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Phylogenetic relationships of species within the tribe Labiostrongylinea (Nematoda: Cloacinidae) from Australian marsupials based on ribosomal DNA spacer sequence data

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ABSTRACT

Parasitic nematodes of the tribe Labiostrongylinea (Family Cloacinidae) occur in the stomachs of a wide variety of potoroid and macropodid marsupials in Australia, Papua Indonesia and Papua New Guinea. The aim of the present study was to infer the evolutionary relationships of the five genera of labiostrongyline nematodes that occur in Australian potoroids and macropodids using sequence data of the nuclear first and second internal transcribed spacers of ribosomal DNA. The phylogenetic analyses resulted in the separation of the Labiostrongylinea into two major groups reflecting coevolution between hosts and parasites. Two nematode species belonging to the genus *Potorostrongylus* formed a sister group to the remaining species of the Labiostrongylinea. This genus occurs exclusively in potoroid marsupials, which are considered to be basal to the macropodid marsupials. The second major group included species of *Labiostrongylus*, *Labiosimplex*, *Labiomultiplex* and *Parazoniolaimus*, all of which occur in macropodids. These species formed two distinct clades, one predominating in the host genera *Thylogale* and *Onychogalea*, and the second in the genus *Macropus*, which includes the more recent macropodids. However, there is also evidence of colonisation by both nematode clades of relatively unrelated hosts. In addition, genetic differences among individuals of *Lm. eugenii* from geographically isolated populations of *M. eugenii*, and among *Ls. longispicularis* from different subspecies of *M. robustus* suggest the existence of sibling species that may have arisen by allopatric speciation. The broad coevolutionary relationship between the labiostrongyline nematodes and their marsupial hosts therefore represents a mixture of potential cospeciation and colonisation events.

1. Introduction

Coevolution between parasites and their hosts is a well-recognised phenomenon [1] even if the relative roles of cospeciation and colonisation in this process are debatable. The cloacinine nematodes (Strongylida: Cloacininae) that occur in the sacculated forestomachs of kangaroos and wallabies (Macropodidae) and rat-kangaroos (Potoroidae) are a diverse assemblage of parasites, comprising 36 genera and more than 255 species arranged in six tribes: Cloacininea, Coronostrongylinea, Labiostrongylinea, Macropostrongylinea, Pharyngostrongylinea and Zoniolaiminea [2]. Cloacinine nematodes have clearly coevolved with their macropodoid hosts (Macropodidae + Potoroidae) as sacculated forestomachs have evolved in macropodoids over the last 30 million years [3–5]. However, the mechanisms involved in the evolution of the numerous groups of cloacinine nematodes are far from clear. Beveridge and Chilton [2] examined the phylogenetic relationships of eight relatively small genera of the subfamily Cloacininae using cladistic analyses of morphological characters. They concluded that, although there was some evidence of cospeciation in the genera examined, colonisation appeared to be the more important mechanism of diversification within this nematode radiation [2]. Their conclusions are potentially limited by the fact that they examined genera comprising a relatively small number of species, and the analyses were conducted exclusively on morphological characters. Using a larger genus or series of closely related genera combined with molecular techniques may provide more significant insights into the evolution of this diverse group of nematodes.

The Labiostrongylinea contains at least 35 species [2,6] that are distinguished from members of the other tribes by differences in the morphology of lips, buccal capsule and oesophagus [7]. The type genus, *Labiostrongylus*, was subdivided by Smales [8,9] into three new

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subgenera (*Labiostrongylus*, *Labiosimplex* and *Labiomultiplex*). Subsequently, *Labiosimplex* and *Labiomultiplex* were elevated to generic status [10]. Currently, the Labiostrongylinea consists of seven genera; *Dorcopsinema*, *Labiostrongylus*, *Labiosimplex*, *Labiomultiplex*, *Parazoniolaimus*, *Paralabiostrongylus* and *Potorostrongylus*[10]. Species of *Potorostrongylus* occur in the stomachs of rat kangaroos (potoroids), while those of the other genera occur in the stomachs of kangaroos and wallabies (macropodids) [11,12]. Two genera, *Dorcopsinema* and *Paralabiostrongylus*, occur only in Papua Indonesia and Papua New Guinea, whereas the other five genera occur predominately in Australia [6,8,9]. A cladistic analysis of the phylogenetic relationships of the seven genera within the Labiostrongylinea was conducted by Smales [10] based on the use of 22 morphological characters. The results revealed the existence of two major clades. One clade comprised the genera *Dorcopsinema* and *Paralabiostrongylus*, while the relationships of the five genera in the second clade could not be resolved [10]. It may be possible to resolve the relationships of these genera using phylogenetic analyses of DNA sequence data.

It has been shown that sequences of the first and second internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA (rDNA) differ among species of cloacinine nematodes [13–16]. These genetic markers have been used to demonstrate the existence of genetically distinct, but morphologically similar (i.e. cryptic) species [15,16], and to examine the phylogenetic relationships within and among genera of a variety of strongylid nematodes [17,18]. For example, the evolutionary relationships of four morphospecies within the genus *Cloacina* (tribe Cloacininea) that parasitize rock wallabies were inferred based on analyses of ITS-1 and ITS-2 rDNA sequence data [16]. The ITS-1 and ITS-2 have also been used as markers to examine genetic variation in two species of the Labiostrongylinea [19,20]. There was genetic divergence among geographically isolated populations of *Labiosimplex australis* on Kangaroo Island (South Australia), Tasmania and mainland Australia based on analyses of ITS-1 and ITS-2 sequence data [19]. The results obtained were consistent with the hypothesis that *Ls. australis* represented a single species, but that the island populations are in the initial stages of allopatric speciation [19]. Genetic divergence in the ITS-2 sequence was also detected between *Labiosimplex longispicularis* which parasitizes *Macropus robustus robustus* in New South Wales and *Ls. longispicularis* from *M. r. erubescens* and *M. rufus* in South Australia [20]. In the present study, sequence data for the ITS-1 and ITS-2 were used to infer the evolutionary relationships of the Australian representatives of the Labiostrongylinea.

