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SHORT COMMUNICATION

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Phylogenetic position of *Dracunculus medinensis* and some related nematodes inferred from 18S rRNA

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Recently, genomes of several parasitic helminths are being sequenced in their entirety (El-Sayed et al. 2004; Ghedin et al. 2004). However, not all medically important helminths received the attention of molecular biologists. Actually, some did not receive any at all. One of them is the guinea worm *Dracunculus medinensis* (Linnaeus 1758), which is supposed to represent the "snake" on the Aeskulap's stick, an ancient symbol of medicine. This causative agent of dracunculosis is still responsible for the suffering of at least tens of thousands of people. It circulates between humans and copepods in a widespread area ranging from Senegal in Africa to eastern India (Mirza 1957; Muller 1971; Cairncross et al. 2002). This dracunculid nematode is a representative of the superfamily Dracunculoidea, which encompasses 166 recognized species that parasitize all classes of vertebrates (Moravec 2004). Since ribosomal RNA sequences are not available for any of these species, inference of phylogenetic relationships of the whole superfamily with other nematode groups is based solely on morphological evidence (Ivashkin et al. 1971; Chabaud 1975). In an effort to place the neglected human parasite *D. medinensis* into a phylogenetic tree, we have sequenced its 18S rRNA gene. Moreover, we have established the sequences of the same gene for two other dracunculoid nematodes parasitizing cold-blooded vertebrates.

Adult female specimen of *D. medinensis* isolated in Ghana from a subcutaneous lesion of a patient in 1991 and stored in 70% ethanol was provided by Dr. P. Bloch, Danish Bilharziasis Laboratory, Charlottenlund, Denmark. Two specimens of *Dracunculus oesophageus* (Polonio 1859) were obtained from the mesentery of an adult colubrid snake *Natrix natrix* (L.), captured in June 2003 in southern Slovakia. Finally, several specimens of *Philometra obturans* (Prenant 1886) were isolated from the gill arteries of pike *Esox lucius* L., captured in April 2003 at Mácha Lake fishpond in northern Bohemia, Czech Republic. Small pieces (2–4 mm) of tissue were snipped from the worm with sterile scissors, mechanically homogenized, and after brief centrifugation resuspended in lysis buffer. Genomic DNA was isolated using the Jetquick DNA isolation kit (Genomed). About 20 ng of genomic DNA was used for PCR amplification of the 18S rRNA gene that was amplified using oligonucleotides D-1F (GCCTATAATGGTGAAACCGCGAAC) and D-1R (CCGGTTCAAGCCACTGCGATTA) and Taq Purple polymerase (Top-Bio). PCR was performed under the following conditions (6 cycles of 95°C for 1 min, 44°C for 1 min and 72°C for 2 min followed by 24 cycles with the annealing temperature increased to 48°C). The amplicons of expected size were gel-purified using Jetquick gel extraction kit (Genomed) and cloned into the pDrive Cloning Vector (Qiagen). Both strands were sequenced using a Beckman Coulter automatic sequencer. Internal oligonucleotides, designed to match the conserved regions, were used to complete the sequences. Sequence fragments were completed with Editseq and Seqman (DNASTAR) and the sequence alignment was created and further edited using ClustalX 1.81 (Thompson et al. 1997) and BioEdit (Hall 1999) software, respectively.

The following 18S rRNA sequences retrieved from the GenBank were used in this work: *Ascaris lumbricoides* U94366, *Goezia pelagia* U94372, *Anisakis* sp. U81575, *Brumptaemilius justini* AF036589, *Daptonema*

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procerus AF047889, *Skrjabingylus chitwoodoorum* AY295819, *Wuchereria bancrofti* AF227234, *Brugia malayi* AF036588. Alignments were analysed by maximum parsimony (MP) and maximum likelihood (ML), performed using PHYLIP (SEQBOOT; Felsenstein 2002), PAUP* (Swofford 2003) and PHYML (Guindon and Gascuel 2003) software. The tree was rooted with a monhysterid (*D. procerus*) and a strongylid (*S. chitwoodorum*).

Phylogenetic analysis (Fig. 1) revealed close relationship between the two species studied, *Dracunculus* and *P. obturans*, while the other spirurids (*B. malayi* and *W. bancrofti*) appeared elsewhere in the tree (Fig. 1). Maximum likelihood placed these spirurids in two clades that also contained the ascarids and a rhigonematid. Both ML and MP strongly support paraphyly of the order Spirurida, with *Dracunculus* spp. and *P. obturans* constituting the crown group (see Fig. 1 for details). The 18S rRNA tree clearly confirmed the morphology-based prediction of close relatedness between Dracunculidae (*Dracunculus* spp.) and Philometridae (*P. obturans*), as already indicated by Ivashkin et al. (1971). This grouping, however, has not been generally accepted (Vismanis and Nikulina 1972; Pan et al. 1990).

