Molecular and Cellular Probes

Phylogenetic relationships of species of the oesophageal parasitic nematode genera *Cyclostrongylus* and *Spirostrongylus* (Strongyloidea: Chabertiidae: Cloacininae) with their wallaby hosts (Marsupialia: Macropodidae)

Neil B. Chilton [a] *, Florence Huby-Chilton [b,c], Anson V. Koehler [b], Robin B. Gasser [b], Ian Beveridge [b]

[a] *Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

[b] *Faculty of Veterinary and Agricultural Science, University of Melbourne, Parkville, Victoria, Australia*

---

* Corresponding author. Tel.: +0000000; fax: +00000000.

E-mail address: neil.chilton@usask.ca

ABSTRACT

A phylogeny for seven species of *Cyclostrongylus* and the monotypic genus *Spirostrongylus* (Nematoda: Chabertiidae), all highly host specific parasites of the oesophagi of wallabies (Marsupialia: Macropodidae), was constructed using sequence data for the first and second internal transcribed spacers (ITS-1 and ITS-2) of the nuclear ribosomal DNA. There was no evidence for co-speciation, or for the sympatric or synxenic speciation of *C. alatus* and *C. perplexus*, both of which are parasites of *Macropus rufogriseus*. Rather, host switching, correlating with geographical distributions, appeared to provide some explanation of the pattern of speciation observed.

Keywords : *Cyclostrongylus*; *Spirostrongylus*; Chabertiidae; *Macropus*; *Wallabia*; Macropodidae; Nuclear ribosomal DNA; Sequence data; Phylogeny

1. Introduction

The phenomenon of niche specialization in helminth parasites is well documented. Nematode parasites, for example, have radiated to occupy virtually all organs of the vertebrate body [1], whereas monogeneans on the gills of fish show distinctive distributions either to facilitate reproduction or possibly to avoid competition [2]. Cestodes often segregate into different regions of the intestine, as in the case of the multiple species of *Raillietina* found in the small intestines of emus (*Dromaius novaehollandiae*) [3], while strongylid nematodes are found in different regions of the caecum and colon of equids [4]. A particularly interesting example of niche specialization is found in the case of the tetraphyllideans of elasmobranchs where the morphology of the scolex is closely adapted to the surface topography of the spiral valve [5]. Such morphological adaptations to specific niches are known from many parasitic nematodes, with members of the Trichostrongyloidea adapted to coiling around the villi of the small intestine [6] or burrowing into the mucosa of the stomach and oesophagus in macropodid marsupials [7].

Two genera of strongylid nematodes of macropodid marsupials, *Cyclostrongylus* and *Spirostrongylus* (Strongloidea Chabertiidae, Cloacininae), occur in the oesophagi of a small group of wallabies belonging to the sub-genus *Prionotemnus* (genus *Macropus*) and the genus *Wallabia* (Marsupialia: Macropodidae), in which the oesophageal mucosa is uniquely modified to contain numerous elongate papillae, around which the nematodes are coiled [8-10] (Figs. 1, 2, 4). Both nematode genera have a body that is spirally coiled in the mid region, and both have alae running the length of the body; these two characters occur in no other genera of the Cloacininae (or the Strongyloidea generally). These ventral or lateral alae are pressed into the papillae presumably to increase traction [10] (Fig. 2). Even when removed from their attachment sites, the nematodes retain their coiled body plan (Fig. 3).

The general pattern observed is that each species of wallaby has a single species of nematode parasite (Table 1). There is a single exception to this general pattern. In the red-necked wallaby, *M. rufogriseus*, while the anterior region of the oesophageal mucosa consists of elongated papillae, the posterior part consists of longitudinal folds [8]. The anterior region is parasitised by *C. perplexus*, which is found coiled around papillae, while in the posterior part, *C. alatus* is found between the folds; the latter species is not coiled and has paired lateral alae rather than a single ventral ala, presumably aiding in attachment in this particularly modified region of the oesophagus [10].

These observations raise several questions regarding the evolution of oesophageal-inhabiting nematodes of macropodids: (i) Given that in most instances there is a single species of nematode in each host species, does this pattern represent an instance of co-speciation? A phylogenetic analysis of morphological characters [11] has suggested that this is the case, but the hypothesis has not been tested. (ii) In *M. rufogriseus*, is the adaptation of *C. alatus* to a region of longitudinal oesophageal folds, an example of sympatric or synxenic speciation [12] within the one host?

