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DURIKAINEMA MACROPI GEN. ET SP. NOV. (MUSPICEOIDEA : ROBERTDOLLFUSIDAE), A remarkable nematode from Macropodidae (Marsupialia)

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SUMMARY. *Durikainema macropi* gen. et sp. nov. (Muspiceoidea : Robertdollfusidae) is described from the mesenteric and hepatic portal veins of *Macropus giganteus* Shaw 1790, *M. agilis* (Gould 1842) and *M. rufogriseus* (Desmarest 1817) (Marsupialia : Macropodidae) from Queensland, Australia.

It is also known from histological sections of hepatic portal veins of *M. robustus* Gould 1841, *M. fuliginosus* (Desmarest 1817) and *Lagorchestes conspicillatus* Gould 1842. The new genus resembles the Enoplina in cephalic and caudal characters and the Dorylaimina in other characters. *Durikainema* resembles *Robertdollfusa* Chabaud and Campana 1950 in its small form, absence of mouth oesophagus and anus, atrophied digestive tube, reduction of female genital apparatus to a uterine pouch, viviparity and cephalic cuticular inflation in larvae.

It differs from this genus in its complex and welldeveloped cephalic structures and its well-developed body musculature in both sexes. *Durikainema* is tentatively placed in the Robertdollfusidae, Muspiceoidea. Larvae develop beyond firststage in the uterus of the female. They have been found in the non-peripheral blood of male and female *M. giganteus*, the lactating mammary gland of female *M. agilis* but not the non-lactating glands of the same female and in the deep capillaries of thigh skin of male *M. agilis*. Transmission of the parasite may be direct by a percutaneous or milk route, or indirect by a haemophagous arthropod.

Durikainema macropi gen. et sp. nov. (Muspiceoidea : Robertdollfusidae), un nématode remarquable des Macropodidae (Marsupialia).

RÉSUMÉ. *Durikainema macropi* gen. et sp. nov. (Muspiceoidea: Robertdollfusidae) est décrit des veines portales du mésentère et du foie de *Macropus giganteus* Shaw 1790, *M. agilis* (Gould 1842) et *M. rufogriseus* (Desmarest 1817) (Marsupialia: Macropodidae), de Queensland (Australie). La même espèce est trouvée sur des coupes histologiques des veines portales du foie de *M. robustus* Gould, 1841, *M. fuliginosus* (Desmarest, 1817) et *Lagorchestes conspicillatus* Gould, 1842. Ce genre nouveau ressemble aux Enoplina par les caractères des extrémités céphalique et caudale, et aux Dorylaimina par les autres caractères. *Durikainema* ressemble à *Robertdollfusa* Chabaud et Campana, 1950, par les caractères suivants : petite taille, bouche, œsophage et anus absents, intestin atrophié, appareil génital de la femelle réduit à une poche utérine, viviparité, présence d'un petit dôme céphalique cuticulaire transparent chez la larve. Ce genre nouveau diffère de

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Robertdollfusa par les caractères suivants : structures céphaliques complexes et bien développées, appareil musculaire des mâles et des femelles bien développé. Nous proposons de placer *Durikainema*, au moins à titre provisoire, dans les Robertdollfusidae (Muspiceoidea). Les larves se développent au-delà du premier stade dans l'utérus de la femelle. Les larves ont également été trouvées dans le sang non-périphérique des mâles et des femelles de *M. giganteus*, dans la glande mammaire lactifère de la femelle *M. agilis* (mais pas dans les glandes mammaires non-lactifères de la même femelle), et dans les capillaires profonds de la peau de la cuisse d'un mâle *M. agilis*. Le cycle évolutif est peut-être direct, par voie cutanée ou par le lait, ou peut-être indirect par un arthropode hématophage.

Introduction

For more than a decade a bizarre nematode has been known from the blood of members of the kangaroo family (Macropodidae) in Australia. However, a dearth of well-preserved specimens and of knowledge of the precise location of the nematode in the host rendered description premature. The recent finding (by R. S.) of living female worms in the distal portal veins of the mesentery of *Macropus agilis* (Gould, 1842) permits description of this remarkable vertebrate parasite. Observations on the natural history of the new parasite augment morphological considerations.

