Antarctic Science 26(1), 15–22 (2014) © Antarctic Science Ltd 2013 doi:10.1017/S0954102013000230

Nematodes from the Victoria Land coast, Antarctica and comparisons with cultured *Panagrolaimus davidi*

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Abstract: Morphological and genetic information was used to determine the species identity and relationships of four species of nematodes from the Victoria Land coast, Antarctica; and between laboratory and field populations of *Panagrolaimus davidi* Timm. The 18S and D3 expansion segments of 28S rRNA were successfully amplified from the four species: *Scottnema lindsayae* Timm, *Plectus murrayi* Yeates, *Eudorylaimus* spp. and *Panagrolaimus davidi*. The four species from field populations mapped to three different nematode clades, indicating that if they are survivors in, rather than colonizers of, Antarctica they originate from several Gondwanan ancestors. The laboratory culture of *P. davidi* is shown to be a different species to that of the field samples of *P. davidi*, which were genetically identical to each other. There are several possible explanations for this observation, which require further investigation.

Received 2 December 2012, accepted 17 March 2013, first published online 13 May 2013

Key words: *Eudorylaimus* sp., field and laboratory strain, *Plectus murrayi*, *Scottnema lindsayae*

Introduction

In a recent paper we described nematode distributions at Cape Hallett and Gondwana Station, Antarctica (Raymond *et al.* 2013). Four species were found: *Panagrolaimus davidi* Timm, *Scottnema lindsayae* Timm, *Eudorylaimus* sp. and *Plectus* sp. In this paper we describe morphological and genetic studies that determine species or genus identity and relationships.

There are 57 species of Antarctic nematodes (all endemic) of which 25 are from continental Antarctica (Andrassy 2008a, Kito & Ohyama 2008). Identification using morphological characters has often been problematic, as illustrated by Antarctic *Eudorylaimus* species. Andrassy recently identified two new species and clarified some past misidentifications (Andrassy 2008a). As more specimens become available, variation initially ascribed to population-level differences has led to two additional *Eudorylaimus* morphospecies being described: *E. glacialis* Andrassy and *E. quintus* Andrassy (Andrassy 2008a).

The 18S rRNA gene has been widely used for the identification of nematode species, construction of phylogenies and for barcoding (Floyd *et al.* 2002). This gene has some highly conserved regions facilitating primer design, but enough variation to make it useful for taxonomy (Blaxter *et al.* 1998, Powers 2004). Primers have been developed that amplify ~1 kb of 18S sequence specifically from nematodes, reducing contamination problems (Floyd *et al.* 2005). 18S sequences have been used to build a number of nematode phylogenies that have led to significant improvements in our understanding of this phylum (Blaxter *et al.* 1998).

Genetic data are used in phylogeographical studies to give insight into the historic processes that explain current geographical distributions. In terrestrial Antarctica, phylogeographical studies have the potential to help explain the relative roles of vicariance and dispersal in the evolution of the extant fauna, add to, or contest, the geological evidence for the extent of historical glaciation events, and give a time-frame for the evolution of the observed physiological adaptations of the fauna.

There are few phylogeographical studies on Antarctic nematodes. Courtright *et al.* (2000) applied a phylogeographical approach to populations of *S. lindsayae* in the McMurdo Dry Valleys and Adams *et al.* (2007) reported no differences in ITS1 region sequences between *S. lindsayae* from the McMurdo Dry Valleys and from the Beardmore Glacier, which are 700 km apart. Studies on *E. antarcticus* (Steiner) populations suggest that there are at least three cryptic species (Barrett *et al.* 2006).

A study employing 18S sequences from various *Panagrolaimus* species and strains suggests that *P. davidi* (CB1 culture strain) is a recent colonizer of Antarctica (Lewis *et al.* 2009). We investigate this further by sequencing genes from field populations of *P. davidi*. We also investigated the phylogenetics of species from Cape Hallett and Gondwana Station that have previously only been morphologically defined. Before applying molecular techniques it was important to confirm that the species match current morphological descriptions. The 18S and D3 genes were sequenced. Together with morphological measurements and images, these provide an identification for each species. This analysis also provides an opportunity to identify any cryptic species.

