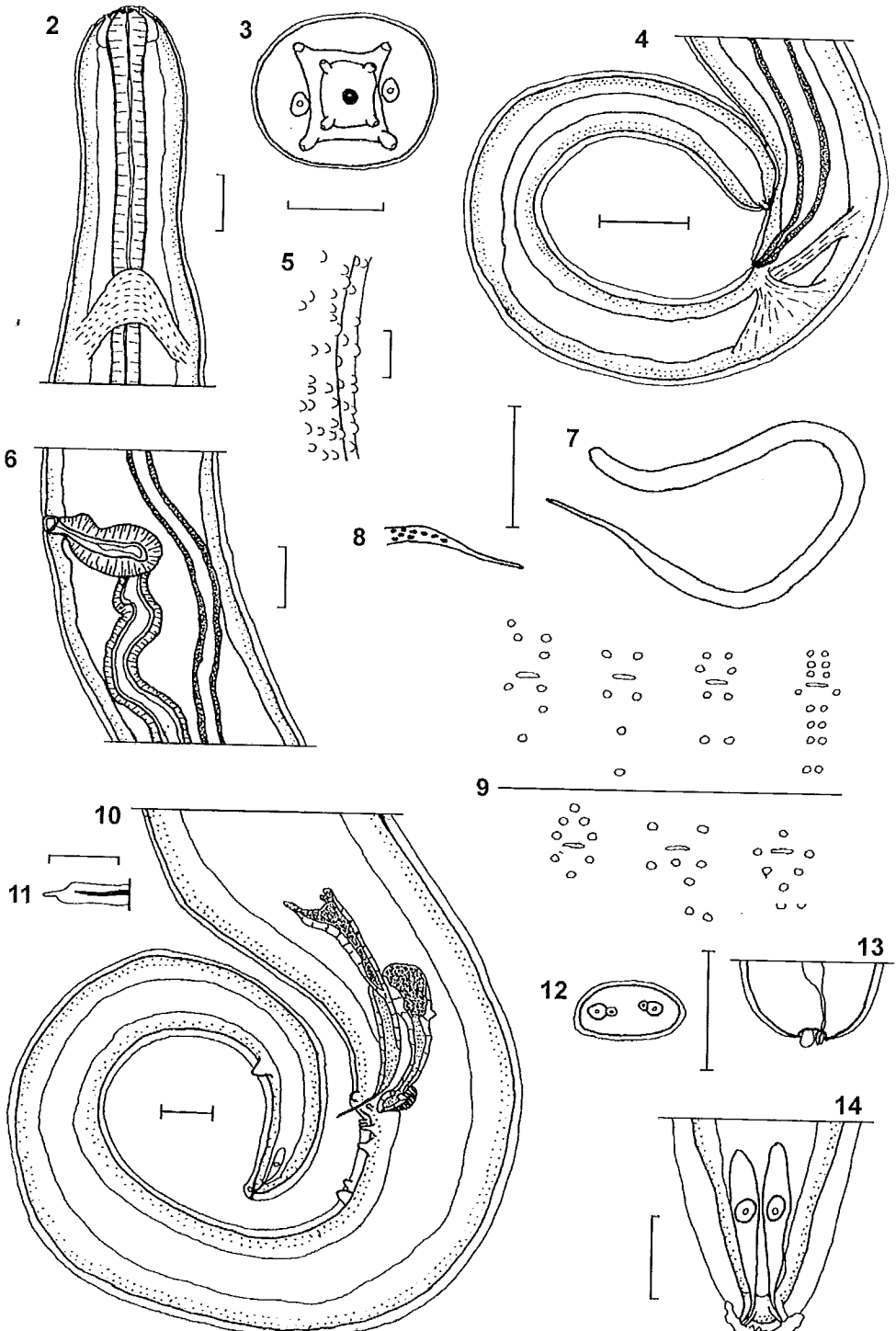
Nematodes were fixed at room temperature in 70% ethanol with 5% glycerine and cleared in lactophenol for examination. *En face* preparations of the cephalic extremity were prepared by the method of Anderson (1958). The same glycerine jelly hanging-drop



Fig. 1. *Breinlia (Breinlia) ventricola* sp. nov. in right ventricle of red kangaroo, *Macropus rufus*. scale bar

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technique was used to study the posterior ends of some males and females.

