PORTLAND PRESS

Research Article

Gene-based molecular characterization of *cox1* and *pnad5* in *Hymenolepis nana* isolated from naturally infected mice and rats in Saudi Arabia

Dina M. Metwally[1,2], Huda A. Al-Enezy[1], Isra M. Al-Turaiki[3], Manal F. El-Khadragy[1,4], Hany M. Yehia[5,6] and Tahani T. Al-Otaibi[7]

[1]Department of Zoology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia; [2]Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt; [3]Department of Information Technology, Faculty of Computer and Information Science, King Saud University, Riyadh, Saudi Arabia; [4]Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt; [5]Department of Food Science and Nutrition, Faculty of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; [6]Department of Food Science and Nutrition, Faculty of Home Economics, Helwan University, Cairo, Egypt; [7]Department of Biology, Faculty of Arts and Sciences, Nairiyah, Hafr Al-Batin University, Saudi Arabia

Correspondence: Dina M. Metwally (mdbody7@yahoo.com)

OPEN ACCESS

Mice and rats are animals commonly used in research and laboratory testing. Compared with other animal species, they harbor many more zoonotic agents. *Hymenolepis nana* (*H. nana*) is a common tapeworm that parasitizes both humans and rodents. Although this tapeworm is of socio-economic importance worldwide, information related to its mitochondrial genome is limited. The present study examined the sequence diversity of two mitochondrial (mt) genes, subunit I of cytochrome oxidase (*cox1*) and NADH dehydrogenase subunit 5 (*pnad5*), of *H. nana* in mice and rats from two geographical regions of Saudi Arabia (Makkah and Riyadh). Partial sequences of *cox1* and *pnad 5* from individual *H. nana* isolates were separately amplified using polymerase chain reaction (PCR) and sequenced. The GC contents of the sequences ranged between 31.6–33.5% and 27.2–28.6% for *cox1* and *pnad5*, respectively. The genomic similarity among specimens determined via *cox1* primer and *pnad5* primer was 97.1% and 99.7%, respectively. Based on these primers, our data did not indicate any differences between *H. nana* from rat and mice isolates. Results demonstrated that the present species are deeply embedded in the genus *Hymenolepis* with close relationship to other *Hymenolepis* species, including *H. nana* as a putative sister taxon, and that the isolates cannot be categorized as belonging to two different groups with origins in Makkah and Riyadh.

Introduction

Hymenolepis nana, a common tapeworm that is distributed worldwide, is found mostly in young children in developing countries [1]. Human hymenolepiasis, caused by *H. nana* and *H. diminuta,* is a globally prevalent zoonosis. It is endemic in Asia, Southern and Eastern Europe, Central and South America, and Africa, and produces many health problems such as headaches, weakness, anorexia, abdominal pain, and diarrhea [2]. The mature worm lives in the small intestine of humans, mice, and rats [3]. It is mostly transmitted by contamination with fecal matter containing eggs or via insect vectors acting as intermediate hosts. *Hymenolepis nana,* is also able to complete its entire life cycle in a single host, and is therefore capable of auto-infection [4]. *Hymenolepis nana,* in different rodents, such as rats and mice, is morphologically similar to human *H. nana.* Thus, establishing the identity of these two species is epidemiologically important [5]. Despite revised nomenclature, speciation and host specificity of *H. nana* continues to be problematic [6]. Hence, a biological, taxonomic and epidemiological investigation of *H. nana* in various hosts may be useful in order to better understand endemic strains [6].

Hymenolepidids have been categorized into several genera based on morphological characteristics [7, 8]. Mitochondrial (mt) genomes are small (usually less than 20,000 bp), circular and maternally inherited [9]. The property of having a high copy number per cell makes them attractive and more amenable targets for studies related to characterization, population genetics, and phylogenetics [10]. Mitochondrial DNA (mtDNA) sequences are reliable genetic markers that have been useful in studies on population genetics and systematics [11]. Genetic diversity of *H. nana* has been studied using genetic makers, such as the mt cytochrome oxidase subunit 1(*cox1*) and the entire first and second internal transcribed spacer (ITS-1 and ITS-2) regions of nuclear ribosomal DNA (rDNA) [6, 12, 13]. These studies indicated the presence of genetic variation in *H. nana* from different domestic and wildlife host species, as well as from different areas, suggesting that *H. nana* comprises 'cryptic' species, which are morphologically identical but genetically distinct. Although mitochondrial (mt) genes, such as NADH dehydrogenase subunit 5 (*pnad5*), small subunit ribosomal RNA (*rrnS*) and ATPase subunit6 (*atp6*), of *H. nana* in mice from different geographical regions of China have been studied, information on the sequence variability in other mt genes of *H. nana* isolates, is rare [14].

The objective of the present study was to analyze *cox1* and *pnad5* in *H. nana* isolated from naturally infected mice and rats in Makkah and Riyadh, Saudi Arabia.

This work was based on my previous study, "Gene-based molecular analysis of *cox1* in Echinococcus granulosus cysts isolated from naturally infected livestock in Riyadh, Saudi Arabia," which was a part of a major research project. This project is conducted by the Zoology Department, Faculty of Science, King Saud University. The project aims to analyze genetic sequences of different parasites that are found spread out over Saudi Arabia, in order to help differentiate between the genetic sequences of local parasites and parasites of other regions, both inside and outside Saudi Arabia. Such information is expected to facilitate the development of methods for the prevention and control of these parasites.