Materials and methods

Sample sources

Nematodes were isolated from soil samples collected as part of a study of the distribution of nematodes in the Cape Hallett and Gondwana Station areas (Victoria Land coast, Antarctica). For site details and extraction methods see Raymond *et al.* (2013). An additional soil sample was obtained from the Adélie penguin colony at Cape Bird, Ross Island (collected by J. Banks). Laboratory cultures of *Panagrolaimus davidi* isolated from Ross Island in 1988 (Wharton & Brown, 1989) and later designated as strain CB1 (Lewis *et al.* 2009) were grown in liquid culture (Wharton *et al.* 2000).

Nematode preparation and morphological measurements

Nematodes were fixed using hot formalin/acetic acid fixative, identified to species level under a dissecting microscope, processed into glycerol by the Seinhorst method and permanent mounts prepared using the wax-ring method (Hooper 1986a). Slides were observed on a Zeiss Axiophot photomicroscope and images captured using a Canon Powershot A640 digital camera. Measurements were made using the image processing program Image J vers. 1.38x (http://rsb.info.nih.gov/ij/) and de Man ratios calculated (Hooper 1986b). Morphological measurements were compared with those from published descriptions for *Scottnema lindsayae*, *Panagrolaimus davidi*, *Plectus murrayi* Yeates 1970 and *Eudorylaimus* spp. (Timm 1971, Andrassy 1998, 2008a).

DNA extraction and sequencing

DNA was extracted from live nematodes as described by Floyd *et al.* (2002). Individual nematodes were identified to species level and transferred to 0.2 ml PCR (polymerase chain reaction) tubes containing 20 µl of 0.25 M NaOH. Each tube was then centrifuged briefly to ensure submersion of the nematode and stored at -80°C. Tubes were incubated at 25°C for 3–5 h, heated in a thermocycler (Eppendorf Mastercycler Gradient, Eppendorf, Hamburg, Germany) to 95°C for 3 min and allowed to cool to room temperature before the addition of 4 µl of 1 M HCl, 10 µl of 0.5 M Tris-HCl (pH 8.0) and 5 µl of 2% Triton X-100. The samples were mixed briefly and centrifuged. They were then reheated to 95°C for 3 min and allowed to cool to room temperature. This digest was used directly in the PCR reactions as template DNA (Floyd *et al.* 2002).

Nematode-specific primers for the nuclear 18S small subunit (SSU) ribosomal RNA gene have been successfully employed for a large range of nematode species (Blaxter *et al.* 2005, Floyd *et al.* 2005). Primers Nem_18S_F 5'- CGCGAATRGCTCATTACAACAGC-3' and Nem_18S_R 5'- GGCGATCAGATACCGCCC-3' (Floyd *et al.* 2005) were used to amplify an internal fragment of around 900 bp of 18S.

The nuclear D3 expansion segment of the 28S large subunit (LSU) rRNA gene and approximately 140 bp of 3' core sequence have successfully been amplified in a range of nematode species using two primers situated in conservative flanking regions: D3A 5'- GACCCGTCTTG AAACACGGA-3' and D3B 5'-TCGGAAGGAACCAG CTACTA- 3' (Nunn *et al.* 1996, Shannon *et al.* 2005). These primers have previously been used on *Eudorylaimus* sp. (Barrett *et al.* 2006) and *P. davidi* CB1 (Shannon *et al.* 2005) and were therefore used in this study. All primers were ordered online from Sigma Aldrich (www.sigmaaldrich.com).

The desired D3 PCR product of about 320 bp was reliably amplified in suitable quantities, resulting in good quality sequences. The D3 PCR products were therefore only sequenced using the forward primers. Sequences were aligned and trimmed to equal length, giving a sequence length of 275 bp without gaps.