Measurements of 9 adult males and 9 adult females are presented in millimetres as the mean followed by the range in parentheses, those of 10 microfilariae from the *vagina uterina* of female worms are in micrometres. Drawings were made with the aid of a drawing tube. Type-specimens are deposited in the South Australian Museum (SAM) Australian Helminth Collection (AHC), Adelaide and the Queensland Museum (QM), Brisbane. Additional material is in the Western Australia Museum (WAM), in CSIRO Sustainable Ecosystems and in the School of Veterinary and Biomedical Sciences, Murdoch University

***Breinlia (Breinlia) ventricola* sp. nov.**  
(FIGS 2-14)

*Material examined:*

*Holotype*

♂ from right ventricle of *Macropus rufus* (Desmarest, 1822), Mindaroo Station, 50 km southwest of Onslow (21° 41' S, 115° 08' E) Western Australia, coll. F. Stephens, 9 May, 2002, SAM AHC [IDW]32174.

*Allotype*

♀ same data, SAM AHC [IDW]32175.

*Paratypes*

4♂♂, from right ventricle and pulmonary arteries of *M. rufus*, Warrawagina Station (20° 51' S, 120° 41' E) Western Australia, coll. F. Stephens, 4 June, 2002, SAM AHC 32176, 4♀♀ same data AHC 32177, 4♂♂ same data, QM G222333-222336, 4♀♀ same data, QM G222337-222340.

*Other material examined*

Fragments of specimens from type localities in the Parasite Collection of CSIRO Sustainable Ecosystems, Canberra, W/L HC# N5259; 1♀ from *M. rufus* near Mt. Augustus (24° 19' S, 116° 50' E) in WAM V4382, 2♀♀ same data in Department of Veterinary Biology and Biomedical Sciences, Murdoch University, Perth, X02/07.

*Prevalence*

Observed in 19 of 550 (3.5%) red kangaroos examined.

*Site in host*

Right ventricle and pulmonary arteries.

*Etymology*

The suffix *-icola* means "inhabiting" and the specific epithet reflects the location of the nematodes in the right ventricle of the host.

*Description*

Long, robust nematodes with attenuated anterior and helically coiled posterior extremities. Oral opening small, round, not bounded by prominent refractile membrane. Cephalic extremity large, oval. Four pairs of submedian papillae arranged in outer circle of 4 large and inner circle of 4 slightly smaller papillae. Rectangular cuticular peribuccal field present, appearing as slight elevation of cuticle joining bases of papillae of inner circle. Distance between papillae on lateral surface greater than on dorsal and ventral surfaces. Second cuticular peribuccal field present, rectangular in shape, formed by slight elevation of cuticle joining bases of papillae of outer circle. Distance between papillae on lateral surfaces greater than on dorsal and ventral surfaces. Internolateral papillae absent. Amphids lateral, large but not raised, opening at level of outer circle of papillae; amphidial pouches large. Buccal capsule minute, narrow, with delicate wall. Small, refractile pre-pharyngeal ring present. Oesophagus divided into short anterior muscular and long posterior glandular regions. Excretory pore not observed. Cuticle with conspicuous transverse striations. Refractile cuticular bosses observed on ventral surface of males only. Lateral cords and hypodermal nuclei inconspicuous. Lateral alae absent. Deirids not observed.

