Veterinary Parasitology 164 (2009) 248–256

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Population genetic structure of the parasitic nematode *Camallanus cotti* inferred from DNA sequences of ITS1 rDNA and the mitochondrial *COI* gene

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ARTICLE INFO

Article history:
Received 15 August 2008
Received in revised form 23 April 2009
Accepted 28 April 2009

Keywords:
Population genetic structure
ITS1 rDNA
COI gene
Fish
Camallanus cotti

ABSTRACT

The population genetic structure of fish parasitic nematode, *Camallanus cotti*, collected from the Yangtze River, Pearl River and Minjiang River in China was investigated. From these parasites, the ~730 bp of the first internal transcribed spacer of ribosomal DNA (ITS1 rDNA) and the 428 bp of mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene were sequenced. For the ITS1 rDNA data set, highly significant Fst values and low rates of migration were detected between the Pearl River group and both the Yangtze River (Fst = 0.70, $P < 0.00001$; Nm = 0.21) and Minjiang River (Fst = 0.73, $P < 0.00001$; Nm = 0.18) groups, while low Fst value (Fst = 0.018, $P > 0.05$) and high rate of migration (Nm = 28.42) were found between the Minjiang and the Yangtze rivers. When different host/locality populations (subpopulations) within each river were considered, subpopulations between the Yangtze River and Minjiang River had low Fst values ($\leq$0.12) and high Nm values (>3.72), while Pearl River subpopulations were significantly different from the Yangtze River and Minjiang River subpopulations (Fst $\geq$ 0.59; Nm < 1). The *COI* gene data set revealed a similar genetic structure. Both phylogenetic analyses and a statistical parsimony network grouped the Pearl River haplotypes into one phylogroup, while the Yangtze River and Minjiang River haplotypes formed a second group. These results suggested that the Yangtze River and Minjiang River subpopulations constituted a single reproductive pool that was distinct from the Pearl River subpopulations. In addition, the present study did not find host-related genetic differentiation occurring in the same drainage.

1. Introduction

The species-rich phylum Nematoda includes more than described 26,000 species, both free-living and parasitic (Hugot, 2002). The parasitic nematodes represent one of the most important groups of metazoan parasites of fish. These parasites infect practically all body organs of fish and many are known to be the agents of serious diseases of fishes, domestic animals, and man (Moravec, 1994). Their significance as important fish pathogens is increasing with the rapid development of marine, brackish-water, and freshwater aquaculture in different countries, where nematodes can be the cause of considerable economic loss (Moravec, 2000). The phylum Nematoda has now received more and more attention.

Camallanus cotti is a common intestinal parasitic nematode of freshwater fish. Originally, *C. cotti* was reported by Fujita from Lake Biwa in Japan and considered a native parasite of Asia (Moravec and Nagasawa, 1989),

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0304-4017/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.vetpar.2009.04.030

but now it has been found in fish species in Hawaii, Europe, North America and Australia (Levsen and Berland, 2002b; Moravec et al., 2003). The cosmopolitan distribution results from extensive trade of ornamental fishes and subsequent release from the aquariums and from the introduction of exotic poeciliids for mosquito control (Levsen and Berland, 2002b). In the wild, the definitive host fish acquires this nematode by ingesting cyclopoid copepods carrying the infectious third-stage larvae (Levsen and Berland, 2002b). This nematode feeds on its host's blood or tissue fluid. Multiple infection of *C. cotti* can cause rectal inflammation, anemia, emaciation, and even death, especially in small fish (Stumpp, 1975). Additionally, infection can change the host's sexual behavior (McMinn, 1990). The distribution and pathological effects of the nematode are of global concern. Extensive studies have been carried out in relation to the nematode's taxonomy, faunal investigation, pathology, and population dynamics (e.g. Moravec and Nagasawa, 1989; McMinn, 1990; Moravec et al., 2003; Menezes et al., 2006; Wu et al., 2007). Despite these extensive researches, little is known about its population genetic structure.