Polymerase Chain Reaction (PCR) amplifications were carried out in a thermocycler with the following conditions: 1 cycle of 2 min at 94°C, 40 cycles of: 15 s at 94°C, 30 s at 45°C and 2 min at 72°C, and 1 cycle of 2 min at 72°C. All PCR reactions were carried out in 50 µl volumes containing 1 unit of 1U/µl BIOTAQTM red DNA polymerase (Bioline), 5 µl of 10x NH4 Buffer (Bioline), 5 µl of 50 mM MgCl2, 1.2 µl of 100 mM dNTP, 5 pmol of each primer and 3–5 µl genomic DNA from single nematode extractions.

Table I. Measurements and de Man ratios for Antarctic nematodes from Gondawana Station, Cape Bird and laboratory culture.

Species	Sex	*N*	length µm	width µm	oesophagus µm	tail µm	to vulva[5] µm	a	b	c	V
S. lindsayae [2]	F	7	512 ± 44[1]	29 ± 1.8	131 ± 7	39 ± 2	366 ± 7	17.3 ± 0.5	3.9 ± 0.2	13.0 ± 0.7	62.7 ± 0.1
	M	5	568 ± 25	30 ± 1	134 ± 5	47 ± 3		19.1 ± 0.8	4.2 ± 0.1	12.1 ± 0.3	
P. murrayi [2]	F [3]	16	817 ± 12	31 ± 1	184 ± 1	97 ± 2	384 ± 3	26.5 ± 0.3	4.4 ± 0.1	8.5 ± 0.2	47 ± 0.4
P. davidi	F	6	715 ± 25	31 ± 1	168 ± 2	42 ± 1	443 ± 13	22.8 ± 0.5	4.3 ± 0.2	17.0 ± 0.4	62 ± 0.4
Gondwana	M	3	722 ± 9	31 ± 1	158 ± 2	39 ± 3		23.4 ± 0.1	4.6 ± 0.2	18.8 ± 1.2	
P. davidi	F	20	863 ± 14	42 ± 1	174 ± 2	41 ± 1	548 ± 8	21.0 ± 0.3	5.0 ± 0.1	21.0 ± 0.2	63.6 ± 0.5
Cape Bird	M	20	772 ± 8	35 ± 0.4	165 ± 1	37 ± 1		23.4 ± 0.1	4.6 ± 0.1	18.8 ± 1.2	
P. davidi CB1[4]	F [3]	20	950 ± 17	39 ± 1	146 ± 1	59 ± 1	566 ± 9	24.4 ± 0.3	6.5 ± 0.1	16.0 ± 0.2	59.6 ± 0.3
Eudorylaimus sp.[2]	M	1	1343	42	327	37		31	4.1	36	

[1] mean ± SE, [2] Gondwana Station, [3] only females found, [4] laboratory culture strain, [5] distance from anterior to vulva

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Table II. Origin of nematodes and results of 18S and D3 sequencing of Antarctic nematodes.

Species	Sample origin	18S (861 bp) Haplotypes (n=2)	Genbank accession #	D3 (275 bp) Haplotypes (n=6)	Genbank accession #
P. davidi field populations	Luther lakes (CH) Adelie Cove (GS) Lake 17 (GS)[1] Cape Bird (RI)	1 (all sequences identical)	HQ270131	1 (all sequences identical)	HQ270138
P. davidi CB1 lab culture	isolated from RI (Cape Bird?)	1 (all sequences identical)	HQ270130	1 (all sequences identical)	HQ270137
Eudorylaimus sp.	Redcastle (CH) Luther lakes (CH) Mario Zucchelli (GS)	2 (Luther lakes and Mario Zucchelli populations identical, 3 variable sites between these and Redcastle)	Haplotype_LL HQ270134 Haplotype_MZ HQ270135 Haplotype_RC HQ270136	2 (Redcastle and Mario Zucchelli populations identical, 1 variable site between these and Luther lakes)	Haplotype_MZ HQ270142 Haplotype_LL HQ270141
P. murrayi	Luther lakes (CH) Lake 17 (GS) Mario Zucchelli (GS)[1]	1 (all sequences identical)	HQ270132	1 (all sequences identical)	HQ270139
S. lindsayae	Redcastle (CH) Gondwana Station (GS)	1 (all sequences identical)	HQ270133	1 (all sequences identical)	HQ270140

[1] D3 sequence only, CH = Cape Hallett area, GS = Gondwana Station area, RI = Ross Island.

