International Journal for Parasitology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

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

Paralogues of nuclear ribosomal genes conceal phylogenetic signal within the invasive Asian fish tapeworm lineage: evidence from next generation sequencing data ☆

Jan Brabec [a,*], Roman Kuchta [a], Tomáš Scholz [a], D. Timothy J. Littlewood [b]

[a] Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic
[b] Department of Life Sciences, Natural History Museum, Cromwell Road, SW7 5BD London, United Kingdom

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 20 March 2016
Accepted 23 March 2016
Available online xxxx

Keywords:
Schyzocotyle acheilognathi
Asian fish tapeworm
Bothriocephalus acheilognathi
Invasive parasite
Mitochondrial genome
Ribosomal RNA
Illumina sequencing
Phylogeny

ABSTRACT

Complete mitochondrial genomes and nuclear rRNA operons of eight geographically distinct isolates of the Asian fish tapeworm *Schyzocotyle acheilognathi* (syn. *Bothriocephalus acheilognathi*), representing the parasite's global diversity spanning four continents, were fully characterised using an Illumina sequencing platform. This cestode species represents an extreme example of a highly invasive, globally distributed pathogen of veterinary importance with exceptionally low host specificity unseen elsewhere within the parasitic flatworms. In addition to eight specimens of *S. acheilognathi*, we fully characterised its closest known relative and the only congeneric species, *Schyzocotyle nayarensis*, from cyprinids in the Indian subcontinent. Since previous nucleotide sequence data on the Asian fish tapeworm were restricted to a single molecular locus of questionable phylogenetic utility—the nuclear rRNA genes-separating internal transcribed spacers—the mitogenomic data presented here offer a unique opportunity to gain the first detailed insights into both the intraspecific phylogenetic relationships and population genetic structure of the parasite, providing key baseline information for future research in the field. Additionally, we identify a previously unnoticed source of error and demonstrate the limited utility of the nuclear rRNA sequences, including the internal transcribed spacers that has likely misled most of the previous molecular phylogenetic and population genetic estimates on the Asian fish tapeworm.

1. Introduction

Schyzocotyle acheilognathi (Yamaguti, 1934), commonly known as the Asian fish tapeworm (AFT), represents a significant, commercially important fish pathogen with a global impact (Scholz et al., 2012). Belonging to an evolutionarily derived lineage of bothriocephalidean cestodes restricted to freshwater fish (Brabec et al., 2015a), *S. acheilognathi* is perhaps best known amongst the 134 currently recognised species of the order Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008 and among cestodes generally. Its notoriety and familiarity are underpinned by its exceptionally low host specificity, its ability to colonise a broad variety of freshwater fish taxa, its status as an invasive species, and its human-assisted history of dissemination throughout all continents of the globe except Antarctica (see Choudhury and Cole, 2012; Scholz et al., 2012 for reviews). It has also been recorded as a potential human parasite (Yera et al., 2013). Adult worms inhabit intestines of a wide range of freshwater fish hosts (rarely also amphibians, reptiles and birds) where they may cause notable harm (Scholz et al., 2012).

Schyzocotyle acheilognathi was originally described in Japan by Yamaguti (1934) from Lake Ogura as *Bothriocephalus acheilognathi*, but historical evidence suggests that the pathogen's distribution range may have been first limited to the Amur River in mainland eastern Asia (Choudhury and Cole, 2012), although opinions exist suggesting the parasite's origin in Africa (Scholz et al., 2012). Since then, AFT has been described under at least 23 different names and most recently transferred to the genus *Schyzocotyle* Akhmerov, 1960 by Brabec et al. (2015a). In each case, eastern Asia is well documented as the primary focus from where AFT spread throughout the globe (see http://www.cabi.org/isc/datasheet/91669 and references therein for up-to-date information on AFT distribution). AFT was originally a parasite of cyprinid fish and while it remains predominantly confined to cyprinids in Eurasia and Africa (Retief et al., 2007), it managed to colonise a range of non-cyprinids

☆ Nucleotide sequence data reported in this paper are available in DDBJ/EMBL/GenBank databases under the accession numbers KX060587–KX060604.