The first question is significant in that attempts to demonstrate events in the co-speciation of cloacinine nematodes from macropodid marsupials have so far been extremely limited in their success [11] [13-14]. Consequently, the observations of host associations made here offer a prospect of demonstrating co-speciation in the pair of nematode genera. Second, although it has been claimed that sympatric speciation in parasitic nematodes is highly likely [15], few convincing cases of this phenomenon have been reported to date [12]. As the observed host-parasite associations between oesophageal parasites and their wallaby hosts present prima facie

evidence for these phenomena, it was decided to investigate these host-parasite associations in more detail using molecular methods.

2. Materials and methods

Nematodes were obtained from the oesophagi of wallabies (Fig. 5A, Table 2), which had been collected as fresh road-kills or from road-kills frozen prior to examination. The geographical distributions of the various species of wallabies are shown in Figs. 5A and 5B. In instances where a nematode species occurred across a large geographical region, an attempt was made to include samples from different Australian states, particularly any occurring in the island state of Tasmania. Nematodes were washed in saline and then frozen in liquid nitrogen and stored at -80° prior to examination. Additional samples of nematodes from each host were fixed in Berland's fluid (glacial acetic acid and formalin [16]) for morphological examination.

Frozen nematodes were thawed. The head and tail of each worm were removed, fixed in lactophenol and mounted permanently in polyvinyl lactophenol as voucher specimens, with the mid-body region being used for genetic analyses. Nematodes were identified from published descriptions [10] [17]. Voucher specimens have been deposited in the South Australian Museum (SAM), Adelaide (Table 2). Host nomenclature follows van Dyck & Strahan [18].

Genomic DNA was isolated from the mid-body section of each nematode using a small-scale sodium-dodecyl-sulphate/proteinase K extraction procedure [19], followed by purification using a mini-column (Wizard™ Clean-Up, Promega). The region of rDNA comprising the ITS-1, 5.8S rRNA gene, ITS-2 and flanking sequences (= ITS+) was amplified by the polymerase chain reaction (PCR) [20] using primers NC16 (forward; 5’-AGTTCAATCGCAATGGCTT-3’) and NC2 (reverse; 5'-TTAGTTTCTTTTCCTCCGCT-3'). PCR was performed in a 50 µl volume

for 30 cycles at 94°C for 30 sec (denaturation), 55°C for 30 sec (annealing) and 72°C for 30 sec (extension), followed by one cycle at 72°C for 5 min (final extension). Negative (no-DNA) controls were included in each set of reactions. Amplicons were purified using mini-columns (Wizard™ PCR-Preps, Promega), and the ITS+ was sequenced in both directions using the same primers (separately) as used for PCR. The sequences generated in the present study have been deposited in GenBank (Table 2). Sequences were initially aligned using Muscle [21] and alignments were adjusted manually using the program Mesquite v.2.75 [22]. The ITS+ sequences of *Oesophagostomoides longispicularis* (Phascolostrongylinae), from the colon of wombats (*Vombatus ursinus*), and *Paramacropostrongylus typicus* (Phascolostrongylinae), from the stomach of the western grey kangaroo (*Macropus fuliginosus*), were used as outgroups in phylogenetic analyses, as the Phascolostrongylinae is the sister group to the Cloacininae, to which both *Cyclostrongylus* and *Spirostrongylus* belong [10] [23].

Phylogenetic analyses of sequence data were conducted by Bayesian inference (BI) using Monte Carlo Markov Chain analysis in the program MrBayes v.3.2.2 [24]. The likelihood parameters set for the BI analysis of sequence data were based on the Akaike Information Criteria test in jModeltest v.2.1.5 [25]. The number of substitutions was set at 6, with a gamma-distribution. For the tree, posterior probability (pp) values were calculated by running 2,000,000 generations with four simultaneous tree-building chains. Trees were saved every 100th generation. At the end of each run, the standard deviation of split frequencies was <0.01, and the potential scale reduction factor approached one. For each analysis, a 50%-majority rule consensus tree was constructed based on the final 75% of trees produced by BI. Analyses were run three times to ensure convergence and insensitivity to priors. Phylogenetic analyses were also carried out using a neighbor-joining (NJ) and maximum parsimony (MP) methods in the