Materials and methods
Sample collection

During the period between March and April of 2017, a total of 100 BALB/c mice (50 from Makkah and 50 from Riyadh) and 120 *Rattusu norvegis* rats (70 from Makkah and 50 from Riyadh) were obtained from the Female Center for Scientific and Medical Colleges, Riyadh, Saudi Arabia. The animals were kept in wire-bottomed cages in a room under conditions of standard illumination with a 12-h light–dark cycle, at a temperature of $25 \pm 1°C$ for 1 week, until the commencement of treatment. Animals were provided with tap water and a balanced diet ad libitum. Mice were killed via decapitation. Worms were collected and extracted from all mice and rats, washed with normal saline and examined under a microscope to determine the type of worm. Worms were stored at $-20°C$ until molecular analysis. All experiments were conducted according to specifications of the animal ethics committee outlined by the University of Sattam Bin Abdulaziz University (IRB number: SAU-2017-LAB-523/PI), which also included the joint efforts of Parasitology Department, Sattam Bin Abdulaziz University, and the College of Science, King Saud University.

DNA extraction

Worms obtained from mice and rats were washed with distilled water and ethanol before they were centrifuged. Genomic DNA (gDNA) was then extracted using a High Pure PCR Template Preparation Kit (Qiagen GmbH, Hilden, Germany Cat. No.51304). Amplification of *cox1* and *pnad5* was performed using specific primers (*cox1*: F:5′ AGAGTGATCCGGTGATATGGTGA 3′ R:5′ ACCATTCACCCTTGGTATAAGCAGA 3′, *pnad5*: F:5′ GAAGCGTTAATTATGGGTT 3′ R:5′ GATTACAAGTTGATAGAGCCC 3′) [14] in a 40 μl reaction mixture containing 8 μl of master mix, 25.6 μl of deoxynucleotides (dNTPs), 2.4 μl of primers, and 4 μl of DNA template. The PCR program consisted of an initial denaturation step at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, extension at 72°C for 10 min, and a final extension step at 72°C for 10 min. PCR products were analyzed via 1% agarose gel electrophoresis.

DNA sequencing and phylogenetic analysis

PCR products of *cox1* and *pnad5* were purified and sequenced using both forward and reverse complements by Genetic Analyzer at the Central Lab of King Saud University. A multiple sequence alignment was generated for the samples using the ClustalW [15] algorithm with a gap opening penalty of 10 and a gap extension penalty of 1. All sequences were truncated slightly using an error probability method with a limit of 0.05 at both ends. A BLAST search was performed for each sequence to locate related sequences. A phylogenetic tree was generated using MrBayes 3.2.6

PORTLAND
PRESS

Table 1 Genetic sequences from the isolated *H. nana* with variable lengths and GC contents (*cox1*)

Name	Host location	Host species	%GC	Post-Trim	Length
1MR	Riyadh, Saudi Arabia	*Mus musculus*	31.80%	756	790
2MR	Riyadh, Saudi Arabia	*Mus musculus*	31.80%	757	786
3MM	Makkah, Saudi Arabia	*Mus musculus*	32.00%	754	785
8MM	Makkah, Saudi Arabia	*Mus musculus*	31.80%	773	789
11MR	Riyadh, Saudi Arabia	*Mus musculus*	31.70%	765	793
12MR	Riyadh, Saudi Arabia	*Mus musculus*	32.20%	760	786
13MM	Makkah, Saudi Arabia	*Mus musculus*	31.60%	754	787
14MM	Makkah, Saudi Arabia	*Mus musculus*	32.00%	762	791
15MM	Makkah, Saudi Arabia	*Mus musculus*	31.90%	767	789
15MR	Riyadh, Saudi Arabia	*Mus musculus*	31.90%	755	786
16MM	Makkah, Saudi Arabia	*Mus musculus*	32.30%	748	787
16MR	Riyadh, Saudi Arabia	*Mus musculus*	31.80%	756	786
18MR	Riyadh, Saudi Arabia	*Mus musculus*	32.00%	758	776
19MR	Riyadh, Saudi Arabia	*Mus musculus*	32.20%	757	777
20MM	Makkah, Saudi Arabia	*Mus musculus*	31.90%	761	791
20MR	Riyadh, Saudi Arabia	*Mus musculus*	32.00%	760	788
24MR	Riyadh, Saudi Arabia	*Mus musculus*	32.00%	759	785
26MR	Riyadh, Saudi Arabia	*Mus musculus*	31.90%	759	788
27MM	Makkah, Saudi Arabia	*Mus musculus*	31.60%	779	787
32MR	Riyadh, Saudi Arabia	*Mus musculus*	32.10%	757	787
36MR	Riyadh, Saudi Arabia	*Mus musculus*	32.20%	758	786
37MM	Makkah, Saudi Arabia	*Mus musculus*	31.90%	763	789
42MM	Makkah, Saudi Arabia	*Mus musculus*	31.80%	759	787
23RM	Makkah, Saudi Arabia	*Rattus norvegius*	33.50%	759	786
40RM	Makkah, Saudi Arabia	*Rattus norvegius*	33.40%	203	293

[16], a Bayesian inference algorithm. Bootstrap method was used for resampling with the number of replicates set to 1000.