Materials and Methods

Blood of freshly shot animals was collected into tubes containing EDTA and stored at 4 °C for 1-3 days. Blood was diluted 1:10 (v/v) in a solution of 0.04% NH_4OH to lyse red blood cells. Nematodes found in this solution were dead. Some were removed and fixed in hot 10% neutral buffered formalin, others were studied in wet mounts of 1% orcein in 45% acetic acid. Living specimens were recovered from distal portal veins of *M. agilis* and fixed in hot 10% neutral buffered formalin. Worms were cleared in lactophenol or glycerine for study. Gross transverse sections of female worms were cut with an ocular scalpel and mounted in a hanging drop of glycerine jelly. Histological sections were cut at 4 μm and stained with haematoxylin and eosin.

Measurements were made from camera lucida drawings and are presented in microns unless otherwise stated, the range followed by the mean in parenthesis. Illustrations were made with the aid of a Leitz drawing device.

Durikainema gen. nov.

Diagnosis

Dorylaimina Chitwood and Chitwood, 1950; Muspiceoidea Roman, 1965; Robertdollfusidae Chabaud and Campana, 1950. Adenophorean nematodes with

well-developed elongate cephalic papillae or bristles, well-developed amphids, pronounced cephalic cuticular inflation. Mouth, buccal capsule, oesophagus and anus absent; intestine and rectum atrophied. Platymyarian, meromyarian. Females amphidelphic, viviparous, genital apparatus in gravid females reduced to uterine pocket; uterus connecting to exterior by short narrow vagina without sphincter; vulval aperture small. Male with single spicule; pre- and post-anal papillae; long attenuated tail. Larvae numbering more than 200 in gravid female; long; surrounded by second cuticular layer; this outer cuticular layer inflated in cephalic region; musculature well-developed; digestive tube atrophied; genital primordia unknown. Parasites of blood vessels of macropodid marsupials.

Type species: *Durikainema macropi* sp. n.

Durikainema macropi sp. n.

DESCRIPTION *(fig 1 to 16)*

Short slender nematodes; male shorter and narrower than female. Cuticle thin with fine transverse striations; bacillary bands absent. Cephalic extremity with large cuticular inflation through which pass elongate papillae or bristles. Four pairs elongate submedian papillae/bristles, internolateral cephalic one of each pair longer and broader than externolabial ones of respective pairs. Two small elongate lateral externolabial papillae/bristles. Amphids large, oval, located at level of bases of submedian papillae/bristles, opening below cephalic inflation; amphidial pouches large; minute setae at or immediately posterior to each amphidial opening. Oral opening absent; six minute elongate possibily fused lip-like structures surrounding central core of tissue; core with central depression distally, increasing in diameter proximally. Buccal capsule absent. Oesophagus atrophied; often three strands of tissue (? hypodermal) uniting in anterior oesophageal region and passing posteriorly as single or double strand containing a few nuclei but without an observable lumen. Trophosome not observed. Strands of tissue disappear near anterior extremity of ovary or testis; strands not observed in mid-body region of whole mounts, gross or histological transverse sections. Atrophied intestine containing some nuclei present in tail region of female near proximal extremity of posterior ovary; rectum indefinite, not patent; anus absent. Nerve ring, dorsal and ventral nerve chords, phasmids, excretory cells, vesicle or pore not observed. Body musculature composed of single muscle cell per quadrant; cell containing numerous contractile elements and large nucleus with prominent nucleolus. Cytoplasmic region of cell hypertrophied and containing copious quantity of granular material in non-gravid females; not hypertrophied, inconspicuous and non-granular in gravid females.