The volume was then made up to 50 µl with sterile distilled water.

The presence and size of the amplification products were checked by electrophoresis on 1% agarose gels stained with Sybr Safe (Invitrogen). PCR amplification products were purified using a High Pure PCR Purification Kit (Roche Diagnostics GmbH). The DNA concentration of the clean product was determined with an ND-1000 spectrophotometer (NanoDrop Technologies Inc.) and samples were sent to the Allan Wilson Centre Genomic Services (www.allanwilsoncentre.ac.nz) for cycle sequencing and scanning.

Alignment and phylogenetic analyses

Sequence alignment and phylogenetic analyses were performed with MEGA4 (Tamura *et al.* 2007). For all sequences, sequencer files were checked for errors and any primer sequences removed. For the 18S sequences, alignment was performed in the ClustalW function of MEGA4, using the nematode 18S sequence alignment dataset available from Blaxter as a reference (Blaxter 1998). This alignment had been created by hand with respect to a secondary structure model (Blaxter *et al.* 1998). For the D3 sequences, alignments were also done using ClustalW, with gap opening penalties set at 15 and gap extension penalties at 6.66 (Tamura *et al.* 2007). Alignments were then checked through visually and minor manual adjustments were made before the alignments were trimmed to the same length.

Maximum parsimony (MP) and neighbour joining (NJ) phylogenetic analyses were performed on both sequence alignments using MEGA4 (Tamura *et al.* 2007). For both MP and NJ analyses, bootstrap consensus trees were computed with 1000 bootstrap replicates.

Results

Morphological measurements and de Man ratios of *Scottnema lindsayae*, *Panagrolaimus davidi*, *Plectus murrayi* and *Eudorylaimus* sp. are summarized in Table I. This table also compares *P. davidi* from Gondwana with fresh material from Cape Bird and with the culture strain, *P. davidi* CB1.

The rDNA small subunit (18S) and the D3 expansion region of the 28S rDNA subunit genes were successfully amplified and sequenced from the four species. For each species, two (18S) and six (D3) individuals from each of two geographically distinct populations (Cape Hallett and Gondwana Station) were sequenced. A third population of *P. davidi* from Cape Bird, Ross Island was sequenced, as was the laboratory strain *P. davidi* CB1 (Table II).

Two haplotypes were identified for *Eudorylaimus* sp. 18S sequences: the two individuals from the Redcastle population had different sequences to those from Luther lakes (unofficial name) and Mario Zucchelli Station, with three variable sites in the sequences. There was one variable site in the 275 bp D3 sequence between populations of *Eudorylaimus* sp. The D3 sequences of Redcastle and Mario Zucchelli Station individuals were identical to each other but different to those from Luther lakes. This pattern was the same for all six individuals from each of the three populations.

Whereas all individuals from the three (18S) or four (D3) field *P. davidi* populations had identical 18S and D3 sequences, large differences were identified between these