Results

Amplification of *cox1* and *pnad5*

Partial PCR amplification of *cox1* and *pnad5* yielded the expected 800 bp fragments for all DNA samples from both mice and rats.

Analysis of *Cox1*

The sequences of 25 samples, including those of 10 Makkah mice, 13 Riyadh mice and 2 Makkah rats, were analyzed. The final sequences were 776–793 nucleotides in length (Table 1). A BLAST search was performed for each sequence to locate related sequences. All samples except one showed a pairwise identity of 99–99.60% and a 62–100% coverage relative to the genome of *H. nana,* Japan, with the accession number LM402005. In addition, all samples showed a pairwise identity of 99.00–99.60% and a 62–100% coverage relative to *H. nana* mt genome with accession numbers LM403673 and AP017666. All samples showed a pairwise identity of 97.90–98.60% and a 62–100% coverage to *H. nana* mt genome with the accession number KT951722.

All samples showed a pairwise identity of 98.80–99.60% and a 62.60–86.48% coverage with *H. nana* mt *cox1* gene, encoding cytochrome *c* oxidase subunit 1, partial *cds*, isolate: *H. nana* with accession number LC063187.

All samples showed a pairwise identity of more than 97.60% and 54% coverage with *H. nana cox1* partial *cds*, mitochondrial with accession numbers GU433102, GU433103, and GU433104. All of the samples showed a pairwise identity of 98.80–99.60% and 62.60–74% coverage to *H. nana cox1* mitochondrial gene, partial *cds* with accession number AB033412.

The multiple sequence alignment of the 25 samples and related sequences retrieved from Genbank was generated. The sequence LM402005 was set as the reference sequence. We found the following one-nucleotide substitutions (SNP) transitions: T to C at position 9998 of the reference sequence in 25% of the samples; C to T at position 10264 of the reference sequence in 92% of the samples; G to A at position 10495 of the reference sequence in all samples;
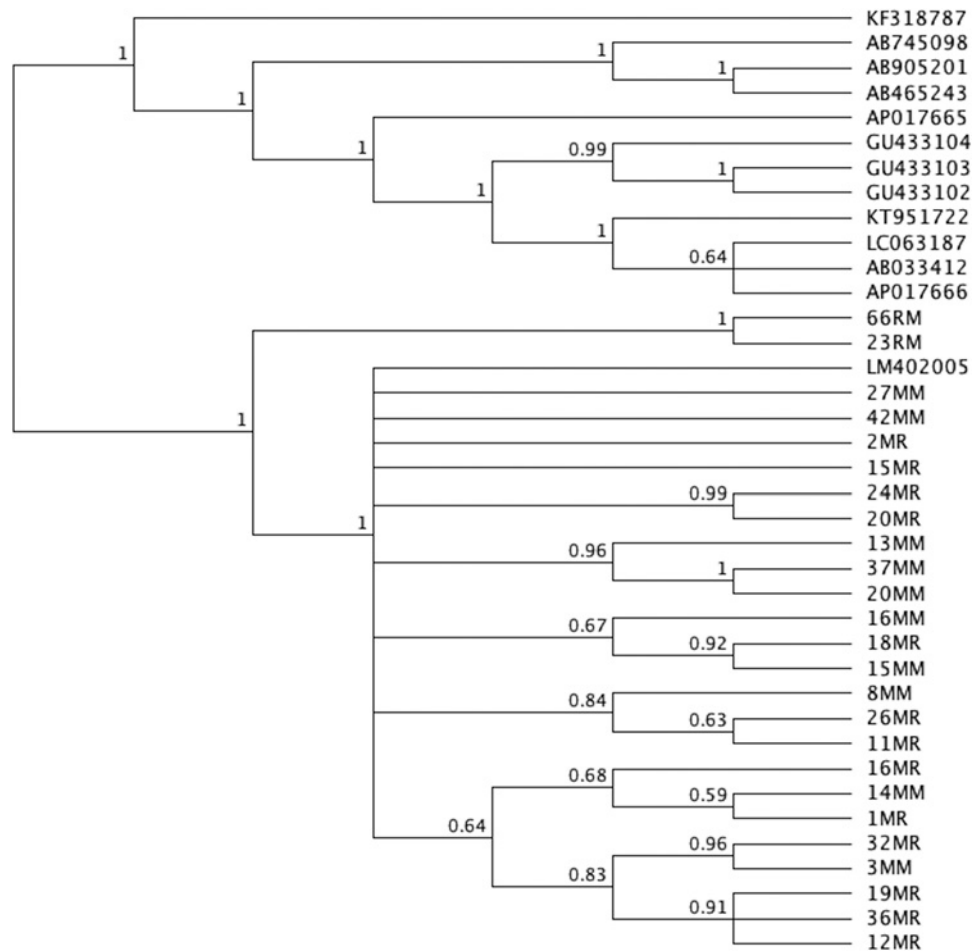
Figure 1. Phylogenetic tree of the 25 mice and rats samples in the present study along with similar sequences published in Genbank

and A to C at position 10591 of the reference sequence in all samples. In addition, one insertion of T was found at position 10766 of the reference sequence in 80% of the samples. Finally, two deletions of A were observed at positions 10760 and 10004, in 31% and 55% of the samples respectively (note: the alignment is provided in FASTA and Nexus formats).