MALE (1 specimen from non-peripheral blood of *M. giganteus*):

Body 2 mm long; maximum diameter 12 μm in mid-body region. Spicule 21 μm long, bifid proximally, bulbous in distal half and with rounded knob-like distal extremity. Gubernaculum absent. Single median pre-anal papilla; five median

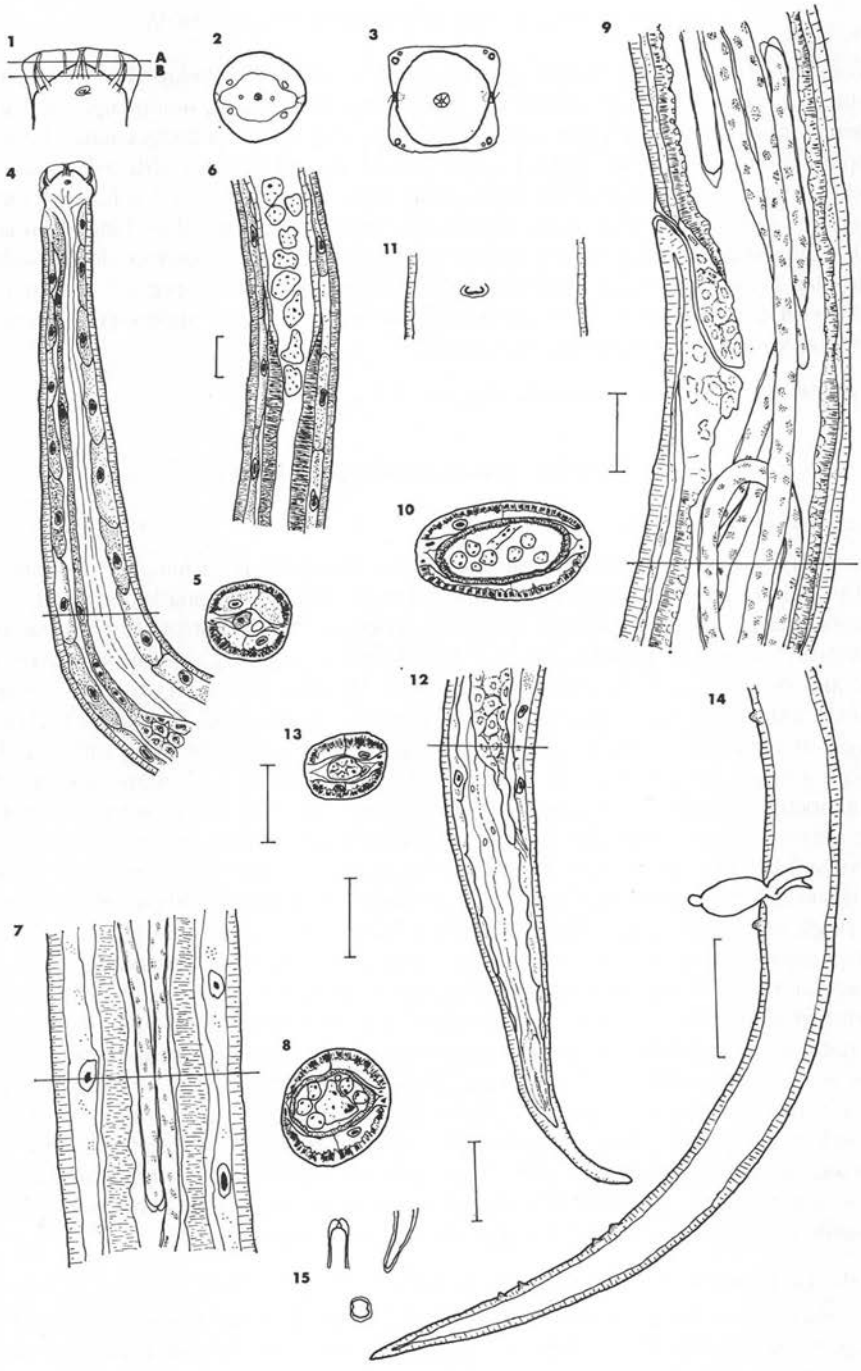

FIG. 1 to 15

post-anal papillae, one immediately post-anal, four closer to tail tip and arranged in tandem in two groups of two. Tail 118 μm long, tapering to gently rounded tip. Specimen lost, consequently reproductive system unknown.

