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CRYPTIC SPECIES IN POPULATIONS OF *GLOBOCEPHALOIDES TRIFIDOSPICULARIS* KUNG (NEMATODA: TRICHOSTRONGYLOIDEA), PARASITIC IN MACROPODID MARSUPIALS

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Summary

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The technique of allozyme electrophoresis was applied to populations of the nematode *Globocephaloides trifidospicularis* Kung from *Macropus giganteus* and *M. rufogriseus* from Tasmania. Fixed genetic differences were found at four of 24 (17%) loci examined. Because the nematodes and their hosts are in sympatry, these populations constitute two distinct biological species. By comparison, both populations of *Globocephaloides* differed at 58% of loci from the related genus and species *Amphicephaloides thylogale*, parasitic in *Thylogale billardierii* in Tasmania.

KEY WORDS: *Globocephaloides*; *Amphicephaloides*; *Macropus*; *Thylogale*; allozyme electrophoresis; cryptic species

Introduction

Globocephaloides trifidospicularis Kung, 1948 is a common duodenal parasite of a number of macropodid marsupial species from south-eastern Australia (Beveridge 1979). Because it feeds on the blood of the host, this nematode species is capable of causing mortality in eastern grey kangaroo (*Macropus giganteus*) populations, particularly in juvenile animals (Arundel *et al.* 1990). In a taxonomic revision of the sub-family Globocephaloidinae, Beveridge (1979) concluded that a single, morphologically variable species occurred in *Macropus giganteus, M. fuliginosus, M. rufogriseus, M. eugenii, M. parryi* and *Wallabia bicolor* in the south-eastern region of the continent.

During an investigation into mortalities in *M. giganteus* on Maria Island, Tasmania (42°38'S, 148°05'E), in which *G. trifidospicularis* was involved, it was observed that *M. rufogriseus*, also parasitised by *Globocephaloides*, remained unaffected (D. L. Obendorf unpubl. data). These observations suggested that the nematodes infecting the two macropodid hosts might belong to different species. The present study aimed to test this hypothesis using the technique of allozyme electrophoresis, a powerful means of testing the existence of sibling species (Richardson *et. al.* 1986).

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Materials and Methods

Specimens of *Globocephaloides* were collected from the duodena of *M. giganteus* from Mt William, Tas. (40°55'S, 148°15'E), and from *M. rufogriseus* from Trevallyn, Tas. (41°27'S, 147°05'E). Nematodes were washed in saline and frozen in the wells of micro-titre plates at -80°C until processing. As an outgroup, specimens of a related genus and species *Amphicephaloides thylogale*, a duodenal parasite of *Thylogale* spp., were utilised; they were derived from *Thylogale billardierii* from the Launceston area, Tas.

Because of their small size, pools of nematodes rather than individuals were examined. Homogenates were prepared by adding an equal volume of homogenising solution (Richardson *et al.* 1986) to thawed samples, sonicating and centrifuging at 5000g for 10 min at 4°C. Electrophoresis was conducted on cellulose acetate ("Cellogel", Chemetron, Milan) according to the methods of Richardson *et al.* (1986).

Forty-five enzymes were screened for suitability as enzyme markers. Thirty-two enzymes showed activity following histochemical staining in at least one sample of either *Globocephaloides* or *Amphicephaloides*. The enzyme names, abbreviations and Enzyme Commission codes (E.C.) for these enzymes are as follows: acid phosphatase (ACP, E.C. 3.1.3.2.), adenosine deaminase (ADA, E.C. 3.5.4.4.), alcohol dehydrogenase (ADH, E.C. 1.1.1.1.), adenylate kinase (AK, E.C. 2.7.4.3), aldolase (ALD, E.C. 4.1.2.13), diaphorase (DIA, E.C. 1.8.1.4), enolase (ENOL, E.C. 4.2.1.11), esterase (EST, E.C. 3.1.1.1), fumarate hydratase (FUM, E.C. 4.2.1.2), guanine deaminase (GDA, E.C. 1.4.1.3), glutamate dehydrogenase (GDH, E.C. 1.4.1.3), aspartate