The Phylogenetic tree was generated using MrBayes. *Dicrocoelium dendriticum* (Accession number KF318787) was used as the outgroup. The Phylogenetic tree with posterior probability values is shown (Figure 1). All the samples in the present study were grouped in a clade with *H. nana* genome assembly *H. nana* Japan with accession number LM402005 (note: the tree is provided in Nexus format).

Analysis of *pnad5*

Sequences of 31 samples, including, 10 Makkah mice, 17 Riyadh mice and 4 Makkah rats, were analyzed. The final sequences were 816–846 nucleotides in length (Table 2). A BLAST search was performed for each sequence in order to locate related sequences. All samples showed a pairwise identity of 98.70–99.50% and coverage 82.40–100% with *H. nana* sequences with accession numbers: LM403673, LM402005, KT951722, and AP017666.

These samples were also similar to the sequence of *H. nana* isolate *y1 pnad5*, partial *cds*, mitochondrial (accession number KT589891), *H. nana* isolate *shz1 pnad5*, partial *cds, mitochondrial* (accession number KT589901), and *H. nana* isolate *s2* gene*, partial *cds,* mitochondrial (accession number KT589905) with identity of 98.70–99.40% and coverage of 81–89%.

A multiple sequence alignment was generated for the 31 samples and related sequences using ClustalW algorithm. The sequence LM402005 was set as the reference sequence. The SNPs are shown (Table 3).

Table 2 Genetic sequences from the isolated *H. nana* with variable lengths and GC contents (*pnad5*)

Name	Host location	Host species	%GC	Post-Trim	Length
1MM	Makkah, Saudi Arabia	*Mus musculus*	27.80%	814	834
1MR	Riyadh, Saudi Arabia	*Mus musculus*	28.20%	801	838
2MR	Riyadh, Saudi Arabia	*Mus musculus*	27.60%	800	837
3MM	Makkah, Saudi Arabia	*Mus musculus*	27.70%	798	837
8MM	Makkah, Saudi Arabia	*Mus musculus*	28.20%	803	837
10MR	Riyadh, Saudi Arabia	*Mus musculus*	27.90%	802	834
11MR	Riyadh, Saudi Arabia	*Mus musculus*	27.90%	799	838
12MR	Riyadh, Saudi Arabia	*Mus musculus*	27.80%	802	837
13MM	Makkah, Saudi Arabia	*Mus musculus*	27.60%	799	836
15MR	Riyadh, Saudi Arabia	*Mus musculus*	27.70%	803	838
16MM	Makkah, Saudi Arabia	*Mus musculus*	27.50%	798	816
16MR	Riyadh, Saudi Arabia	*Mus musculus*	27.20%	807	843
19MR	Riyadh, Saudi Arabia	*Mus musculus*	27.80%	802	834
20MR	Riyadh, Saudi Arabia	*Mus musculus*	27.60%	801	837
21MM	Makkah, Saudi Arabia	*Mus musculus*	27.70%	802	839
23RM	Riyadh, Saudi Arabia	*Mus musculus*	28.20%	799	837
25MR	Riyadh, Saudi Arabia	*Mus musculus*	27.50%	803	834
26MR	Riyadh, Saudi Arabia	*Mus musculus*	27.40%	803	819
27MM	Makkah, Saudi Arabia	*Mus musculus*	27.60%	801	836
27MR	Riyadh, Saudi Arabia	*Mus musculus*	27.40%	797	820
32MR	Riyadh, Saudi Arabia	*Mus musculus*	28.10%	802	839
35MR	Riyadh, Saudi Arabia	*Mus musculus*	28.40%	754	846
36MR	Riyadh, Saudi Arabia	*Mus musculus*	28.10%	805	837
37MM	Makkah, Saudi Arabia	*Mus musculus*	28.60%	746	840
40MM	Makkah, Saudi Arabia	*Mus musculus*	27.70%	818	840
40MR	Riyadh, Saudi Arabia	*Mus musculus*	27.50%	806	835
41MR	Riyadh, Saudi Arabia	*Mus musculus*	28.60%	811	844
42MM	Makkah, Saudi Arabia	*Mus musculus*	27.80%	800	837
66RM	Makkah, Saudi Arabia	*Rattus norvegius*	28.00%	801	837
40RM	Makkah, Saudi Arabia	*Rattus norvegius*	27.40%	801	819
22RM	Makkah, Saudi Arabia	*Rattus norvegius*	27.90%	800	838
23RM	Makkah, Saudi Arabia	*Rattus norvegius*	28.2%	799	837

Table 3 Variations found in the sequences in the present study relative to sequence LM402005