FEMALE (6 specimens from non-peripheral blood of *M. giganteus*):

Body 3.26-3.96 (3.69) mm; maximum diameter 38-50 (45) μm in mid-body region. Distance cephalic extremity to anterior ovary 83-155 (132) μm, caudal extremity to posterior ovary 100-146 (127) μm. Vulva 1.85-2.27 (2.04) mm from cephalic extremity. Genital apparatus of early gravid females (numerous embryonating eggs but few larvae) clearly demarcated into ovaries, oviducts and uteri. Oviduct with narrow muscular wall; uterus with thicker muscular wall and with narrow glandular epithelium only in region immediately anterior and posterior to vulva. Vulval opening small, maximum diameter 4-7 (6) μm, vagina with extremely short narrow lumen; without musculature; with thin cuticular lining in distal one-third. Vagina and vulva possibly non-functional in gravid females (more than 200 larvae *in utero*); ovaries and oviducts degenerate; uteri and pseudocoele filled with larvae; latter occurring within 9 μm and 23 μm of cephalic and caudal extremities respectively. Postcephalic cuticle and hypodermal musculature thin in gravid females; larvae possibly released by rupturing or penetrating region immediately posterior to head of female.

(17 specimens from mesenteric portal veins of *M. agilis*):

Body 2.85-4.35 (3.80) mm; maximum diameter 41-52 (47) μm in mid-body region. Distance cephalic extremity to anterior ovary 79-175 (136) μm, caudal extremity to posterior ovary 95-155 (120) μm. Vulva 1.65-2.66 (2.17) mm from cephalic extremity. Maximum diameter of vulval opening 6-9 (8) μm. Larvae in gravid females occurring within 4 μm and 17 μm of cephalic and caudal extremities respectively.

LARVAE (10 specimens from blood *M. giganteus*, stained 1% orcein in 45% acetic acid):

Body 848-950 (901) μm long; maximum diameter 6.2-6.6 (6.4) μm in mid-body region. Body surrounded by second layer of cuticle, latter inflated in cephalic

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FIG. 1 to 15. — *Durikainema macropi* gen. et sp. nov.

Fig. 1. Schematic representation cephalic end female, lateral view. — Fig. 2. Schematic representation cephalic end female, *en face* view at A. — Fig. 3. Schematic representation cephalic end female, *en face* view at B. — Fig. 4. Anterior end female, lateral view. — Fig. 5. Transverse histological section at level noted in Fig. 4. — Fig. 6. Early-gravid female ; thin-walled oviduct with ova, muscular uterus, lateral view, near mid-body. — Fig. 7. Gravid female ; uterus containing larvae, lateral view, anterior one-quarter of body. — Fig. 8. Transverse histological section at level noted in Fig. 7. — Fig. 9. Gravid female : vulva, vagina, muscular and glandular uterus containing larvae, lateral view, near mid-body. — Fig. 10. Transverse histological section at level indicated in Fig. 9. — Fig. 11. Vulval aperture, female, ventral view. — Fig. 12. Caudal end female, lateral view. — Fig. 13. Transverse histological section at level indicated in Fig. 12. — Fig. 14. Caudal end male, lateral view. — Fig. 15. Larvae from uterus, head, tail, transverse section in glycerine.
Scales 20 μm.

region. Cephalic structures and genital primordium not observed. Musculature well developed, platymyarian, meromyarian. Two prominent depressions in body wall in transverse sections (presumed lateral fields). Nuclei of musculature stained prominently with Giemsa, brilliant cresyl blue or acetic orcein. Digestive tube atrophied. Tail bluntly rounded.