* Corresponding author. Tel.: +420 387 775 484; fax: +420 385 310 388.
E-mail address: brabcak@paru.cas.cz (J. Brabec).

http://dx.doi.org/10.1016/j.ijpara.2016.03.009
0020-7519/

mainly in North American and Australian regions (Dove and Fletcher, 2000; Salgado-Maldonado and Pineda-López, 2003; Méndez et al., 2010; Scholz et al., 2012). Currently it has been documented from more than 200 species of freshwater fish belonging to 10 orders and 19 families (Scholz et al., 2012), even though the parasite might not be able to reach maturity in some of the hosts (Dove and Fletcher, 2000). In the cases of some predatory fish, amphibians, reptiles and birds harbouring adults of *S. acheilognathi*, the hosts might in fact represent postcyclic hosts in which the adult parasites manage to re-establish after being preyed on together with their definitive host (Hansen et al., 2007; Scholz et al., 2012).

Despite the relative abundance of published surveys of the life history, geographical distribution and host ranges of AFT, studies utilising molecular data remain surprisingly scarce and largely limited to non-coding internal transcribed spacer sequences (ITS1, ITS2) separating the nuclear rRNA genes. Liao and Lun (1998) and Feng and Liao (2000) were the first to use molecular data to study genetic polymorphism of AFT, using a random amplified polymorphic DNA (RAPD) approach in both cases. However, datasets from their studies were uncomfortably small, with inferred results insufficiently convincing to support their conclusions that AFT from *Opsariichthys bidens* Günther should be considered a separate species, and that AFT parasitising grass and common carp, *Ctenopharyngodon idella* (Valenciennes) and *Cyprinus carpio* Linnaeus, in China represents two unrelated groups, possibly separate species. In order to obtain further insights into the intraspecific diversity of AFT, Luo et al. (2002, 2003) utilised information from ITS1 and ITS2 sequences and microsatellite loci situated within this region, respectively, on a set of *S. acheilognathi* specimens predominantly from China. Conclusions from both studies should be treated with caution. Luo et al. (2002) interpreted their results of phylogenetic analysis incorrectly, and in fact failed to find any convincing associations among the ITS genotypes. Luo et al. (2003) ignored the fact that they had genotyped eight physically tightly linked microsatellite loci of a multi-copy region that might display intragenomic variation, a feature leading to the violation of Mendelian inheritance assumptions (Selkoe and Toonen, 2006).

Bean et al. (2007) used ITS together with partial ssrDNA sequences to evaluate genetic distances of North American AFT representatives. Chaudhary et al. (2015) and Salgado-Maldonado et al. (2015) compared ITS and rDNA sequences, respectively, to test identity of their isolates to those characterised previously. lsrDNA sequences of *S. acheilognathi* were utilised as part of a taxonomic re-evaluation of bothriocephalidean cestodes from African fishes (Kuchta et al., 2012). This study revealed that AFT is closely related to the clade comprising all African bothriocephalids. The first published mitochondrial (mt) sequence of *S. acheilognathi* was a partial fragment of cytochrome *c* oxidase subunit 1 (*cox*1) obtained from the only documented clinical case (Yera et al., 2013). Recently, a multi-gene phylogenetic study of cestodes of the order Bothriocephalidea (Brabec et al., 2015a) confirmed that African bothriocephalids form a derived monophyletic clade and that AFT together with its congeneric species, *Schyzocotyle nayarensis* (Malhotra, 1983), form its sister lineage. The authors also confirmed that the genus *Bothriocephalus* Rudolphi, 1808 represents an artificial taxonomic entity (originally revealed by Škeříková et al. (2004)) and subsequently transferred *B. acheilognathi* Yamaguti, 1934 to *Schyzocotyle* as *S. acheilognathi*. The present study aims to evaluate the utility of the traditionally exploited genomic loci, the nuclear rRNA operon and mt genome sequences, to study diversification of the AFT and gain first insights into the phylogeography of this invasive species.