Name	Minimum	Maximum	Length	Change	Coverage	Polymorphism type	Variant frequency
C	5616	5616	1	A -> C	4	SNP (transversion)	25.00%
CA	6442	6443	2	TC->CA	3 -> 4	Substitution	33.3% -> 50.0%
	5634	5634	1	(A)3 -> (A)2	29	Deletion (tandem repeat)	37.90%
C	6439	6439	1	A -> C	5	SNP (transversion)	40.00%
T	6440	6440	1	A -> T	5	SNP (transversion)	40.00%
	5621	5621	1	#NAME?	20	Deletion	45.00%
T	6426	6426	1	A -> T	5	SNP (transversion)	60.00%
	6439	6439	1	#NAME?	5	Deletion	60.00%
	6440	6440	1	#NAME?	5	Deletion	60.00%
	5617	5618	2	(AA)3 -> (AA)2	10	Deletion (tandem repeat)	80.00%
A	5885	5885	1	G -> A	31	SNP (transition)	100.00%
A	5956	5956	1	G -> A	31	SNP (transition)	100.00%
G	6273	6273	1	A -> G	31	SNP (transition)	100.00%
T	6295	6295	1	C -> T	31	SNP (transition)	100.00%
	6427	6432	6	#NAME?	5	Deletion	100.00%
C	6435	6435	1	A -> C	5	SNP (transversion)	100.00%
	6438	6438	1	#NAME?	5	Deletion	100.00%
T	6441	6441	1	C -> T	5	SNP (transition)	100.00%
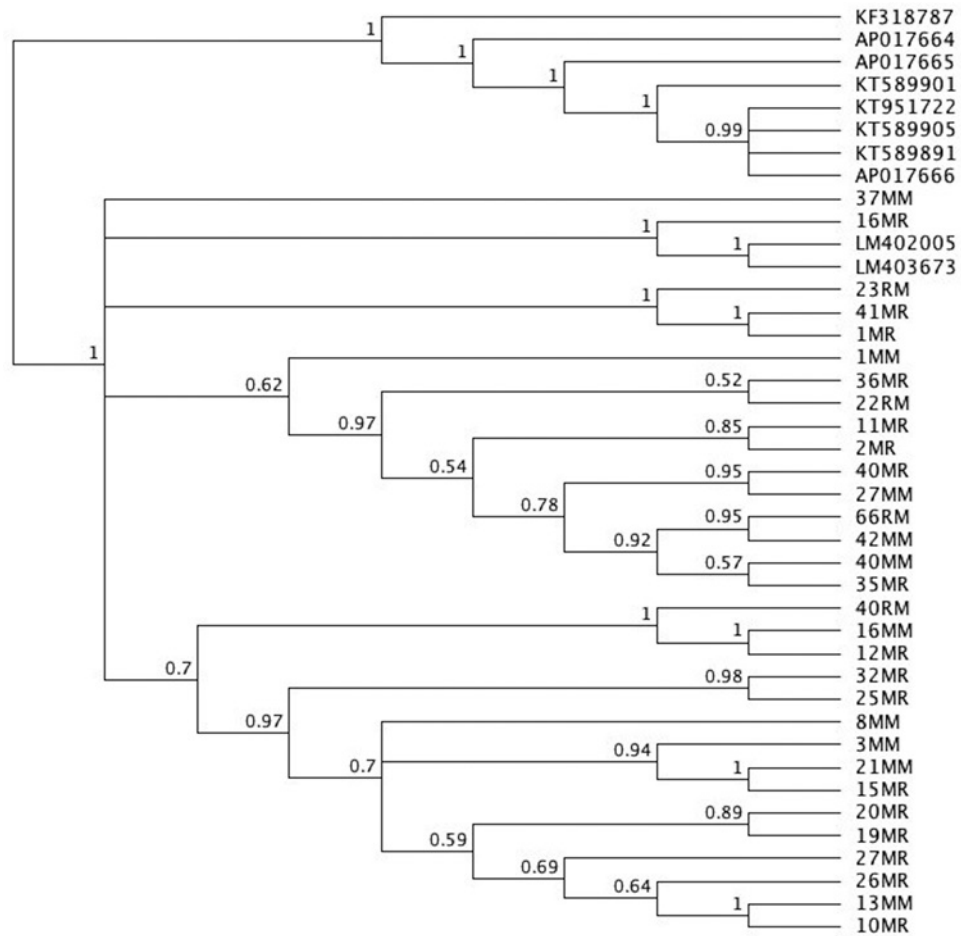
Figure 2. Phylogenetic tree of 31 mice and rats samples in the current study along with similar sequences published in Genbank

MrBayes was used in order to generate the Phylogenetic tree. *Dicrocoelium dendriticum* (accession number KF318787) was used as the outgroup. The Phylogenetic tree with posterior probability values is shown (Figure 2). The sequences placed in a clade of *H. nana* sequences LM402005 and LM403673 (note: the tree is provided Nexus format).

Discussion

Laboratory animal models, especially rodents of the family, *Muridae*, share important links in food chains in their ecosystems, due to their life style and great biotic potential [17–19]. Compared with most animal species, rodents have a greater ability to harbor many zoonotic agents [20–23]. Due to their broad distribution and close contact with different animals as well as with humans, rodents may act as reservoir hosts for vector-borne disease agents [24]. In conventional animal facilities, rodent colonies either are frequently infected with helminth parasites or become infected in places where they are maintained while waiting to be experimented on [2,25,26]. Based on morphological characters, the Hymenolepidid species that were analyzed had all the characteristic features of genus *Hymenolepis* and were identified as *H. nana*.

Molecular phylogenetic approaches in association with traditional morphological techniques are used extensively for identification, phylogenetic analysis, and differentiation of highly similar Hymenolepidid species infecting laboratory rodents [27–33]. Mitochondria play an essential role in metabolism, apoptosis, illness, and aging [34]. They facilitate oxidative phosphorylation, ATP production and other biochemical functions. Mitochondria contain their own genome, consisting of mitochondrial DNA (mtDNA), often used as a part of molecular phylogenetics studies [35]. Mitochondrial genomes of helminths display unique characteristics such as all genes being coded on the same strand [36].