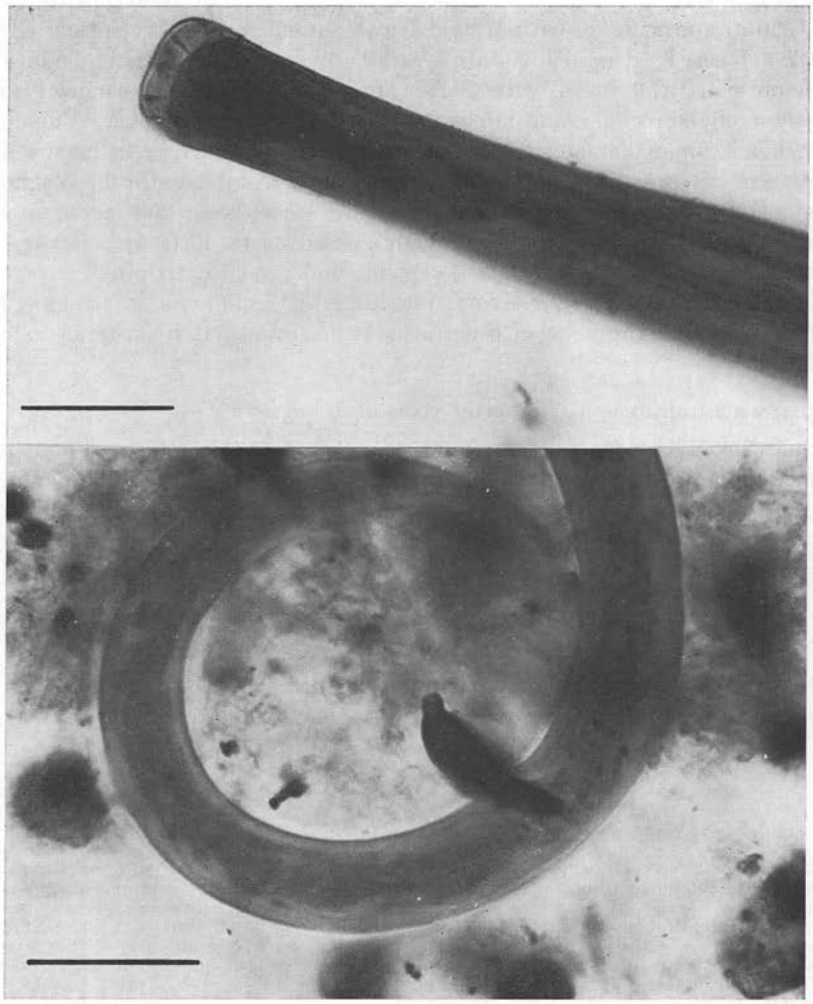

FIG. 16. — *Durikainema macropi*, *Above*, cephalic end of female illustrating lateral extranolabial papillae/bristles and lip-like structures surrounding central core of tissue, lateral view. *Below*, caudal end of male illustrating spicule, lateral view. Specimens in wet mounts of 1 % orcein in 45 % acetic acid ; Scales 20 µm.

(10 specimens from vagina of females from *M. agilis*, unstained):

Body 802-938 (885) μm long; maximum diameter 6.0-6.6 (6.3) μm in mid-body region. Other features as noted above.

Type specimens from *M. giganteus*

Holotype: Female, South Australian Museum (Adelaide) No. V2849

Allotype: Female, South Australian Museum (Adelaide) No. V2850

Paratypes: South Australian Museum (Adelaide) Nos. V2851-V2854 (4 females);

Other specimens from *M. agilis*

Muséum national d'Histoire naturelle, Paris No. 105MC. bocal N438(4 females).

United States National Museum of Natural History (USNM) Helm. Coll. No. 76546 (2 females).

South Australian Museum (Adelaide) No. V2855-V2858 (4 females).

Host of types and locality: Macropus giganteus Shaw 1790, Durikai (west of Warwick) southeast Queensland, Australia.

Other hosts and localities: M. agilis (Gould, 1842), Townsville, north Queensland; *M. rufogriseus* (Desmarest, 1817), Durikai, southeast Queensland; *M. robustus** Gould, 1841, Allan (near Warwick), southeast Queensland; *M. fuliginosus** (Desmarest, 1878), Hindmarsh Valley, South Australia; *Lagorchestes conspicillatus*** Gould, 1842, Inkerman Station (near Ayr), north Queensland; Rutland Plains, Cape York Penninsula, north Queensland. All localities in Australia.

Site of infection: Living adult females free in lumen of portal veins of mesentery and large portal veins of liver; degenerate females in small terminal vessels of liver and associated with eosinophilic phlebitis. Larvae recovered from non-peripheral blood of *M. giganteus* and *L. conspicillatus* (after shooting), from lactating mammary gland but not from non-lactating glands of same female *M. agilis* and from deep capillaries in thigh skin of male *M. agilis*.