Obviously, the understanding on *C. cotti* population genetic structure and gene flows within and among the populations is required to explain its worldwide distribution and to form effective control strategies. The available literature reveals that the population genetic structure of nematode is determined mainly by the effective sizes (i.e. breeding) of the populations (Ne) and the rates of gene flow among them (Blouin et al., 1995; Hawdon et al., 2001). For the nematode parasites of vertebrates, effective sizes and rates of gene flow are largely affected by host mobility and parasite life history traits (Blouin et al., 1995, 1999). So far, most researches related to nematode parasite focus on nematodes associated tightly with human activities, such as parasites of humans, domestic animals, and they show high genetic diversity within populations, but low differentiation among locations, indicating high levels gene flow among populations (Blouin et al., 1992, 1995, 1999; Hawdon et al., 2001). For example, the trichostrongylid parasites of sheep and cattle have little genetic structure, consistent with high gene flow among populations, where extensive host movement results in large Ne (Blouin et al., 1995). However, in contrast to many other groups of parasitic nematodes, to our knowledge, there is little literature relating population genetics of fish nematode (but for Mejía-Madrid et al., 2007; Derycke et al., 2008).

To resolve population genetic structure, variation in the nuclear first internal transcribed spacer of ribosomal DNA (ITS1 rDNA) and the mitochondrial *cytochrome c oxidase subunit I* (*COI*) has been studied in a wide range of organisms (e.g. Blouin et al., 1995; Presa et al., 2002; Mejía-Madrid et al., 2007; Nieberding et al., 2008). Because the mitochondrion is inherited maternally, mitochondrial markers offer the advantage of a single haplotype for each sample. Additionally, many mitochondrial markers evolve quickly. In contrast, nuclear markers offer a biparental view of genetic variation and provide higher resolution of the relationships between closely related species. The present study was therefore designed to shed light on the genetic structure of *C. cotti* populations in its fish definitive hosts from the Yangtze River, Pearl River and Minjiang River in south-central China using ITS1 rDNA and *COI* gene markers.

2. Materials and methods

2.1. Sample collection, identification and DNA extraction

C. cotti adults were collected from host fishes from eight localities in the three main drainage systems of south-central China during the period from February 2004 to August 2006 (Fig. 1, Table 1). The recovered nematodes were

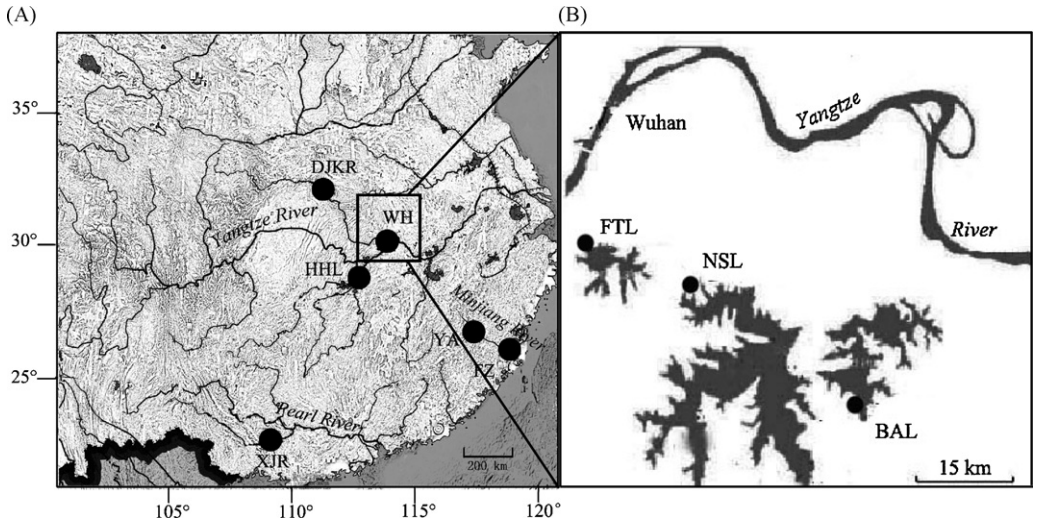

Fig. 1. (A) Sampling localities of *Camallanus cotti* in the Yangtze River, Pearl River and Mingjiang River. Abbreviations of sampled localities are as follows: DJKR, Danjiangkou Reservoir, Hubei Province, China; HHL, Honghu Lake, Hubei Province, China; FTL, Futou Lake, Hubei Province, China; NSL, Niushan Lake, Hubei Province, China; BAL, Baoan Lake, Hubei Province, China; WH, Wuhan, Hubei Province, China; XJR, Xijin Reservoir, Guangxi Province, China; FZ, Fuzhou, Fujian Province, China; YA, Yongan, Fujian Province, China. Names of drainage systems mentioned in the text were labelled and italicized. (B) Close-up view of sampling sites from the Yangtze River near Wuhan.