2. Materials and methods

2.1. Samples

Adult nematodes of 26 morphospecies within the Australian representatives of the tribe Labiostrongylinea were collected from the stomachs of several species of macropodid and potoroid marsupials (Table 1). Nematodes were washed in physiological saline, snap frozen in liquid nitrogen and then stored at −70 °C until required for molecular analysis. Upon thawing, the anterior and posterior ends of each nematode were excised, placed on a microscope slide, fixed in glacial acetic acid, stored in 70% ethanol and deposited as voucher specimens in the South Australian Museum (SAM) (Table 1). Each nematode was identified to species using morphological criteria [6,8,9,11,12].

2.2. DNA extraction, PCR and sequencing

Genomic (g) DNA was extracted from the remaining part of each nematode (i.e. excluding the anterior and posterior ends) by SDS/Proteinase K treatment and then subjected to column purification using Wizard DNA Clean Up columns (Promega) according to the manufacturer's protocol. The region of the ribosomal DNA comprising

Table 1
Nematodes used in phylogenetic analyses, their hosts, collecting locality, coordinates and registration numbers for voucher specimens.

Nematode species	Host species	Locality[a]	Coordinates	SAM registration nos.
Labiostrongylus grandis	*M. robustus robustus*	Warrawee Stn, Qld	20°18′S 146°31′E	AHC34948
L. labiostrongylus	*Macropus agilis*	Cape Ferguson, Qld	19°28′S 147°06′E	AHC34949
Labiosimplex aridus	*M. rufus*	Cunnamulla, Qld	28°07′S 145°68′E	AHC30060
Ls. australis	*M. rufogriseus*	Launceston, Tas	41°27′S 147°10′S	AHC34751
Ls. australis	*M. rufogriseus*	Miles, Qld	26°40′S 150°11′S	AHC23781
Ls. australis	*M. dorsalis*	Miles, Qld	26°40′S 150°11′S	AHC30028
Ls. australis	*M. eugenii*	Kangaroo Island, SA	35°50′S 137°28′E	AHC34757
Ls. bancrofti	*M. parryi*	Dawes, Qld	24°86′S 151°11′E	AHC23045
Ls. bipapillosus	*M. giganteus*	Moonie, Qld	27°71′S 150°36′E	AHC30064
Ls. clelandi	*M. bicolor*	Traralgon Creek, Vic	38°24′S 146°31′E	AHC19909
Ls. communis	*M. bicolor*	Rockhampton, Qld	23°39′S 150°51′E	AHC23070
Ls. dendrolagi	*Dendrolagus lumholtzi*	Mt. Baldy, Qld	17°17′S 145°27′E	AHC34945
Ls. flanneryi	*Thylogale stigmatica*	Julatten, Qld	16°59′S 145°33′E	AHC34946
Ls. irma	*M. irma*	Collie, WA	33°36′S 116°15′E	AHC32959
Ls. kungi	*M. fuliginosus*	Naracoorte, SA	36°58′S 140°44′S	AHC23060
Ls. longispicularis	*M. robustus robustus*	Coonabarabran, NSW	31°16′S 149°17′E	AHC30042
Ls. longispicularis	*M. robustus eurebscens*	Spear Ck Stn, Pt. Augusta, SA	32°34′S 137°59′E	AHC23052
Ls. longispicularis	*M. rufus*	Wallerberdina, SA	31°43′S 138°06′E	AHC23059
Ls. major	*M. fuliginosus*	Kersbrook, SA	34°46′S 138°48′E	AHC23057
Ls. robustus	*M. robustus robustus*	Coonabarabran, NSW	31°16′S 149°17′E	AHC45458
Ls. thetidis	*T. stigmatica*	Lamington Nat Pk, Qld	28°15′S 153°08′E	AHC30074
Ls. thomasae	*M. eugenii*	Perup, WA	34°19′S 116°24′E	AHC32908
Labiomultiplex billardierii	*T. billardierii*	Launceston, Tas	41°27′S 147°10′S	AHC34951
Lm. contiguus	*M. parryi*	Thangool, Qld	24°21′S 150°56′E	AHC34944
Lm. eugenii	*M. eugenii*	Kangaroo Island, SA	35°50′S 137°28′E	AHC34758
Lm. eugenii	*M. eugenii*	Perup, WA	34°19′S 116°24′E	AHC33040
Lm. onychogale	*Onychogalea fraenata*	Dingo, Qld	23°38′S 148°19′E	AHC24427
Lm. thylogale	*T. stigmatica*	Lake Barrine NP, Qld	17°14′S 145°32′E	AHC34753
Lm. uncinatus	*M. dorsalis*	Rockhampton, Qld	23°39′S 150°51′E	AHC34947
Parazoniolaimus collaris	*M. bicolor*	Bondo State Forest, NSW	35°42′S 150°11′E	AHC45459
Potorostrongylus tropicus	*Bettongia tropica*	Davies Ck, Lamb Range, Qld	17°00′S 145°34′E	AHC30448
Po. finlaysoni	*Potorous tridactylus*	Lamington Nat Pk, Qld	28°15′S 153°08′E	AHC30450

[a] Qld = Queensland, NSW = New South Wales, Vic = Victoria, Tas = Tasmania, SA = South Australia and WA = Western Australia.