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The complete mitochondrial genome sequence of *Oncicola luehei* (Acanthocephala: Archiacanthocephala) and its phylogenetic position within Syndermata

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ABSTRACT

In the present study, we determined the complete mitochondrial genome sequence of *Oncicola luehei* (14,281 bp), the first archiacanthocephalan representative and the second complete sequence from the phylum Acanthocephala. The complete genome contains 36 genes including 12 protein coding genes, 22 transfer RNA (*tRNA*) genes and 2 ribosomal RNA genes (*rrnL* and *rrnS*) as reported for other syndermatan species. All genes are encoded on the same strand. The overall nucleotide composition of *O. luehei* mtDNA is 37.7% T, 29.6% G, 22.5% A, and 10.2% C. The overall A + T content (60.2%) is much lower, compared to other syndermatan species reported so far, due to the high frequency (18.3%) of valine encoded by GTN in its protein-coding genes. Results from phylogenetic analyses of amino acid sequences for 10 protein-coding genes from 41 representatives of major metazoan groups including *O. luehei* supported monophyly of the phylum Acanthocephala and of the clade Syndermata (Acanthocephala + Rotifera), and the paraphyly of the clade Eurotatoria (classes Bdelloidea + Monogononta from phylum Rotifera). Considering the position of the acanthocephalan species within Syndermata, it is inferred that obligatory parasitism characteristic of acanthocephalans was acquired after the common ancestor of acanthocephalans diverged from its sister group, Bdelloidea. Additional comparison of complete mtDNA sequences from unsampled acanthocephalan lineages, especially classes Polyacanthocephala and Eoacanthocephala, is required to test if mtDNA provides reliable information for the evolutionary relationships and pattern of life history diversification found in the syndermatan groups.

1. Introduction

The Acanthocephalan (thorny headed worms) is a phylum of endoparasites with a worldwide distribution and approximately 1200 described species. These parasites use vertebrates (fishes, amphibians, reptiles, birds, and mammals) as definitive hosts, arthropods (insects and crustaceans) as intermediate hosts, and in some cases, fishes, reptiles, and amphibians are used as paratenic (transport) hosts [1,2]. The phylum is currently represented by 4 classes, i.e., Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala, and Polyacanthocephala [3–6]. This classification is based mainly on morphological features such as the location of the lacunar system (network of cavities in the epidermis), the persistence of ligament sacs in females, the number and shape of cement glands in males, the number and size of proboscis hooks, and host taxonomy and ecology [3,7–9]. Molecular and morphological phylogenetic hypotheses for acanthocephalans show substantial congruence and support the monophyly of these classes [6,10–12].

Rotifers, which are microscopic organisms that inhabit freshwater and marine habitats, are now established as close relatives of acanthocephalans. The phylum Rotifera is currently divided into 3 classes, Bdelloidea, Monogononta and Seisonidea. Bdelloid rotifers lack males and reproduce strictly by parthenogenesis, representing one of the few likely instances of ancient asexuality among animals [13]. Monogononts represent the largest group of rotifers, and are characterized by a heterogonic life history involving an alternation between generations of parthenogenesis and sporadic sexual reproduction. Seisonidea is a marine group represented by only 3 described species that reproduce by amphimixis and has symbiotic lifestyles including

Abbreviations: *atp6* and *atp8*, genes for ATP synthase subunits 6 and 8; BI, Bayesian inference; bp, base pair; BP, bootstrap percentage; BPP, Bayesian posterior probability; *cob*, gene for cytochrome oxidase *b*; *cox1–cox3*, genes for cytochrome oxidase *c* subunit 1–3; dNTP, deoxyribonucleotide triphosphate; kb, kilo base; ML, maximum likelihood; mtDNA, mitochondrial DNA; *nad1–6* and *nad4L*, genes for NADH dehydrogenase subunits 1–6 and 4L; NCR, non-coding region; nt, nucleotide; PCR, polymerase chain reaction; pp, posterior probability; *rrnS* and *rrnL*, genes for small and large mitochondrial ribosomal RNA subunits; tRNA, transfer RNA.

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some association as ecto-commensals of marine leptostracan crustaceans of the genus *Nebalia* [14–16]. Currently the phyla Rotifera + Acanthocephala are recognized as a clade named Syndermata [17]; this clade has been supported by some morphological [18–20] and several molecular phylogenetic analyses (SSU rDNA [5,21]; SSU + LSU rDNAs and mitochondrial *cox1* [6]). Although syndermatan monophyly has been continuously supported by morphological and molecular investigations, the internal phylogeny within Syndermata (e.g., Eurotatoria monophyly/paraphyly, relationships among major syndermatan classes including those of acanthocephalans) is still matters of debate [6,15,22–24] but with certain recent combined analysis of molecular and morphological data supporting the inclusion of Acanthocephala as a rotiferan subgroup [25]. Full resolution of this question requires analysis of additional molecular data, including mtDNA genomes.

With a very few exceptions, metazoan mtDNA are typically circular DNA molecules, ranging in size from 14 kb to 42 kb, encoding 37 genes that consist of 13 protein coding genes (but *atp8* is lacking in most flatworm and nematode species), 2 ribosomal RNA genes (*rrnS* and *rrnL*), and 22 transfer RNA (*tRNA*) genes. Although there is a growing body of recent reports for extensive gene rearrangement even among closely related species, the gene content of mitochondrial genomes is generally relatively conserved across most metazoans. Based on the view that gene order is rather stable and that gene rearrangement (i.e., gene order changes) resulting from convergent evolution appears relatively uncommon (in certain taxonomic groups), comparison of mitochondrial gene order pattern has been proposed as a reliable tool for resolving deep node phylogenetic relationships [26–28]. More than 2400 complete mitochondrial genome (mt genome) sequences are available from different metazoan groups (www.ncbi.nlm.nih.gov./genomes/OGANELLES/mztax_short.html). Despite this wealth of mitochondrial genome information from diverse metazoans, there are some phyla for which mtDNA is still underrepresented or unavailable to date [29,30]. This lack of genome information for as yet unsampled animal groups has rendered development of phylogenetic hypotheses based on mitochondrial sequences far less comprehensive. Mitochondrial genomes from underrepresented animal groups can provide a wealth of genetic information for understanding both animal and mitochondrial genome evolution.