In the so far most extensive 18S rRNA-based phylogenetic analysis of the phylum Nematoda, the orders Ascaridida, Spirurida, Rhigonematida and Oxyurida constituted a well-supported monophyletic assembly, which is composed of vertebrate- and arthropod-parasitic taxa (Blaxter et al. 1998). While the inspection of morphological features supported close relationship between spirurids and ascaridids, to the exclusion of Rhigonematida (Blaxter et al. 1998; Anderson 2000), the only rhigonematid included in our trees (*B. justini*) appeared inserted between representatives of both orders (Fig. 1). Wider sampling will eventually test the stability of this branching order.

Our phylogenetic analysis also enabled addressing the long-debated issue of whether or not *D. medinensis* is confined to humans only, since, in addition to man, the Guinea worm has also been reported from many species of mammalian and reptilian hosts (reviewed by Muller 1971). Therefore, uncertainty still exists whether these findings actually relate to *D. medinensis* or to another congeneric species confined to animal hosts. On the other hand, some records of other *Dracunculus* spp. from non-human hosts may well concern *D. medinensis*. Moreover, the validity of the other 11 nominal species of the genus *Dracunculus*, all histozoic parasites of mammals and reptiles, is frequently questioned because of their inadequate descriptions and/or morphological similarity (Mirza 1957; Muller 1971; Moravec and Little 2004). However, the 18S rRNA sequences presented herein show beyond any doubt that the dracunculid

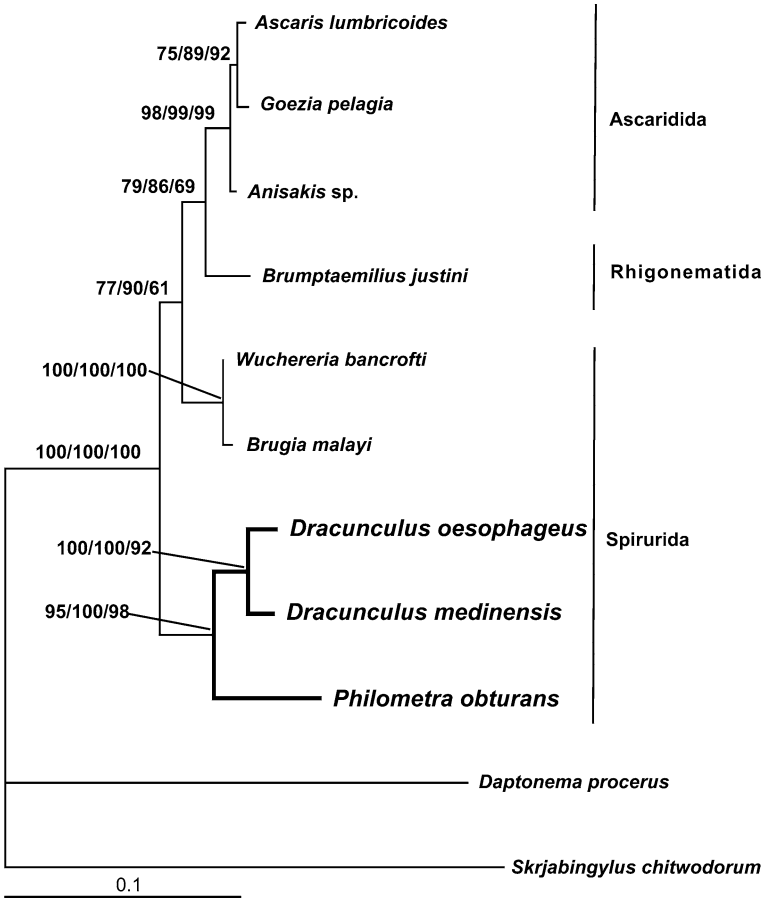

Fig. 1 Phylogenetic position of *D. medinensis*. Maximum likelihood (ML) tree (Loglk = −5383.09470) inferred from an alignment of eleven 18S rRNA gene sequences and 1,603 sites (206 parsimony informative) using a general time reversible model for base substitution with discrete gamma distribution (GTR + Γ + I) and all parameters estimated from dataset (α = 0.300; Pinvar = 0.000) as implemented in PhyML 2.1b. Tree was rooted with the monhysterid *D. procerus* and the strongylid *S. chitwoodorum*. *Numbers above branches* indicate bootstrap supports for MP/MPg/ML, where MP and MPg represents maximum parsimony with gaps treated as missing data and with gaps considered as a fifth state, respectively, by use of PAUP 4.10b and ML stands for maximum likelihood analysis using model mentioned above. All bootstrap values were calculated from 1,000 replicates

D. oesophageus, which is morphologically almost indistinguishable from the human Guinea worm, represents a different species. To describe the diversity within the genus *Dracunculus*, it will be necessary to obtain sequence data from more species and in particular from specimens of the Guinea worm originating from Arabia and India. The available sequences represent first entry in the databases for this group and, we hope, will stimulate the interest of molecular biologists in these important parasites.

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