In the present study, mt genes of *H. nana* were amplified using species-specific primers. A descriptive analysis of *H. nana* mt genes may enable the use of genetic markers in the diagnosis of hymenolepiasis and facilitate epidemiological studies of *H. nana* at a molecular level. Furthermore, the use of mtDNA markers to examine genetic variability in cryptic/sibling species and larval stages of *H. nana* may be vital as morphological descriptions of *H. nana* are still rare [6, 37, 38]. For purposes of the present study, genomic DNA was extracted from 31 specimens of *H. nana,* from two different geographical locations in Saudi Arabia. The lengths of *cox*1 and *pnad5* sequences, obtained separately from the specimens, were 850 bp, and the GC contents of the sequences were 31.6–33.5%, for *cox1*, and 27.2%-28.6% for *pnad5*. The range of genomic similarity determined among specimens by *cox1* primer was 97.1% and by *pnad5* primer was 99.7%.

The inter-specific sequence differences between *cox1* and *pnad5* were found to be low and recorded between *H. nana* (present isolates) and *H. nana* (accession number LM402005, LM403673, KT951722, AP017666, LC063187, GU433102, GU433103, GU433104, AB033412, KT589891, KT589901, and KT589905). These results agreed with those of a previous study that reported lower divergence values between the *Hymenolepis* species that are most related to each other [39].

In the phylogenetic tree, *H. nana* isolates did not exhibit an obvious geographical distinction based on the sequences of the two mtDNA regions. All *H. nana* isolates from Makkah and Riyadh grouped together, indicating that all *H. nana* samples from Makkah and Riyadh were strongly related. Furthermore, isolates from both mice and rats displayed genomic similarity. These results were similar to those of a previous study [14]. Subsequent analyses of genetic sequences of Hymenolepid species have strongly supported monophyly with strong bootstrap values within the cestoda clade. These results substantiated those obtained in previous studies indicating that Hymenolepididae species of the genus *Hymenolepis* may be monophyletic in origin [40–43].

Supported by existing data, the present study, investigated the placement of Hymenolepid species within Hymenolepididae. Results indicated that the present species were deeply embedded in the genus *Hymenolepis* with close relationships to other *Hymenolepis* species, including previously described *H. nana,* as a putative sister taxon. Our results indicate that more indepth phylogenetic studies, which include more taxa and different molecular markers of Hymenolepid species, may be needed in the future. A recent field study provided useful tools for the rapid identification and phylogenetic analysis of Hymenolepidids infecting laboratory rodents. In addition, *cox1* and *pnad5* of *H. nana* that were analyzed by the present study yielded a unique sequence that confirmed their taxonomic position within the family of Hymenolepid species. Also, laboratory rodents should be considered potential natural reservoirs of different parasite species, which require further monitoring in order to improve the awareness of researchers, in order to prevent possible transmission of parasitic zoonosis from laboratory animals.

Funding

Author Contribution

Conceptualization: D.M.M. Data curation: H.M.Y. Formal analysis: H.A.A. Funding acquisition: M.F.E. Investigation: D.M.M. Methodology: H.A.A. and T.T.A. Project administration: D.M.M. Resources: H.A.A. and T.T.A. Software: I.M.T. Supervision: D.M.M. Validation: D.M.M. Visualization: H.M.Y. Writing: original draft: D.M.M. Writing, review and editing: MFE, DMM, and IMT.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviation

cox1, cytochrome oxidase.

References

1 Willcocks, B., McAuliffe, G.N. and Baird, R.W. (2015) Dwarf tapeworm (*Hymenolepis nana*): characteristics in the Northern Territory 2002-2013. *Aust. Paediatr. J.* **51**, 982–987

2 Thompson, R.A. (2015) Neglected zoonotic helminths: *Hymenolepis nana*, *Echinococcus canadensis* and *Ancylostoma ceylanicum*. *Clin. Microbiol. Infect.* **21**, 426–432, https://doi.org/10.1016/j.cmi.2015.01.004

3 Marquardt, W.C., Demaree, R.S. and Grieve, R.B. (2000) *Parasitology and Vector Biology*, Harcoart Academic Press, London

4 Sirivichayakul, C., Radomyos, P., Praevanit, R., Jojjaroen-Anant, C. and Wisetsing, P. (2000) *Hymenolepis nana* infection in Thai children. *J. Med. Assoc. Thailand* **83**, 1035–1038

5 Ferretti, G., Gabriele, F. and Palmas, C. (1981) Development of human and mouse strain of *Hymenolepis nana* in mice. *Int. J. Parasitol.* **11**, 425–430, https://doi.org/10.1016/0020-7519(81)90060-6

6 Macnish, M.G., Morgan-Ryan, U.M., Monis, P.T., Behnke, J.M. and Thompson, R.C.A. (2002) A molecular phylogeny of nuclear and mitochondrial sequences in *Hymenolepis nana* (Cestoda) supports the existence of a cryptic species. *Parasitology* **125**, 567–575, https://doi.org/10.1017/S0031182002002366