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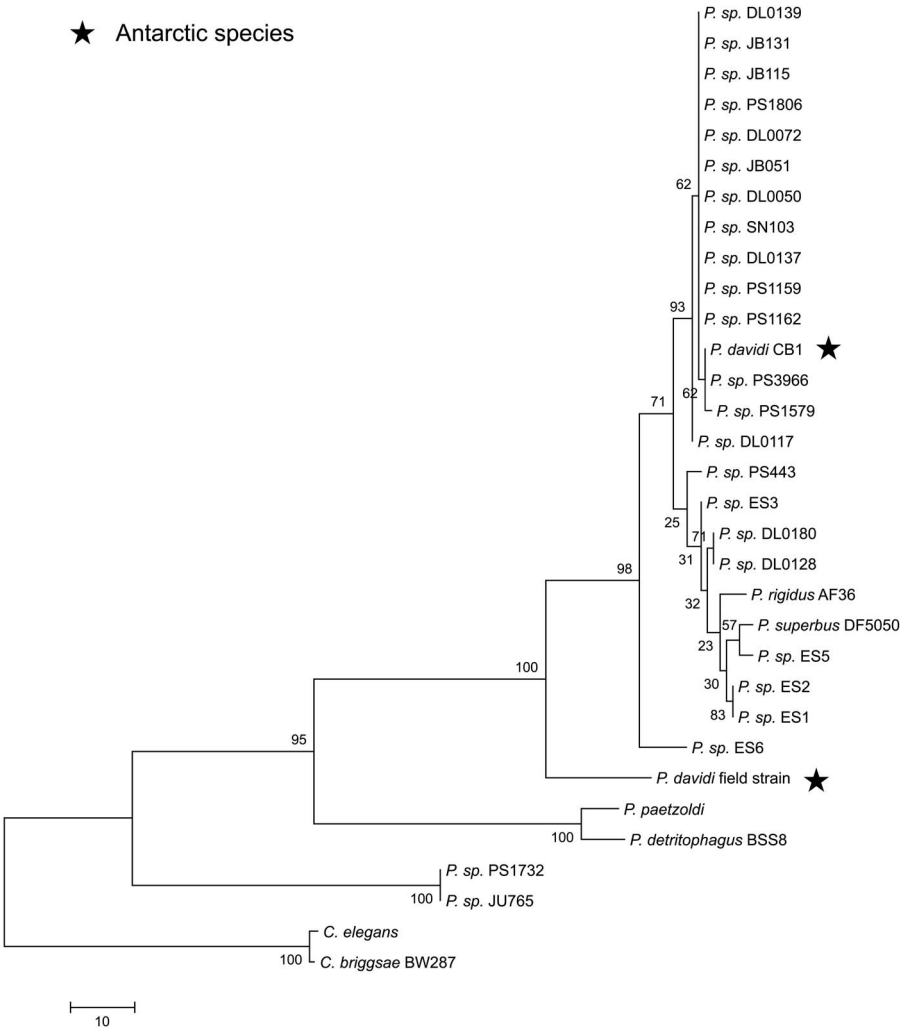

Fig. 1. Evolutionary relationships of 30 *Panagrolaimus* species, including *P. davidi* field strain and *P. davidi* CB1 (asterisks), inferred from 18S sequence data from this study and that of Lewis *et al*. (2009). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with the branch lengths calculated using the average pathway method (Nei & Kumar 2000). There were a total of 362 positions in the final dataset, out of which 143 were parsimony informative.

sequences and those from *P. davidi* CB1. In an alignment of 18S sequences between a representative individual from a field population and one from *P. davidi* CB1, 81 out of 861 sites were different (9.4%). D3 sequences from field populations varied at 30 sites (10.5%) when aligned to those of *P. davidi* CB1.

A tree inferred using a 1000 bootstrap replicate neighbour-joining model groups the species into the same five main clades as in the study of Blaxter *et al.* (1998), with the Antarctic species from this study representing different major lineages (Fig. S1 which will be found at http://dx.doi.org/10.1017/S0954102013000230).

Discussion

Morphological measurements

Identifications of *Scottnema lindsayae*, *Panagrolaimus davidi*, *Plectus murrayi* and *Eudorylaimus* sp. were confirmed by reference to published descriptions. The original species descriptions are from the McMurdo Sound area, and this study supports observations that their range extends over the Victoria Land coast (Adams *et al.* 2006).

Gondwana Station (GS) *S. lindsayae* were considerably shorter than those from populations near Lacroix Glacier and the Strand Moraines (Timm 1971). However, the de Man ratios are similar, and the GS individuals are within the range of reported 'a' values. Our GS specimens match Andrassy's description (Andrassy 1998) better than the original species description (Timm 1971), and the GS de Man indices are within reported ranges (Andrassy 1998, Boström *et al.* 2011), although some are a little shorter in length. This suggests that there can be considerable variation in the body length of *S. lindsayae* individuals, which may be an age effect rather than a population-level difference.