software package PAUP v4.0b2 [26]. NJ analyses were run with and without alignments that had been filtered using GBlocks [27] to remove ambiguous sections of the alignment. For the MP analyses, heuristic searches were carried out with random addition of sequences (n=100), tree-bisection-reconstruction (TBR) branch swapping, the MulTrees option in effect, MaxTrees set at 4,000 and saving all equally parsimonious trees. All characters were equally weighted and unordered. Alignment gaps were treated as missing values in the analyses. The tree length (L), consistency index excluding uninformative characters (CI) and retention index (RI) were recorded for each analysis. The relative support for clades in NJ and MP analyses was determined using 1,000 bootstrap replicates. Parasite phylogeny was compared with a molecular phylogeny of the hosts [28]. *Macropus dorsalis* was missing from the latter study and was interpolated based on a comprehensive data set for the Macropodidae [29]. *Macropus parma* was included even though no nematode parasites were collected from it (see below) so that all members of the host clade were shown in the co-phylogeny.

3. Results

The DNA sequences of the ITS-1, 5.8S rRNA gene and ITS-2 for *S. spirostrongylus* and the six species of *Cyclostrongylus* were 378-382 bp, 153 bp and 211-223 bp, respectively. The ITS-1 and ITS-2 sequences of these nematodes and the two outgroup taxa were aligned over 632 positions, of which 116 were informative in the MP analyses. Three equally most parsimonious trees (L = 435, CI = 0.75, and RI = 0.77) were produced from the MP analyses. The topologies of the strict consensus trees and the NJ tree are not shown; however, they were very similar to that produced by the BI analysis (Fig. 6). Clades considered to have adequate support were those with bootstrap percentages greater than 70% in the MP and NJ trees and posterior probabilities

of greater than 0.95 in the BI tree. The results of the phylogenetic analyses placed *Spirostrongylus* as the sister genus to *Cyclostrongylus*. Within the *Cyclostrongylus* clade, *C. leptos* from *M. dorsalis* and *C. elegans* from *M. parryi* formed a well-supported clade (1.0 in the BI analysis, 96% in the NJ tree and 86% in the MP tree) sister to the remaining clades within the genus, although support for this relationship was poorer (0.92 in the BI analysis, 78% in the NJ tree and 70% in the MP tree). *Cyclostrongylus irma* from *M. irma* and *C. kartana* from *M. eugenii* formed a poorly supported clade that was sister to the clade consisting of *C. alatus* from *M. rufogriseus*, *C. wallabiae* from *W. bicolor* and *C. perplexus* from *M. rugogriseus* (Fig. 6). Within this clade, *C. alatus* was the sister species to *C. wallabiae* and *C. perplexus*. Based on this phylogeny, the two synhospitalic species (i.e. two or more related parasite species occurring together on the same host species or host individual [30]), *C. alatus* and *C. perplexus*, both found in the oesophagus of *M. rufogriseus*, are not sister species.

Comparison of the parasite phylogeny, derived from the ITS+ sequence data, with the currently available host phylogeny provides little evidence for co-speciation between parasite and host (Fig. 6). *Spirostrongylus*, the sister species in the parasite tree to the genus *Cyclostrongylus*, occurs in a host, *M. agilis*, which is in a terminal branch of the host tree. By contrast, the basal host in the macropodid phylogeny, *W. bicolor*, is parasitised by one of the nematodes, *C. wallabiae*, occurring in the crown of the nematode phylogenetic tree. Similarly, *C. elegans*, which occurs in a basal branch of the phylogeny of *Cyclostrongylus*, is found in a host, *M. parryi*, which occurs in one of the terminal branches of the host phylogeny, whereas *C. perplexus*, found in a terminal branch of the nematode phylogeny, occurs in a host, *M. rufogriseus*, which occurs more basally in the host phylogeny. Owing to a lack of molecular data

for the phylogenetic relationships of *M. dorsalis*, no comment can be made concerning the phylogenetic relationship of *C. leptos* with its host.

4. Discussion

At the outset of this study, two questions were posed relating to the oesophageal-inhabiting nematodes of wallabies and these are dealt with here in turn. The first question posed was whether the pattern of nematode distribution, given their high degree of host specificity, indicates a pattern of co-speciation with hosts.