7 Widmer, V., Boyko, G. and Mariaux, J. (2013) A new genus of the family Hymenolepididae (Cestoda) from *Sephanoides sephaniodes* (Apodiformes, Trochilidae) in Northern Patagonia (Chile). *Acta Parasit.* **58**, 105–111, https://doi.org/10.2478/s11686-013-0117-y

8 Makarikov, A.A., Mel'nikova, Y.A. and Tkach, V.V. (2015) Description and phylogenetic affinities of two new species of Nomadolepis (Eucestoda, Hymenolepididae) from Eastern Palearctic. *Parasitol. Int.* **64**, 453–463, https://doi.org/10.1016/j.parint.2015.06.009

9 Boore, J. L. (1999) Animal mitochondrial genomes. *Nucleic Acids Res.* **27**, 1767–1780, https://doi.org/10.1093/nar/27.8.1767

10 McManus, D.P., Le, T.H. and Blair, D. (2004) Genomics of parasitic flatworms. *Int. J. Parasitol.* **34**, 153–158, https://doi.org/10.1016/j.ijpara.2003.11.003

11 Li, J.Y., Liu, G.H., Wang, Y., Song, H.Q., Lin, R.Q., Zou, F.C. et al. (2013) Sequence variation in three mitochondrial DNA genes among isolates of *Ascaridia galli* originating from Guangdong, Hunan and Yunnan provinces, China. *J. Helminthol.* **87**, 371–375, https://doi.org/10.1017/S0022149X12000326

12 Okamoto, M., Agatsuma, T., Kurosawa, T. and Ito, A. (1997) Phylogenetic relationships of three hymenolepidid species inferred from nuclear ribosomal and mitochondrial DNA sequences. *Parasitol* **115**, 661–666, https://doi.org/10.1017/S0031182097001741

13 Mirjalali, H., Kia, E.B., Kamranrashani, B., Hajjaran, H. and Sharifdini, M. (2016) Molecular analysis of isolates of the cestode *Rodentolepis nana* from the great gerbil, Rhombomysopimus. *J. Helminthol.* **90**, 252–255, https://doi.org/10.1017/S0022149X15000115

14 Cheng, T., Gao, D. Z., Zhu, W. N., Fang, S. F., Chen, N., Zhu, X. et al. (2016) Genetic variability among *Hymenolepis nana* isolates from different geographical regions in China revealed by sequence analysis of three mitochondrial genes. *Mitochondrial DNA Part A* **27**, 4646–4650, https://doi.org/10.3109/19401736.2015.1101595

15 Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680, https://doi.org/10.1093/nar/22.22.4673

16 Huelsenbeck, J.P. and Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755, https://doi.org/10.1093/bioinformatics/17.8.754

17 Rosas, G.A. (1997) Diagnóstico: parasitosis intestinal por Aspiculuris tetraptera. *Anim. Experiment.* **2**, 9–11

18 Gonçalves, L., Pinto, R.M., Vicente, J.J., Noronha, D. and Gomes, D.C. (1998) Helminth parasites of conventionally maintained laboratory mice - II. Inbred strains with an adaptation of the anal swab technique. *Memorias Do Instituto Oswaldo Cruz.* **93**, 121–126, https://doi.org/10.1590/S0074-02761998000100023

19 Perec-Matysiak, A., Okulewicz, A., Hildebrand, J. and Zaleśny, G. (2006) Helminth parasites of laboratory mice and rats. *Wiadomosci parazytologiczne* **52**, 99–102

20 Neifer, S., Kremsner, P.G., Weinig, M., Harms, G., Sahlmüller, G., Bienzle, U. et al. (1991) Interferon-gamma treatment in mice experimentally infected with *Trichinella spiralis*. *Parasitol. Res.* **77**, 437–442, https://doi.org/10.1007/BF00931641

21 Durden, L.A., Hu, R., Oliver, Jr, J.H. and Cilek, J.E. (2000) Rodent ectoparasites from two locations in northwestern Florida. *J. Vector Ecol.* **25**, 222–228

22 Mehlhorn, H., Schmahl, G. and Mevissen, I. (2005) Efficacy of a combination of imidacloprid and moxidectin against parasites of reptiles and rodents: case reports. *Parasitol. Res.* **97**, S97–S101, https://doi.org/10.1007/s00436-005-1451-2

23 Pakdel, N., Naem, S., Rezaei, F. and Chalehchaleh, A.A. (2013) A survey on helminthic infection in mice (*Mus musculus*) and rats (*Rattus norvegicus* and *Rattus rattus*) in Kermanshah, Iran. *Vet. Res. Forum.* **4**, 105–109

24 Mahmoud, A.E., Attia, R. A.H., Eldeek, H.E.M., Abdel, B.L. and Oshaish, H.A. (2009) Oxyurid nematodes detected by colonoscopy in patients with unexplained abdominal pain. *Parasitol. Unit J.* **2**, 93–102

25 Sato, Y., Ooi, H.K., Nonaka, N., Oku, Y. and Kamiya, M. (1995) Antibody production in *Syphacia obvelata* infected mice. *J. Parasitol.* **8**, 559–562, https://doi.org/10.2307/3283853

26 Rehbinder, C., Baneux, P., Forbes, D., van Herck, H., Nicklas, W., Rugaya, Z. et al. (1996) FELASA recommendations for the health monitoring of mouse, rat, hamster, gerbil, guinea pig and rabbit experimental units. *Lab. Animal.* **30**, 193–208, https://doi.org/10.1258/002367796780684881