Plectus antarcticus de Mann (Timm 1971) has been renamed *Plectus murrayi* by authors who consider that *P. antarcticus* is found in the maritime Antarctic only (Andrassy 1998, Convey *et al.* 2008, Maslen & Convey 2006). The GS specimens are within the range of reported measurements. No males were found, consistent with their rarity (Andrassy 2008b). *Plectus frigophilus* Kirjanova is

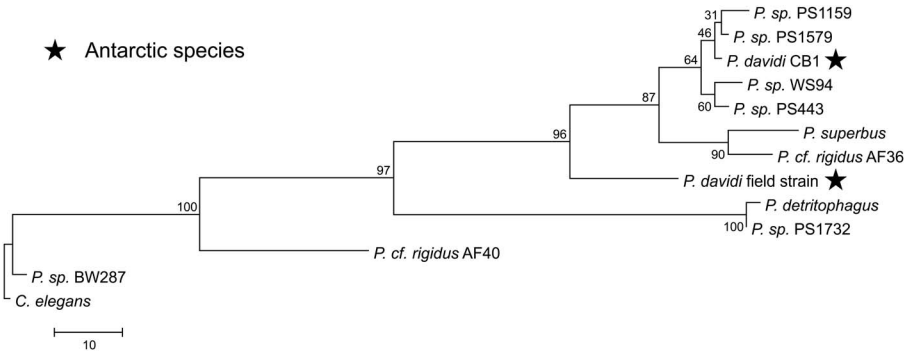

Fig. 2. Evolutionary relationships of 12 *Panagrolaimus* species, including *P. davidi* field strain and *P. davidi* CB1 (asterisks), inferred from D3 sequence data from this study and that of Shannon *et al*. (2005). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths calculated using the average pathway method (Nei & Kumar 2000). There were a total of 269 positions in the final dataset, out of which 116 were parsimony informative.

the other *Plectus* species found in Victoria Land, being much larger than *P. murrayi* and preferring more aquatic habitats (Andrassy 1998). We did not distinguish between *P. murrayi* and *P. frigophilus* but our specimens appear to all belong to the same species (confirmed by genetic studies below). Yeates *et al*. (2009) considered that *Plectus* sp. collected from Cape Hallett were *P. murrayi*. We conclude that the *Plectus* that we collected are *P. murrayi* also.

Field samples of *Panagrolaimus davidi* from Cape Bird, have been compared to *P. davidi* CB1 from laboratory cultures (Wharton 1998). The culture was originally isolated from Ross Island. The strain designation (CB1) presumes its origin is Cape Bird but material was also collected from Cape Royds and Crater Hill (Wharton & Brown 1989). This nematode has been maintained in culture since November 1988 (Wharton & Brown 1989). Gondwana Station individuals are considerably shorter in length than the species description (Timm 1971), but the de Man indices are all within the range reported in the literature (Wharton 1998). There are significant differences between all measurements from the field-collected *P. davidi* and *P. davidi* CB1, which also had a longer and more pointed tail. The most obvious difference was the lack of males in *P. davidi* CB1 and no males have been observed since the culture was established (Wharton 1998). Gondwana Station individuals are closer in morphology to those from wild populations than to *P. davidi* CB1. The characteristic dorsal metastomal tooth of *P. davidi* was observed in both males and females of the GS population, and in *P. davidi* CB1 strain.

Among Antarctic *Eudorylaimus* species, *E. glacialis*, *E. shirasei* Kito, Shishida & Ohyama and *E. antarcticus* have been described from Victoria Land (Adams *et al.* 2006). *Eudorylaimus* sp. was the least abundant species in GS soils (Raymond *et al.* 2013). Few *Eudorylaimus* individuals are found in surveys and species descriptions are based on very few individuals (Andrassy 2008a). In our study, morphological measurements were carried out on a single GS male and we did not have enough material to identify to species level.