The clear indication from the molecular data derived from the nematodes is that there has been little or no co-speciation with hosts. The conclusions that can be drawn are limited, to some extent, because the molecular phylogenetic data currently available for *M. dorsalis* do not include the genes used in other studies [28] and also because we were unable to obtain nematodes from *M. parma*, currently considered to be rare [31], for molecular analyses. In spite of these limitations, the overall pattern is not one of co-speciation. The current data are not congruent with the morphological phylogeny and host associations published previously [11]. However, since then, a more refined estimate of host associations has become available [28] and a new species, *C. irma*, has been described [17], which was previously considered to be *C. wallabiae* [11].

Consequently, it is difficult to make comparisons with the earlier analysis based on morphology. Nevertheless, the overall conclusion of the study based on a morphological examination of seven other cloacinine genera [11], was that host switching appeared to be the major mechanism of speciation within the Cloacininae. The sole exception to this pattern was the

genus *Cyclostrongylus.* The molecular data presented here suggest that, contrary to previous studies, this genus also complies with the overall pattern observed to date of evolution by host switching rather than co-speciation.

Reviewing current hypotheses on the evolution of the strongylid nematodes of Australasian marsupials, this result may not be surprising. The phascolostrongyline nematodes inhabiting the large intestine and, to a lesser extent the stomachs of herbivorous marsupials, probably represent the ancestors of the stomach-inhabiting cloacinine nematodes [10] [32]. The invasion of the oesophagus of wallabies as a parasitological niche is therefore likely to be secondary to the colonization of the stomach. On this basis, since the tribe Pharyngostrongylinea, to which both *Cyclostrongylus* and *Spirostrongylus* putatively belong, occur primarily as inhabitants of the macropodid stomach [10], oesophageal parasitism is a secondary development. If recent, then it could post-date the evolution of the hosts as the separation of *Wallabia* from *Notamacropus* is thought to have occurred about 5.3 mya [28]. If this were the case, co-speciation would not be expected to be the principal mode of parasite evolution. Hence, a scenario in which the invasion of the oesophagus by *Spirostrongylus* in one species of *Notamacropus* and the evolution of multiple species of *Cyclostrongylus* in *Notamacropus* and *Wallabia* primarily by host switching seems plausible.

It is possible that the current geographical distribution of the hosts may better explain the parasite phylogeny established here. *Spirostrongylus spirostrongylus*, a sister taxon to species in the genus *Cyclostrongylus*, is parasitic in the oesophagus of *M. agilis*, a wallaby found across northern Australia, extending to central Queensland (Fig. 5B), although a disjunct population exists on Stradbroke Island in southern Queensland, suggesting that it once had a wider distribution [33]. One highly supported clade of *Cyclostrongylus* includes *C. elegans* and *C.*

leptos, which are parasitic in *M. parryi* and *M. dorsalis* respectively. Both of these host species occur in north-eastern Australia (northern New South Wales and Queensland) [34-35] and both are sympatric in some areas with *M. agilis*. Consequently, it is easy to postulate a scenario of host switching between *M. dorsalis* and *M. parryi*.

There was a weak sister taxa relationship of *C. irma* in *M. irma* and *C. kartana* in *M. eugenii*. These host species are essentially southern in their distribution. *Macropus eugenii* occurs in South Australia and the south-west of Western Australia, where it is sympatric with *M. irma* [36 -37]. The crown clade contains nematodes parasitic in *M. rufogriseus* and *W. bicolor*. *Macropus rufogriseus* extends into central northern New South Wales and Queensland, where it is sympatric with both *M. dorsalis* and *M. parryi* [36 - 38]. In addition, *M. rufogriseus* once occurred on Kangaroo Island [39], where it was sympatric with *M. eugenii*. Consequently, this series of host species are or were sympatric, providing opportunities for a series of host switching events in the north and in the south. This hypothesis needs to be treated with caution, as the detailed phylogeography of these hosts is currently unknown, and the distribution of *W. bicolor* (the host of *C. wallabiae*) along the entire eastern region of Australia [40] is not entirely consistent with this proposal.