The Acanthocephala is one phylum represented by only a single mitochondrial genome. The mitochondrial genome sequence of *Leptorhynchoides thecatus* is the only species reported for Acanthocephala [31]. Within Syndermata there have been two reports for rotifers (a monogonont *Brachionus plicatilis* [32] and a bdelloid *Rotaria rotatoria* [24]), thus there are three complete genomes available representing Syndermata. Considering the remarkable diversity in their ecology (parasitic life cycles versus free-living, sexual versus asexual reproduction) and number of described species (approximately 1200 acanthocephalan [8] and 2000 rotifer species [33]), the relative lack of genome information necessitates further mitogenomic investigation. In the present study, we report the complete mitochondrial genome sequence of *Oncicola luehei*, the first archiacanthocephalan species for which the entire genome sequence has been determined, and the second representative of the phylum Acanthocephala. *O. luehei* is an endoparasite of the intestine of small mammals, such as coatis and opossums, that are distributed in regions of North, Central and South America. In molecular phylogenies based on nuclear ribosomal DNA and the mitochondrial *cox*1 gene, *Oncicola* spp. are members of a clade with other Archiacanthocephala; this class is the sister group to a clade consisting of Palaeacanthocephala, Polyacanthocephala and Eoacanthocephala [6]. Therefore, the *Oncicola* mtDNA sequence, which is considered representative for the archiacanthocephalan lineage, was used along with 40 published metazoan species to assess phylogenetic relationships within the syndermatan clade and among major metazoan phyla.

2. Materials and methods

2.1. Sampling and molecular techniques

Specimens of the species *O. luehei* Travassos, 1917 were isolated from the intestine of its final host animal *Didelphis virginiana* (opossum) captured from Veracruz, México. After isolation from the host intestine, the specimens were thoroughly washed, kept in 70% ethanol and stored at −20 °C until genomic DNA extraction. Total genomic DNA was extracted using the Masterpure DNA extraction kit (Epicentre Biotechnologies Co.) according to the manufacturer's protocol. Initially, three partial gene fragments for *cox1*, *cob*, and 16S (*rrnL*) were amplified using universal primer sets (Cox1F/Cox1R) or primer sets (Syn-CytbF/Syn-CytbR, and Syn-16S-F/Syn-16S-R) designed from conserved regions of the published acanthocephalan and two rotifer species (Table 1). PCR reactions for these partial fragments were carried out in a 50 μl reaction volume consisting of 10 units of *Taq* polymerase (Roche), 2.5 mM dNTP mixture, 2.5 mM $MgCl_2$, and 20 pmol of each primer with the following amplification conditions: 1 cycle of the initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 1 min, primer annealing at 45 °C for 30 s and elongation at 72 °C for 1 min, and the final extension at 72 °C for 10 min. The nucleotide sequences obtained from these partial gene fragments were then used to design *O. luehei* specific primers for long PCR amplification (Table 1). These overlapping long PCR products (approximately 1.8 kb, 5.5 kb, and 10 kb in size, respectively; Fig. 1), covering the entire mitochondrial genome, were amplified using the long PCR primer sets and the Expand Long Template PCR System (Roche) with the following amplification conditions: 1 cycle of initial denaturation (2 min at 94 °C), 30 cycles of denaturation–primer annealing–elongation (15 s at 94 °C, 30 s at 50–60 °C, and 10 min at 68 °C), and 1 cycle of the final extension (10 min at 68 °C). The amplified long PCR products were gel-isolated, and extracted using the TOPO Gel Purification reagents supplied with the TOPO XL cloning kit (Invitrogen Co.). After gel purification, each of the long PCR products was ligated using the TOPO XL cloning kit and then transformed into competent *Escherichia coli*. Cycle sequencing

Table 1
PCR primers used in the study of *Oncicola luehei*.

Primer	DNA sequence (5′-3′)	Estimated size of PCR products	Primer source
LCO1490	GGTCAACAAATCATAAAGATATTGG	~680 bp	[63]
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		
Syn-16S-F	GACYGTRCTWAGGTAGCRTRATC	~600 bp	This study
Syn-16S-R	AWRDRATRATCCAACATCGAGGTA		
Syn-Cytb-F	CTTTTTTAGGGTATGTTTTACC	~600 bp	This study
Syn-Cytb-R	TCWACARYAYAWCCTCC		
Onci-Cox1-F	GTGGGTCTATAGAAGTGGAGCATCTGTGG	~1.8 kb	This study
Onci-16S-R	CTAATGATTACGCTACCTTAGCACAGTC		
Onci-Cob-F	GATGGCTTTGGCAGTGACTATTGTTG	~5.5 kb	This study
Onci-Cox1-R	TACCAAACCCTCCTATTATCACCGGTATTGC		
Onci-16S-F	GACTGTGCTAAGGTAGCGTAATCATTAG	~10 kb	This study
Onci-CO2-R	TACTCCCAATACCACTGATGGC		