Discussion

Durikainema gen. nov. exhibits an assemblage of bizarre morphological characters rendering its placement among the supratаxa of nematode parasites of vertebrates exceptionally difficult. It resembles the Enoplina (*senus* Chitwood and Chitwood 1950) in cephalic characters (10 elongated papillae/bristles, pocket-like amphids, absence of stylet) and long attenuated male tail, and the Dorylaimina (*sensu* Chitwood and Chitwood, 1950) in other characters (somato-intestinal musculature in 4 rows, absence of caudal glands and excretory system, single spicule and paired ovaries).

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* Gravid females seen in histological sections of portal veins of liver.

** As in * and larvae seen in blood.

The term papillae/bristles has been used to describe the cephalic structures; these are presumed to be sensory in function although nerve endings were not observed in them nor was a nerve ring seen in adult worms. In addition a minute seta is present near each amphid.

At the generic level *Durikainema* appears most closely related to *Robertdollfusa* Chabaud and Campana, 1950 described (females only) from the anterior chamber of the eye of *Corvus corone* L. in France. Roman (1965) suggested that *Encephalonema longimicrofilaria* Parukhin and Oshmarin, 1960 from the encephalon of *Pandion haliaetus* (Falconiformes) in Siberia was probably a synonym of *Robertdollfusa paradoxa* Chabaud and Campana, 1950. *Durikainema* resembles *Robertdollfusa* in its small form, atrophied digestive tube, absence of mouth, oesophagus and anus, reduction of female genital apparatus to a uterine pouch connecting to exterior by narrow vagina without sphincter, viviparity and cephalic cuticular inflation in larvae. It differs from *Robertdollfusa* in the presence in males and females of an inflated cephalic extremity bearing ten elongated papillae/bristles, well-developed amphids, and well-developed body musculature. In contrast, cephalic papillae/bristles and amphids are absent and the body musculature is atrophied in *R. paradoxa*. Parukhin and Oshmarin (1960) described 6 cephalic papillae disposed in two circles in *E. longimicrofilaria*.

Chabaud and Campana (1950) noted the affinities of *R. paradoxa* with Muspiceidae on the one hand and with Mermithidae on the other (holomyarian musculature, smooth cuticle, refringent granulations homologous with cordons of *Muspicea*, more primitive form of *R. paradoxa* due to functional vagina). However, none of these characters was assessed as being of phylogenetic significance and the position of the family Robertdollfusidae was viewed as uncertain.

Recently Bain and Chabaud (1979) reviewed the Muspiceidae and argued that the family, composed of *Muspicea* Sambon, 1925, *Riouxgolvania* Bain and Chabaud, 1968, *Lukonema* Chabaud and Bain, 1974 and *Pennisia* Bain and Chabaud, 1979, is actually Dorylaimina *sensu* Chitwood (1950) and closely related to Mermithoidea. They grouped the families Muspiceidae and Robertdollfusidae in the Muspiceoidea and concluded that this superfamily, along with Mermithoidea and Trichuroidea, form a natural group of invertebrate and verbetrate parasites stemming from the Dorylaimoidea.

Of the four genera recognized in Muspiceidae, only *Pennisia* is dioecious, and males do not possess a spicule. The male of *R. paradoxa* is unknown. That of *D. macropi*, known from a single specimen, possesses one spicule and a single row of median pre- and post-anal papillae.

Cephalic structures are recognized as important taxonomic characters in nematodes (Chitwood and Wehr, 1934; Chitwood and Chitwood, 1950; Chabaud, 1955, 1959; Anderson, 1968). The pronounced difference in cephalic structure between *R. paradoxa* and the new species warrants erection of a new genus. Biological differences offer further support for this action. *D. macropi* occurs free in the mesenteric and large hepatic portal veins of a range of Macropodidae, marsupial mammals belonging to the kangaroo family and occuring only in Australia and Papua New Guinea.

The atrophy or hypertrophy of body musculature is viewed as a poor generic character more likely to reflect habitat or mode of life of a parasite species rather than phylogenetic relationships (cf. *Onchocerca* spp., Bain and Beveridge, 1979). The well-developed musculature of *D. macropi* is probably an adaptation to life in blood vessels, permitting the nematode to maintain its position in the portal veins and against the flow of blood. Living adult worms are highly active in blood and in blood clots during dissection of portal veins.