27 Jacobs, K.A., Collins-Racie, L.A., Colbert, M., Duckett, M., Golden-Fleet, M., Kelleher, K. et al. (1997) A genetic selection for isolating cDNAs encoding secreted proteins. *Gene* **198**, 289–296, https://doi.org/10.1016/S0378-1119(97)00330-2

28 Zhu, X.Q., Jacobs, D.E., Chilton, N.B., Sani, R.A., Cheng, N. and Gasser, R.B. (1998) Molecular characterization of Toxocara variant from cats in Kuala Lumpur, Malaysia. *Parasitol* **117**, 155–164, https://doi.org/10.1017/S0031182098002856

29 Vermund, S.H. and Wilson, C.M. (2000) Pinworm (*Enterobius vermicularis*). *Semin. Pediatr. Infect. Dis. WB Saunders* **11**, 252–256, https://doi.org/10.1053/spid.2000.9639

30 Morales-Hojas, R., Post, R.J., Shelley, A.J., Maia-Herzog, M., Coscarón, S. and Cheke, R.A. (2001) Characterization of nuclear ribosomal DNA sequences from *Onchocerca volvulus* and *Mansonella ozzardi* (Nematoda: Filarioidea) and development of a PCR-based method for their detection in skin biopsies. *Int. J. Parasitol.* **31**, 169–177, https://doi.org/10.1016/S0020-7519(00)00156-9

31 Nakano, T., Okamoto, M., Ikeda, Y. and Hasegawa, H. (2006) Mitochondrial cytochrome c oxidase subunit 1 gene and nuclear rDNA regions of *Enterobius vermicularis* parasitic in captive chimpanzees with special reference to its relationship with pinworms in humans. *Parasitol. Res.* **100**, 51–57, https://doi.org/10.1007/s00436-006-0238-4

32 Zhu, X.Q., Amelio, S.D., Gasser, R.B., Yang, T.B., Paggi, L., He, F. et al. (2007) Practical PCR tools for the delineation of *Contracaecum rudolphii A* and *Contracaecum rudolphii B* (Ascaridoidea: Anisakidae) using genetic markers in nuclear ribosomal DNA. *Mol. Cell. Probes* **21**, 97–102, https://doi.org/10.1016/j.mcp.2006.08.004

33 Chang, T.K., Liao, C.W., Haung, Y.C., Chang, C.C., Chou, C.M., Tsay, H.C. et al. (2009) Prevalence of *Enterobius vermicularis* infection among preschool children in kindergartens of Taipei City, Taiwan in 2008. *Korean J. Parasitol.* **47**, 185–187, https://doi.org/10.3347/kjp.2009.47.2.185

34 Lin, M.T. and Beal, M.F. (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* **443**, 787, https://doi.org/10.1038/nature05292

35 Avise, J.C. (2000) *Phylogeography: The History and Formation of Species 56*, p. 7, Harvard University Press

36 von Nickisch-Rosenegk, M., Brown, W.M. and Boore, J.L. (2001) Complete sequence of the mitochondrial genome of the tapeworm *Hymenolepis diminuta*: gene arrangements indicate that Platyhelminths are Eutrochozoans. *Mol. Biol. Evol.* **18**, 721–730, https://doi.org/10.1093/oxfordjournals.molbev.a003854

37 Morgan, J.A.T. and Blair, D. (1995) Nuclear rDNA ITS sequence variation in the trematode genus *Echinostoma*: an aid to establishing relationships within the 37-collar-spine group. *Parasitology* **111**, 609–615, https://doi.org/10.1017/S003118200007709X

38 Sorensen, R.E., Curtis, J. and Mindhela, D.J. (1998) Intraspecific variation in the rDNA ITS loci of 37-collar-spined *Echinostomes* from North America: Implications for sequence-based diagnoses and phylogenetics. *J. Parasitol.* **84**, 992–997, https://doi.org/10.2307/3284633

39 Nkouawa, A., Haukisalmi, V., Li, T., Nakao, M., Lavikainen, A., Chen, X. et al. (2016) Cryptic diversity in hymenolepidid tapeworms infecting humans. *Parasitol. Intl.* **65**, 83–86, https://doi.org/10.1016/j.parint.2015.10.009

40 De Ley, P. and Blaxter, M.L. (2002) Systematic position and phylogeny. *The Biol. Nematodes* **1**, 1–30

41 Bert, W., Messiaen, M., Manhout, J., Houthoofd, W. and Borgonie, G. (2006) Evolutionary loss of parasitism by nematodes? Discovery of a free-living fiG. (20 nematode). *J. Parasitol.* **92**, 645–647, https://doi.org/10.1645/GE-672R.1

42 Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O. et al. (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol. Biol. Evol.* **23**, 1792–1800, https://doi.org/10.1093/molbev/msl044

43 Wijová, M., Moravec, F., Horák, A. and Lukeš, J. (2006) Evolutionary relationships of Spirurina (Nematoda: Chromadorea: Rhabditida) with special emphasis on dracunculoid nematodes inferred from SSU rRNA gene sequences. *Int. J. Parasitol.* **36**, 1067–1075, https://doi.org/10.1016/j.ijpara.2006.04.005