The second question posed was whether *C. perplexus* and *C. alatus*, both parasitic in the oesophagus of *M. rufogriseus*, were an example of within-host or synxenic [41] speciation, and the adaptation to different morphological characteristics of the anterior as opposed to the posterior region of the oesophagus (i.e. papillae in the posterior region as opposed to longitudinal folds in the anterior region). The molecular data presented here provide strong evidence to reject this hypothesis and suggest that these two species are not closely related to one another, representing independent invasions of the oesophagus of *M. rufogriseus*. The analysis presented

here also suggests that the single ventral ala shared by *Spirostrongylus* and most species of *Cyclostrongylus* is the plesiomorphic state for these genera, and that the development of paired lateral alae and the loss of the ventral ala in *C. alatus* is an autapomorphic development. An alternative explanation, that following the development of lateral alae, there was a reversion to the plesiomorphic state of a single ventral ala seems less parsimonious.

Although large numbers of nematodes were not examined in this study, the use of specimens of *C. perplexus* from four states, covering a geographical distance of 1,450 km on the mainland, *C. wallabiae* from three states covering 1,690 km and *C. kartana* from two states covering 1,700 km, without significant genetic differences between the samples, suggests that more extensive sampling would be unlikely to reveal much genetic differentiation within these taxa. In addition, in the case of two species, *C. alatus* and *C. perplexus*, samples were included from the mainland and from Tasmania, from two different sub-species of *M. rufogriseus*. Tasmania has been separated from the mainland for 8,000-10,000 years [39] and there are few genetic differences between *Labiosimplex australis* , another cloacinine nematode, from Tasmania and the mainland [42]. The present study also included duplicate specimens from a single site (*C. perplexus* and *C. irma*) as well as the use of both male and female specimens of the same species (*C. alatus*, *C. irma*, *C. perplexus* and *C. wallabiae*). Within the strongylid nematodes generally, both the ITS-1 and ITS-2 sequences are reliable as genetic markers for specific identification [43], in particular in nematodes from macropodids [44-45].

In conclusion, it appears that the radiation of nematodes within the oesophagi of wallabies has occurred primarily by host switching, with the geographical distributions of the hosts possibly playing a role. Further resolution of the parasite tree (i.e. with inclusion of *C. parma*) would be useful in further supporting these conclusions.

Acknowledgements

The Australian Research Council and the Australian Biological Resources Study provided financial support for this research. Other support from Melbourne Water Corporation and a Victorian Life Sciences Computation Initiative (grant number VR0007) on its Peak Computing Facility at the University of Melbourne, an initiative of the Victorian Government, is gratefully acknowledged (RBG).

References

[1] R.C. Anderson, Nematode parasites of vertebrates, their development and transmission, Commonwealth Agricultural Bureaux International, Wallingford, 2000, pp 650.

[2] K. Rohde, Niche restriction in parasites: proximate and ultimate causes. Parasitology 109 (1994) S69-S94.

[3] M.G. O'Callaghan, M. Davies, R.H. Andrews, The spatial distribution of five species of *Raillietina* infecting the emu, *Dromaius novaehollandiae*, Trans. R. Soc. S. Aust. 130 (2006) 71-78.

[4] L. Stancampiano, L. Mughini Gras, G. Poglayen, Spacial niche competition among helminth parasites in horse's large intestine, Vet. Parasitol. 170 (2010) 88-95.

[5] A. H. McVicar, The distribution of cestodes within the spiral intestine of *Raja naevus* Müller and Henle, Int. J. Parasitol. 9 (1979) 165-176.

[6] M.-C. Durette-Desset, Essai de classification des Nématodes Héligmosomes. Corrélations avec la paléo-biogéographie des hôtes, Bull. Mus. Natl. Hist. Nat. Paris 49 (1971) 1-126.

[7] I. Beveridge, *Woodwardostrongylus petrogale* sp. nov. (Nematoda: Cloacinidae), from the stomachs of rock wallabies (*Petrogale* spp.) from Arnhem Land, Trans. R. Soc. S. Aust. 122 (1998) 73-78.

[8] D.L. Obendorf, The macropodid oesophagus: I. Gross anatomical, light microscopic, scanning and transmission electron microscopic observations of its mucosa, Aust. J. Zool. 32 (1984) 437-445.

[9] D.L. Obendorf, The macropodid oesophagus: III. Observations on the nematode parasites, Aust. J. Zool. 32 (1984) 415-435.

[10] I. Beveridge, A taxonomic revision of the Pharyngostrongylinea Popova (Nematoda: Strongyloidea) from macropodid marsupials, Aust. J. Zool. S83 (1982)1-150.

[11] I. Beveridge, N.B.Chilton, Co-evolutionary relationships between the nematode subfamily Cloacininae and its macropodid marsupial hosts, Int. J. Parasitol. 31(2001) 976-996.