2. Materials and methods

2.1. DNA sources, sequencing and assembly

Eight adult worms of *S. acheilognathi* and one *S. nayarensis* (representing the only congener) were sampled from distant localities across the globe (Table 1). Individual specimens were identified on the basis of their morphology (Malhotra, 1983; Scholz et al., 2012) and stored in absolute ethanol before genomic DNA was extracted from small fragments from each individual with the use of an QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total DNA concentration was measured with an Qubit® 2.0 Fluorometer (Life Technologies, Paisley, UK) and DNA was submitted to the DNA Sequencing Facility of the Natural History Museum (NHM), London, UK for Illumina sequencing. Genomic DNA samples were indexed and libraries prepared using either the TruSeq DNA PCR-free or TruSeq Nano DNA Sample Preparation Kits (Illumina, Inc., San Diego, USA; see Table 2) and run simultaneously using one and a half MiSeq Illumina sequencer runs yielding 250 bp long paired-end reads.

The mt genomes were directly assembled using the MITObim approach of Hahn et al. (2013). Partial *cox*1 sequences of *S. acheilognathi* (KR780792) and *S. nayarensis* (KR780829) characterised by Brabec et al. (2015a) were used as an initial bait to extract the corresponding reads of individual representatives of species of *Schyzocotyle* out of the resultant Illumina genomic readpools. The new *cox*1 reference sequence assembly was then automatically subjected to an iterative set of baiting and mapping steps until the total number of mapped reads became stationary. Resulting mt genome assemblies were then imported into Geneious version 7 (Kearse et al., 2012) where corresponding raw

Table 1
List of specimens sequenced in this study.

Species	Host species	Country, locality, year; Collection number[a]	GenBank accession numbers[b]	
			rDNA	mtDNA
Schyzocotyle acheilognathi	*Zacco platypus*	China, Hubei Province, Danjiangkaou, 2013; CH1052/13	KX060596	KX060587
Schyzocotyle acheilognathi	*Cyprinus carpio*	Czech Republic, Terezínský pond, 2010; PBI-23	KX060601	KX060592
Schyzocotyle acheilognathi	*Labeobarbus nedgia*	Ethiopia, Beshelo River, 2006; ET295	KX060597	KX060588
Schyzocotyle acheilognathi	*Cyprinus carpio*	Japan, Osaka Prefecture, Neyagawa, 2011; JAP1	KX060599	KX060590
Schyzocotyle acheilognathi	*Gila conspersa*	Mexico, Durango, El Cuarto, 2008; MX62	KX060600	KX060591
Schyzocotyle acheilognathi	*Labeobarbus kimberlyensis*	South Africa, Vaal Dam, 2006; SAF3	KX060602	KX060593
Schyzocotyle acheilognathi	*Atherina boyeri*	Turkey, Iznik Lake, 2010; TU1	KX060603	KX060594
Schyzocotyle acheilognathi	*Cyprinella lutrensis*	USA, Texas, Santa Elena Canyon, 2006; USA10	KX060604	KX060595
Schyzocotyle nayarensis	*Raiamas bola*	India, West Bengal, Siliguri, 2011; IND904	KX060598	KX060589

[a] Voucher specimens are deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic.
[b] Full-length assemblies; mt, mitochondrial.

Please cite this article in press as: Brabec, J., et al. Paralogues of nuclear ribosomal genes conceal phylogenetic signal within the invasive Asian fish tapeworm lineage: evidence from next generation sequencing data. Int. J. Parasitol. (2016), http://dx.doi.org/10.1016/j.ijpara.2016.03.009