aminotransferase (GOT, E.C. 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), glucose phosphate isomerase (GPI, E.C. 5.3.1.9), alanine aminotransferase (GPT, E.C. 2.6.1.2), glutathione reductase (GSR, E.C. 1.6.4.7), hexosaminidase (HEX, E.C. 3.5.1.52), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C. 3.4.11.1), lactate dehydrogenase (LDH, E.C. 1.1.1.27), malate dehydrogenase (MDH, E.C. 1.1.1.37), mannose-phosphate isomerase (MPI, E.C. 5.3.1.8), purine nucleoside phosphorylase (NP, E.C. 2.4.2.1), peptidase valine-leucine (PEP-A, E.C. 3.4.13.11), peptidase leucine-glycine-glycine (PEP-B, E.C. 3.4.11.4), phosphoglycerate mutase (PGAM, E.C. 5.2.4.1), phosphoglycerate kinase (PGK, E.C. 2.7.2.3), phosphoglucomutase (PGM, E.C. 5.2.4.2), pyruvate kinase (PK, E.C. 2.7.1.40), triose-phosphate isomerase (TPI, E.C. 5.3.1), U.P.D.-glucose pyrophosphorylase (UGPP, E.C. 2.7.7.9) and xanthine oxidase (XO, E.C. 1.1.3.22).

Representative specimens of the two nematode populations from Maria I. were fixed in 10% formalin and cleared in lactophenol for examination. Measurements were made with an ocular micrometer on five male and five female *Globocephaloides* from the two macropodid hosts.

Representative specimens from both hosts from various areas of Tasmania have been deposited in the South Australian Museum: from *M. giganteus* HC 9187, 16572, 16581-3, 16612; from *M. rufogriseus* HC 9164, 10742, 14843-4, 10799, 16440.

Results

Sufficient staining intensity and resolution for reliable genetic interpretation was observed for 23 enzymes, encoded by a presumptive 24 loci. A further nine enzymes stained but showed sub-optimal activity. Of the 24 enzyme loci scored (Table 1), fixed allelic differences occurred between the two populations of *Globocephaloides* at four loci (*Ada-2, Idh-2, Got, Pep-B*) (17%). Fixed differences between both samples of *Globocephaloides* and *Amphicephaloides* occurred at 14 of the 24 (58%) loci examined (Table 1).

The following enzyme loci were invariant between samples: *Ald, Dia, Gpt, Mdh, Pgam* and *Tpi*.

Detected morphological differences between the two populations of *Globocephaloides* were slight (Table 2). Specimens from *M. giganteus* were marginally longer than those from *M. rufogriseus*, and the females were substantially stouter. The spicules were slightly longer in specimens from *M. giganteus*, but when expressed as a percentage of total body length, were similar to specimens from *M. rufogriseus*. Female nematodes from *M. giganteus* contained substantially more eggs in the anterior and posterior uteri when compared with females from *M. rufogriseus*. In nematodes from *M. giganteus*, there were significantly more eggs in the anterior interus.

Discussion

Although allozyme analyses were limited to results obtained from pools of nematodes rather than individuals (because of their small size), fixed allelic differences were found at four of 24 (17%) of the loci examined for the two populations of *Globocephaloides*. Typically, allopatric gene pools having fixed differences at more than 15% of

TABLE 1. *Summary of allelic differences between pools of* Globocephaloides *from* Macropus giganteus *(A) and* Macropus rufogriseus *(B) and pools of* Amphicephaloides thylogale *from* Thylogale billardierii.

	Enzyme Locus*																	
Species	*Acp*	*Ada-1*	*Ada-2*	*Ak*	*Enol*	*Est*	*Fum*	*Gda*	*Got*	*Gpi*	*Gsr*	*Idh*	*Lap*	*Ldh*	*Mpi*	*Pep-A*	*Pep-B*	*Ugpp*
Globocephaloides A	—	cd‡	b	a‡	d	—	b	—	b	a	a	c	a	b	de	b	c	b
Globocephaloides B	b	bc	c	a	bd	b	b	c	ac	a	a	b	a	b	de	b	b	fi
Amphicephaloides	a	ab	a	b	ac	a	ab	ac	a	b	b	a	b	a	a/b/c§	a	a	fi

* Multiple loci are designated numerically according to increasing electrophoretic mobility.

† Alleles are designated alphabetically, where a is the most cathodally migrating allele.

‡ Heterozygosity is represented by the two homologous alleles, ie ab, bc etc.

§ Where electrophoretic patterns did not conform to expectations for heterozygosity (eg MPI, a monomeric enzyme where heterozygotes should be double-banded) samples were depicted as possessing a mixture (1) of allozymes (eg for the monomeric enzyme MPI, the *Amphicephaloides* sample had a mixture of three allozymes a/b/c).

— Staining intensity and resolution was not sufficient to allow unequivocal genetic interpretation.