[12] K.D. McCoy, Sympatric speciation in parasitic nematodes - what is sympatry? Trends Parasitol. 19 (2003) 400-404.

[13] I. Beveridge, N.B. Chilton, D.M. Spratt, The occurrence of species flocks in the nematode genus *Cloacina* (Strongyloidea: Cloacininae) parasitic in the stomachs of kangaroos and wallabies, Aust. J. Zool. 50 (2002) 597-620.

[14] N.B. Chilton, F. Huby-Chilton, I. Beveridge, L.R. Smales, R.B. Gasser, R.H. Andrews, Phylogenetic relationships of species within the tribe Labiostrongylinea (Nematoda:

Cloacinidae) from Australian marsupials based on ribosomal DNA spacer sequence data, Parasitol. Internat. 60 (2011) 381-387.

[15] P.W. Price, Evolutionary Biology of Parasites, Princeton University Press, USA, 1980.

[16] D.I. Gibson, Materials and methods in helminth alpha-taxonomy, Parasitology 79 (1979) S26.

[17] A. Appan, J. Bergfeld, I.Beveridge, New species of parasitic nematodes from macropodid marsupials in Western Australia, Trans. R. Soc. S. Aust. 128 (2004) 77-84.

[18] S. van Dyck, R.Strahan, The mammals of Australia, 3rd edn., New Holland Publishers, Australia, 2008, pp. 887.

[19] R.B. Gasser, N.B. Chilton, H. Hoste, I. Beveridge, Rapid sequencing of rDNA from single worms and eggs of parasitic helminths, Nucleic Acids Res. 21(1993) 2525-2526.

[20] R.K. Saiki, D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, H.A.Erlich, Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239 (1988) 487- 491.

[21] R.C. Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res. 32 (2004) 1792-1797.

[22] W.P. Maddison, D.R.Maddison, Mesquite: A modular system for evolutionary analysis, Version 2.75; 2011.

[23] N.B. Chilton, R.B. Gasser, I. Beveridge, Phylogenetic relationships of Australian strongyloid nematodes inferred from ribosomal DNA sequence data, Int. J. Parasitol. 27 (1997) 1481-1494.

[24] F. Ronquist, J.P. Huelsenbeck, MrBayes 3: Bayesian phylogenetic inference under mixed models, Bioinformatics 19 (2003) 1572-1574.

[25] D. Posada, jModelTest: phylogenetic model averaging, Mol. Biol. Evol. 25 (2008) 1253-1256.

[26] D.L.Swofford, PAUP: Phylogenetic Analysis Using Parsimony, version 4.0b2, Sinauer Associates Inc., Sunderand, Massachusetts;1999.

[27] G. Talavera, J.Castresana, Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments, Syst. Biol. 56 (2007) 564-577.

[28] R.W. Meredith, M. Westerman, M.S. Springer, A phylogeny and timescale for the living genera of kangaroos and kin (Macropodiformes: Marsupialia) based on nuclear DNA sequences, Aust. J. Zool. 56 (2008) 395-410.

[29] M. Cardillo, O.R.P. Bininda-Edmonds, E, Boakes, A. Purvis, A species-level phylogenetic supertree of marsupials, J. Zool. (London) 264 (2004)11-31.

[30] W. Eichler, Two new evolutionary terms for speciation in parasitic animals, Syst. Zool. 15 (1966) 216-218.

[31] G. Maynes, Parma wallaby: *Macropus parma* Waterhouse, 1845, in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 341-343.

[32] I. Beveridge, The systematic status of Australian Strongyloidea (Nematoda), Bull. Mus. Natl. Hist. Nat. Paris (4ème série), 9 (1987) 107-126.

[33] J.C. Merchant, Agile wallaby: *Macropus agilis* (Gould, 1842), in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 323-324.

[34] P.M. Johnson, Black-striped wallaby: *Macropus dorsalis* (Gray, 1837), in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 329-330.

[35] P.M. Johnson, Whiptail wallaby: *Macropus parryi* Bennett, 1835, in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 343-345.

[36] K.D. Morris, P. Christensen, Western brush wallaby: *Macropus irma* (Jourdan, 1837), in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 340-341.

[37] L. Hinds, Tammar wallaby: *Macropus eugenii* (Desmarest, 1817), in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 330-332.