The absence of a mouth or oral opening and the failure to observe a trophosme in the new species leaves the question of nutriment unresolved. However the marked hypertrophy and presence of large amounts of granular material in the cytoplasm of muscle cells in non-gravid females, and its absence in gravid females suggests that muscle may play a role in storage of nutritional reserves required by developing eggs and larvae.

Larvae *in utero* and those recovered from blood are surrounded by an additional cuticular layer. This is not a sheath or egg membrane as occurs in the microfilaria of some Filarioidea because it is attached in the oral region. Although a genital primordium was not observed in larvae, the surrounding cuticular layer probably represents a moult and the larvae probably develop to the second or third-stage in the uterus of the female. In the Muspiceidae larvae mature to the third-stage in the uterus of the female in the three genera in which they are known. In this family transmission is thought to be direct and by way of a percutaneous *(Lukonema, Riouxgolvania, Pennisia)* or digestive (by licking) *(Muspicea)* route. While larvae of *D. macropi* have been recovered from the non-peripheral blood of freshly shot animals this may not be their normal environment. The finding of larvae in the lactating mammary gland of an *M. agilis* and their absence from the three non-lactating glands of the same wallaby suggests that transmission of *D. macropi* may be direct and by a milk route. However, the presence of larvae in thigh skin capillaries of a male *M. agilis* also suggests the possibility of a direct percutaneous route or transmission by a haemophagous arthropod. Examination of several *Heterodoxus macropus* Le Souef and Bullen, 1902 from the male *M. agilis* revealed no larval forms of *D. macropi*. Chabaud and Campana (1950) suggested that female *R. paradoxa* are protandric hermaphrodites and that transmission probably occurs through carnivorism.

The morphological and biological characters observed in *D. macropi* clearly warrant generic recognition and may deserve further separation at the subfamily or family level. However, in the interests of taxonomic stability and in view of the absence of knowledge of the male or any aspect of the natural history of *R. paradoxa*, we have tentatively placed *Durikainema* gen. nov. alongside *Robertdollfusa* in the Robertdollfusidae.

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BIBLIOGRAPHY

ANDERSON R. C. : The comparative morphology of cephalic structures in the superfamily Filarioidea (Nematoda). *Can. J. Zool.*, 1968, *46*, 181-199.

BAIN O., BEVERIDGE I. : Redescription d'*Onchocerca gibsoni* C. et J., 1910. *Ann. Parasitol.Hum. Comp.*, 1979, *54*, 69-80.

BAIN O., CHABAUD A. G. : Sur les Muspiceidae (Nematoda-Dorylaimina). *Ann. Parasitol. Hum. Comp.*, 1979, *54*, 207-225.

CHABAUD A. G. : Remarques sur la symétrie céphalique des nématodes et hypothèses concernant l'évolution de cette symétrie chez les phasmidiens parasites. *Bull. Soc. Zool. Fr.*, 1955, *80*, 314-323.

CHABAUD A. G. : Phénomène d'évolution régressive des structures céphaliques et classification des nématodes Spiruroidea. *Parassit.*, 1959, *1*, 11-20.

CHABAUD A. G., Campana Y. : Nouveau parasite remarquable par l'atrophie de ses organes : *Robertdollfusa paradoxa* (Nematode *incertae sedis*). *Ann. Parasitol. Hum. Comp.*, 1950, *25*, 325-334.

CHITWOOD B. G., CHITWOOD M. B. : An introduction to nematology. Sect. I. Anatomy. *Monumental Printing Co.*, Baltimore, 1950.

CHITWOOD B. G., WEHR E. E. : The value of cephalic structures as characters in nematode classification, with special reference to the superfamily Spiruroidea. *Z. Parasitenk.*, 1934, *7*, 273-335.

PARUKHIN A. M., OSHMARIN P. G. : *Encephalonema longimicrofilaria* gen. et sp. nov. ; nematode from the encephalon of birds. *Zool. Zhurn.*, 1960, *39*, 434-436. (In Russian).

ROMAN E. : (p. 725 in Grassé) Traité de Zoologie. Anatomie, Systématique, Biologie. Vol. 4, Part. 2. Némathelminthes (Nématodes), 731 p. *Masson*, Paris, 1965.