TABLE 2. *Measurements (in millimetres) of* Globocephaloides trifidospicularis *from* Macropus giganteus *and* M. rufogriseus *from Maria I., Tas. (mean of five measurements in parentheses).*

	M. giganteus	*M. rufogriseus*
Total length ♂	9.2-11.1 (10.1)	7.6-8.6 (8.2)
♀	11.1-13.4 (11.8)	9.9-11.3 (10.7)
Maximum width ♀	0.38-0.51 (0.45)	0.23-0.36 (0.30)
Spicule length ♂	0.54-0.60 (0.56)	0.43-0.47 (0.45)
Spicule length as % of body length	4.82-6.49 (5.61)	5.26-5.72 (5.51)
No. of eggs in anterior uterus	92-139 (119)	13-61 (43)
No. of eggs in posterior uterus	50-132 (103)	14-68 (47)

enzyme loci belong to different biological species (see Richardson *et al* 1986). Thus the data presented here suggest that the two populations of *Globocephaloides* examined represent distinct species. However, there is reason to believe that the two nematode taxa are in fact sympatric, even though our samples were not collected at the same site. The two hosts are sympatric over much of their geographical range in Tasmania (Calaby 1983; Kirsch & Poole 1972), and in addition the same two host species on the mainland have a substantial overlap (74%) in diet (Jarman & Phillips 1989). Since *G. trifidospicularis* infects its host orally (Beveridge 1979) the two populations of nematodes in *M. giganteus* and *M. rufogriseus* are essentially sympatric. The morphological differences noted between nematode specimens occurs irrespective of the locality of collection within Tasmania, suggesting that the segregration is by host rather than by geography, and that collecting from any location in Tasmania would yield similar results. In the case of a sympatry, a single fixed allelic difference is sufficient to indicate a lack of gene flow and hence the presence of distinct biological species (Richardson *et al.* 1986). Thus, the demonstration of four fixed allelic differences between these nematode populations would indicate that they belong to distinct biological species. The much greater proportion of fixed differences for both species of *Globocephaloides* and *Amphicephaloides* (58%) supports the generic distinction made between them at the morphological level (Beveridge 1979).

The genetic differences between the two species of *Globocephaloides* are matched by quite minor morphological differences (Table 2). The latter would probably not be considered significant in the absence of genetic data. The difference in absolute size in spicule length is not marked when considered as a percentage of total body length, and in the case of both male and female nematodes, differences in overall body size may have been discounted as being host-induced. Numbers of eggs in the uteri of female worms are not generally considered a reliable taxonomic character in trichostrongyloid genera, and a variety of factors, including exposure to chemicals (Hotson *et al.* 1970), can influence the number of eggs present. This feature as well as differences in egg number between anterior and posterior uteri have, however, been utilised advantageously for taxonomic purposes in the case of the trichostrongyloid nematodes of monotremes (Durette-Desset & Cassone 1983). It appears therefore that in the case of *G. trifidospicularis*, relatively minor morphological differences may indicate the existence of sibling species.

The results presented here raise questions as to the status of records of *G. trifidospicularis* in other macropodid host species. Unsuccessful attempts to infect worm-free *M. eugenii* with *G. trifidospicularis* derived from *M. giganteus* (I. Beveridge unpubl. data), may indicate that the nematode in *M. eugenii* is an independent sibling species. The status of specimens from *M. parryi* and *W. bicolor* also remains to be investigated. The type host of *G. trifidospicularis* is *M. rufogriseus*, the original description being based on specimens obtained from wallabies housed at the Regent's Park Zoo (Kung 1948). Beveridge's (1979) redescription is based on material from *M. giganteus* from Victoria and therefore probably represents an undescribed species. No new names are proposed here, pending the clarification of the status of *G. trifidospicularis*-like nematodes in other macropodid host species.

In northern Australia, *G. trifidospicularis* is replaced by two congeners, *G. affinis* occurring almost exclusively in the black-striped wallaby, *Macropus dorsalis*, and *G. macropodis* occurring in a wide range of host species (Beveridge 1979; Beveridge *et al.* 1984). The results obtained in this study suggest that a similar analysis of *G. macropodis* may be appropriate.

The slight morphological differences observed here between the populations of *Globocephaloides*

from *M. giganteus* and *M. rufogriseus*, indicate the care which needs to be taken when morphologically similar parasites from different hosts are assigned to the same taxon. The results have significant practical implications, since in the mortalities observed in *M. giganteus* on Maria I., *M. rufogriseus* was not acting as an alternative or reservoir host for the parasite. Plans to reduce the densities of kangaroos in order to control the adverse effects of this nematode parasite probably do not therefore need to take account of sympatric *M. rufogriseus* populations.

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