[38] P.J. Jarman, J.H. Calaby, Red-necked wallaby: *Macropus rufogriseus* (Desmarest, 1817), in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 359-351.

[39] J.H. Hope, R.J. Lampert, E. Edmondson, M.J. Smith, G.F. van Tets. Late Pleistocene faunal remains from Seton rock shelter, Kangaroo Island, South Australia. J. Biogeogr. 4 (1977) 363-385.

[40] J.C. Merchant, Swamp wallaby: *Wallabia bicolor*, in: S. van Dyck, R. Strahan (Eds.), The Mammals of Australia, 3rd edn, New Holland Publishers, Australia, 2008, pp. 404-406.

[41] L. Euzet, C. Combes, Les problèmes de l'espèce dans le règne animal. Mém. Soc. Zool. France 40 (1980) 238-285.

[42] N.B. Chilton, F. Huby-Chilton, L.R. Smales, R.B. Gasser, I. Beveridge, Genetic divergence between island and continental populations of the parasitic nematode *Labiosimplex australis* in Australia, Parasitol. Res. 104 (2008) 229-236.

[43] N.B. Chilton, The use of nuclear ribosomal DNA markers for the identification of bursate nematodes (order Strongylida) and for the diagnosis of infections, Anim. Health Res. Rev. 5 (2004) 173-187.

[44] N.B. Chilton, F. Huby-Chilton, P.M. Johnson, I. Beveridge, R.B. Gasser, Genetic variation within species of the nematode genus *Cloacina* (Strongyloidea: Cloacininae) parasitic in the stomachs of rock wallabies, *Petrogale* spp. (Marsupialia: Macropodidae) in Queensland, Aust. J. Zool. 57 (2009) 1-10.

[45] N.B. Chilton, A. Jabbar, F. Huby-Chilton, A.R. Jex, R.B. Gasser, I. Beveridge, Genetic variation within the *Hypodontus macropi* (Nematoda: Strongyloidea) complex from macropodid marsupial hosts in Australia, Electrophoresis 33 (2012) 3544-3554.

Captions to figures

Figs. 1-4. *Cyclostrongylus wallabiae* in the oesophagus of *Wallabia bicolor*. **Fig. 1.** Mucosal surface of the oesophagus showing the papillated surface with a heavy burden of *C. wallabiae* coiled around papillae. **Fig. 2.** Histological section of the oesophageal mucosa showing numerous papillae with a single *C. wallabiae* coiled around them. **Fig. 3.** Specimen of *C. wallabiae* removed from the oesophagus showing the permanently coiled body form. **Fig. 4.** Scanning electron micrograph of *C. wallabiae* coiled around the oesophageal papillae of *W. bicolor*.

Fig. 5. A. Collection localities for specimens of *Cyclostrongylus* and *Spirostrongylus* used in molecular analyses together with the distribution of *Wallabia bicolor* in eastern Australia. **B.** Geographical distributions of the macropodid hosts of the sub-genus *Prionotemnus* for species of *Cyclostrongylus* and *Spirostrongylus*.

Fig. 6. Phylogenetic relationships of species of *Cyclostrongylus* and *Spirostrongylus* based on a Bayesisan analysis of the sequence data of the ITS+ nuclear ribosomal DNA and the relationships of their hosts. Values above branches indicate posterior probabilities, while those below branches represent NJ and MP bootstrap values (left and right, respectively) that were greater than 70%. Abbreviations of Australian state names are provided in Table 2. Mya = million years ago

Author/s:
Chilton, NB; Huby-Chilton, F; Koehler, AV; Gasser, RB; Beveridge, I

Title:
Phylogenetic relationships of species of the oesophageal parasitic nematode genera Cyclostrongylus and Spirostrongylus (Strongyloidea: Chabertiidae: Cloacininae) with their wallaby hosts (Marsupialia: Macropodidae)

Date:
2016-04-01

Citation:
Chilton, N. B., Huby-Chilton, F., Koehler, A. V., Gasser, R. B. & Beveridge, I. (2016). Phylogenetic relationships of species of the oesophageal parasitic nematode genera Cyclostrongylus and Spirostrongylus (Strongyloidea: Chabertiidae: Cloacininae) with their wallaby hosts (Marsupialia: Macropodidae). MOLECULAR AND CELLULAR PROBES, 30 (2), pp.93-99. https://doi.org/10.1016/j.mcp.2016.01.004.