*Male*

Total length 195 (180 – 220); maximum width 0.84 (0.76 – 0.89) in mid-body region; nerve ring 0.40 (0.37 – 0.42) from anterior extremity; muscular oesophagus 0.79 (0.71 – 0.90) long, glandular

Figs 2-14. *Breinlia (Breinlia) ventricola* sp. nov. 2. Anterior end female, dorsal view. 3. Cephalic extremity female, apical view. 4. Female tail, lateral view. 5. Cuticular rugosities on ventral surface of male in posterior one-third of body. 6. Female vulva and vagina, lateral view. 7. Microfilaria. 8. Tail of microfilaria showing terminal nuclei. 9. Diagrammatic representation of seven patterns of distribution of cloacal papillae in males. 10. Male tail, lateral view. 11. Distal tip of left spicule. 12. Caudal end of male showing pair of lateroventral lappets and medial openings of ducts of caudal glands, apical view. 13. Caudal end of male, latero-ventral view. 14. Caudal end of male with caudal glands and secretion on

oesophagus 2.1 (1.9 – 2.4) long. Spicules unequal, dissimilar, heavily sclerotized, left spicule 0.59 (0.56 – 0.61) long, calamus 0.26 (0.25 – 0.28), lamina 0.16 (0.15 – 0.17), filament 0.17 (0.16 – 0.19) terminating in fine sclerotized rod surrounded by filamentous membrane; right spicule 0.33 (0.32 – 0.34) long, with calciform, spatulate distal extremity. Gubernaculum present, 0.06 (0.06 – 0.07) long, U-shaped in ventral view with deep lateral walls appearing to wrap around lateral edges of right spicule. Tail helically coiled, 1.2 long, terminating in two large lateroventral lappets located close to one another. Two large caudal glands present, opening adjacent and median to lappets. Some males with material secreted from glands adhering to caudal cuticle and masking view of external features of caudal end. Cloacal papillae highly variable in number, ranging from 6 – 16 with 8 the most frequently occurring number, and in pre- and post-cloacal positions, the majority clustered around cloacal aperture; aperture slightly elevated. Some males with one or two subterminal medio-ventral papillae, some with a subterminal medio-dorsal papilla. Cuticle with longitudinally elongate, refractile, cuticular bosses on ventral surface, commencing posterior to oesophago-intestinal junction and terminating approximately 3 mm from cloacal aperture.

#### Female

Total length 233 (205 – 260); maximum width 1.60 (1.30 – 2.20) in mid-body region; nerve ring 0.40 (0.34 – 0.45) from anterior extremity; muscular oesophagus 0.75 (0.63 – 0.87) long, glandular oesophagus 1.50 (0.91 – 2.40) long. Vulva well posterior to oesophago-intestinal junction, 7.20 (5.20 – 9.50) from anterior extremity; vagina 2.40 (1.90 – 2.70) long, thick-walled, muscular. Tail helically coiled, tapering characteristically on the dorsal surface and terminating in two large lateroventral lappets located close to one another. Two large caudal glands present, opening adjacent and median to lateroventral lappets. Some females with material secreted from glands adhering to caudal cuticle and masking view of external features of caudal end. Phasmids not observed. Cuticular bosses absent.

#### Microfilariae from vagina uterina

Body length 265 (263 – 267); maximum width 6 in mid-body region; nerve ring 51 (49 – 53) and excretory vesicle 79 (78 – 80) from anterior extremity; anal vesicle 208 (205 – 210). Body tapering behind vesicle; nuclear column terminating in single row of 3 small, elongate nuclei; tail filamentous. Distance from last nucleus to tail tip 26 (25 – 28). Cuticle with conspicuous transverse striations. Microfilariae

#### Discussion

Two subgenera of *Breinlia* were defined by Anderson and Bain (1976), *B. (Breinlia)* Yorke and Maplestone, 1926 (not Chabaud and Bain, 1976 as listed by Anderson and Bain, 1976) recognised by spicules unequal in length, the presence of a divided oesophagus and the presence of a gubernaculum, and *B. (Johnstonema)* (Yeh, 1957) recognised by spicules equal or subequal in length, the presence of an oesophagus not clearly divided in females, the presence of external labial papillae arranged in the form of a dorsoventrally expanded rectangle, a buccal cavity with thin walls and the absence of a gubernaculum. However, the morphology of the spicules with the left spicule clearly divided into calamus, lamina and filament and the right with a calciform, spatulate distal extremity is more typical of the subgenus *Breinlia* than of *Johnstonema* and on this basis particularly, we have placed this species therein.