Molecular diagnostics for Antarctic nematode taxonomy

The 18S gene is used as a genetic barcode for nematodes and individuals from the same species are expected to have identical 18S sequences (Floyd *et al.* 2002). For *S. lindsayae*, *P. murrayi* and *P. davidi* individuals from field populations identified morphologically as the same species had identical 18S and D3 sequences across populations.

The level of difference at both the 18S and D3 gene loci strongly indicates that *P. davidi* CB1 is a different species from that of field populations of *P. davidi*. The morphological comparisons of *P. davidi* field strains and *P. davidi* CB1 show differences that had previously been considered insignificant (Wharton 1998), may now clearly be of importance in distinguishing these two species. However, they have a very similar appearance, including the presence of the dorsal metastomal tooth that distinguishes *P. davidi* from *P. magnivulvatus* Boström (Boström 1995), the other *Panagrolaimus* species endemic to continental Antarctica (Andrassy 1998). The two species designated as *P. davidi* are thus different species best distinguished by molecular techniques. The only other species of *Panagrolaimus* known to possess a dorsal metastomal tooth is *P. superbus* Fuchs (De Ley *et al.* 1995, Eyualem & Blaxter 2003), which is considered to be almost morphologically identical to *P. davidi* (Abolafia & Pena-Santiago 2005).

Phylogenetic analyses

Panagrolaimus davidi (field strain) and *P. davidi* CB1 (laboratory strain) group together in our phylogeny (Fig. S1 which will be found at http://dx.doi.org/10.1017/S0954102013000230), and form a sister group to

https://doi.org/10.1017/S0954102013000230 Published online by Cambridge University Press

Panagrellus redivivus. These species are members of Clade IV, which includes members of the Rhabditida (Blaxter *et al.* 1998). *Scottnema lindsayae* groups with *Zeldia punctata* Thorne and falls within Clade IV. *Plectus murrayi* is a sister taxon to *P. minimus* Cobb and *P. aquatilis* Andrassy. These taxa are members of the Chromadorida, a diverse group dominated by bacteriovores, which forms a paraphyletic group (C) in Blaxter's phylogeny. All three of the populations of *Eudorylaimus* sp. group closely with *Aporcelaimellus obtusicaudatus* Bastian and *Xiphinema rivesi* Dalmasso. They form members of Clade I, which includes plant parasites from the Dorylaimida (*X. rivesi*) and the intertidal free-living species *A. obtusicaudatus*.

A recent study of the genus *Panagrolaimus* sequenced part of the 18S gene from over 30 species and strains (Lewis *et al.* 2009). Figure 1 shows the position of the two Victoria Land *Panagrolaimus* species (*P. davidi* field strain and *P. davidi* CB1) in Lewis *et al.*'s MP bootstrap consensus tree with *Caenorhabditis elegans* and *C. briggsae* Dougherty & Nigon as outgroups. *Panagrolaimus davidi* CB1 groups with other parthenogenetic species, while *P. davidi* field strain forms a sister group to most of the other species.

The two Victoria Land *Panagrolaimus* species were also related to other species/strains from the genus using the D3 sequences available from the study of Shannon *et al*. (2005). This MP bootstrap consensus tree shows the level of difference between *P. davidi* CB1 and *P. davidi* field strain (Fig. 2).

In the two *Panagrolaimus* phylogenies presented here (Figs 1 & 2), *P. davidi* CB1 appears to be closely related to *P*. sp. PS1579, a parthenogenetic Californian strain from Huntington Gardens, USA (Lewis *et al.* 2009). In the 18S phylogeny, the other closely related species to *P. davidi* CB1 is *P*. sp. PS3966, another parthenogenetic Californian strain from Pasadena (Lewis *et al.* 2009) and in the D3 phylogeny *P*. sp. PS1159, a parthenogenetic strain from North Carolina (Shannon *et al.* 2005). The field strain of *P. davidi*, however, forms a distinct clade well separated from other species and strains of *Panagrolaimus*; including from *P. davidi* CB1. Both 18S and D3 phylogenies show considerable divergence between *P. davidi* CB1 and *P. davidi* field strain.