*Breinlia (B.) ventricola* is distinguished from all other known species of *B. (Breinlia)* ( $n = 19$ ) and *B. (Johnstonema)* ( $n = 4$ ) by its great length and breadth (males twice as long and broad and females one eighth as long and nearly three times as broad as largest species known previously), the close similarity in size between males and females, the presence of a filamentous membrane surrounding the distal tip of the left spicule and the presence of a pair of caudal glands in male and female worms secreting a material which adheres to the caudal end of some fixed specimens masking external cuticular features. In addition, the spicules in species of *B. (Johnstonema)* are stout, subequal and the left spicule is not divided into calamus, lamina and filament. The new species most closely resembles *Breinlia (B.) trichosuri* (Breinl, 1913) from the peritoneal cavity of the common brushtail possum, *Trichosurus vulpecula* (Kerr, 1792) in Queensland, *B. (B.) mundayi* Spratt and Varughese, 1975 from the pericardium of the red-necked wallaby, *Macropus rufogriseus* (Desmarest, 1817), in Tasmania and *B. (B.) boltoni* Spratt and Varughese, 1975) from the peritoneal cavity of the agile wallaby, *M. agilis* (Gould, 1842), from northern Australia. *Breinlia (B.) ventricola* is distinguished from all three by its much greater size and the absence of a pair of internolateral cephalic papillae. Previously, *B. (B.) macropi* from the quokka, *Setonix brachyurus* (Quoy and Gaimard, 1830), was the only filarioid nematode known from marsupials in Western Australia.

The two subgenera of *Breinlia* occurring in Australia are most common in macropodoid marsupials with 14 species previously described from the subgenus *Breinlia*.

Varughese, 1975) in addition to a number of undescribed species (Spratt *et al.* 1991). Additional species of the subgenus occur in dasyurid, peramelid, petaurid, pseudocheirid, phalangerid and phascolarctid marsupials. The subgenera have been interpreted as a group of australian parasites, having migrated relatively recently from Asia in murid rodents and subsequently radiating widely in marsupials (Bain *et al.*, 1983). Species of the subgenus *Breimlia* are known to occur in six species of "old endemic" murid rodents but only in the Kimberley region of Western Australia, all morphologically indistinguishable from species described from pseudocheirid and macropodid marsupials. No species of the subgenus *Johnstonema* are known from murid rodents in Australia. In Australian marsupial hosts, species of both subgenera appear relatively host specific, but those occurring in macropodoids, especially in the family Macropodidae, exhibit the least host specificity. Consequently, one might expect to encounter *B. (B.) ventricola* in other species, particularly of the larger kangaroos and wallaroos. However, it is surprising that such a large nematode has not been encountered previously, particularly

given the large export trade in kangaroo meat. This suggests that the species may not only be specific to the red kangaroo but also may be limited to the geographic region of northwestern Western Australia.

It is expected that many aspects of pathology seen in canine dirofilariasis would occur in red kangaroos infected with *B. ventricola*, because *Dirofilaria immitis* is similar in size and is found in the same sites. Dirofilariasis is characterised by pulmonary hypertension caused primarily by an intense tissue reaction (Knight, 1977). Heavy infections would also be expected to result in impaired heart valve function and physical blockage (Soulsby, 1982). Exercise intolerance is a feature of dirofilariasis and would be likely to result in greater susceptibility to predation in red kangaroos infected with *B. ventricola*.

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