Lewis *et al*. (Lewis *et al.* 2009) applied a molecular clock approach to estimate the divergence date between *P. davidi* CB1 and the closely related *P*. sp. 1579, externally calibrated using Denver *et al*.'s estimates of mutation rates in *C. elegans* (Denver *et al.* 2004), and concluded a rough time to most recent common ancestor of only 140 206 nematode generations. The authors used an estimate that these nematodes may experience ten generations per year in Antarctica to yield a very surprising divergence date of only approximately 14 000 years ago (Lewis *et al.* 2009).

In our study, sequence data were compared from 24 wild *P. davidi* individuals from Cape Hallett, Gondwana Station and Cape Bird, the latter is thought to be the original source of *P. davidi* CB1. All individuals had identical 18S and D3 sequences, and the *P. davidi* CB1 sequence was not found in any of the wild populations, including the Cape Bird samples.

There are several explanations for the differences between *P. davidi* field strain and *P. davidi* CB1. The laboratory culture may have become contaminated with a different species. Since it was established in 1988, the culture strain has been maintained in isolation, with all efforts made to avoid contamination. Only one *Panagrolaimus* species has been recorded from New Zealand, *P. australis* Yeates (Yeates 2010). However *P. australis* has a distinctly different morphology to *P. davidi* CB1 and is strictly gonochoristic in culture (Yeates 1969). There could be contamination with an undescribed parthenogenetic *Panagrolaimus* strain from New Zealand, since new *Panagrolaimus* strains/species are easy to isolate and the diversity of the genus may be considerably undersurveyed (Lewis *et al.* 2009). Although contamination of our culture with a temperate species cannot be ruled out it seems unlikely that the extreme cold tolerance abilities of *P. davidi* CB1 (Smith *et al.* 2008) would have evolved in an environment where they are unlikely to be exposed to very low temperatures, but it is to be expected for an Antarctic species.

The remarkably close genetic relationship of *P. davidi* CB1 to three North American *Panagrolaimus* strain (*P*. sp. PS1579, *P*. sp. PS3966 and *P*. sp. PS1159) needs to be considered, particularly given the amount of human activity in this area of Antarctica. Lewis *et al*. (2009) concluded that *P. davidi* CB1 colonized Antarctica in the very recent past and that it must have either evolved its extreme cold-tolerance very rapidly, or arrived in a "pre-adapted" state. Anhydrobiotic strains of *Panagrolaimus* form a single phylogenetic lineage, which includes *P. davidi* CB1 and its two closest relatives: *P*. sp. PS1579 and *P*. sp. PS1159 (Shannon *et al.* 2005). The colonization of polar regions by anhydrobiotic nematodes from other parts of the world, or *vice versa*, is a possibility. However, the field strain of *P. davidi* is well separated from other species and strains genetically, suggesting that it is an endemic Antarctic species.

Although the culture process will clearly have had both phenotypic and genetic effects, it does not seem possible that genetic differences of the level seen between *P. davidi* field strain and *P. davidi* CB1 (>9% at two nuclear loci) could have arisen solely from the culture process. *P. davidi* CB1 could be an Antarctic species that is less common than *P. davidi* field strain in the wild, but rapidly dominates in culture due to its parthenogenetic reproductive mode. We are currently conducting an extensive survey of Ross Island sites to test this hypothesis.

Acknowledgements

This work was generously supported by a Fanny Evans Post-Graduate Scholarship for Women and a scholarship

from Antarctica New Zealand and Kelly Tarlton's, awarded to MRR. We are grateful for the logistical support of Antarctica New Zealand (event KO66), technical support from Karen Judge and Tania King, and field assistance from Justine Marshall, Francesca Cunninghame and Konstanze Gebauer. We also thank Jonathan Banks for the Cape Bird sample, Graham Wallis, Sven Bostrøm and an anonymous referee for their comments on the manuscript.

Supplemental Material

A supplemental figure will be found at http://dx.doi.org/10.1017/S0954102013000230

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https://doi.org/10.1017/S0954102013000230 Published online by Cambridge University Press