Microscopy and Microanalysis (2020), 1–15
doi:10.1017/S1431927620000161

Microscopy AND Microanalysis

Micrographia

Morphological, Molecular, and Pathological Appraisal of *Hymenolepis nana* (Hymenolepididae) Infecting Laboratory Mice (*Mus musculus*)

Ebtsam Al-Olayan[1], Maha Elamin[1], Eman Alshehri[1], Abeer Aloufi[1,2], Zainab Alanazi[1], Mina Almayouf[1], Lamia Bakr[3] and Rewaida Abdel-Gaber[1,4]*

[1]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia; [2]Research Chair of Vaccines, Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia; [3]Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt and [4]Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt

Abstract

Hymenolepis nana, typically a parasite found in conventionally established mouse colonies, has zoonotic potential characterized by auto-infection and direct life cycle. The objective of this study was to determine the rate of parasite infection in laboratory mice. The hymenolepidide cestode infected 40% of the 50 mice sampled. The rate of infection in males (52%) was higher than in females (28%). Morphological studies on the cestode parasite showed that worms had a globular scolex with four suckers, a retractable rostellum with 20–30 hooks, and a short unsegmented neck. In addition, the remaining strobila consisted of immature, mature, and gravid proglottids, irregularly alternating genital pores, lobulated ovaries, postovarian vitelline glands, and uteri with up to 200 eggs in their gravid proglottids. The parasite taxonomy was confirmed by using molecular characterization based on the sequence analysis of the mitochondrial cytochrome *c* oxidase subunit 1 (mt*COX1*) gene. The parasite recovered was up to 80% identical to other species in GenBank. High blast scores and low divergence were noted between the isolated parasite and previously described *H. nana* (gb| AP017666.1). The phylogenetic analysis using the *COX1* sequence places this hymenolepidid species of the order Cyclophyllidea.

Key words: *Hymenolepis* species, laboratory animals, molecular analyses, morphological characterization, rodents

(Received 25 October 2019; revised 22 November 2019; accepted 6 February 2020)

Introduction

Laboratory animal models are widely used in biological experiments (Perec-Matysiak et al., 2006). The most common of them used in different research fields are rodents as mice and rats (Mehlhorn et al., 2005). They are a vital component of different ecosystems, acting as prey, or carriers of disease and reservoirs (Pakdel et al., 2013), and also known to harbor several ecto- and endoparasites; thus, posing a threat to human health (Mohd Zain et al., 2012). For many endoparasites, wild rodents act as definitive and/or intermediate hosts (Okoye & Obiezue, 2008). Parasitic eggs are dispersed in rodent droppings in agricultural fields, stored grains, and various edible commodities in houses, resulting in disease spread (Khatoon et al., 2004). The ability of rodents to act as vectors is significantly increased, owing to their physiological similarities with humans (Kataranovski et al., 2010). Increased rodent populations in an area could be directly linked to increased human zoonotic diseases (Stojcevic et al., 2004).

***Author for correspondence:** Rewaida Abdel-Gaber, E-mail: rewaida@sci.cu.edu.eg, eolayan@ksu.edu.sa

Cite this article: Al-Olayan E, Elamin M, Alshehri E, Aloufi A, Alanazi Z, Almayouf M, Bakr L, Abdel-Gaber R (2020) Morphological, Molecular, and Pathological Appraisal of *Hymenolepis nana* (Hymenolepididae) Infecting Laboratory Mice (*Mus musculus*). *Microsc Microanal.* doi:10.1017/S1431927620000161

Hymenolepididae Ariola, 1899 is a diverse family of cyclophyllidean tapeworms that infects approximately 620 bird species and 230 mammal species (Czaplinski & Vaucher, 1994). *Hymenolepis* Weinland, 1858) is a genus characterized by having an unarmed scolex and a rudimentary rostellar apparatus. It is mainly a parasite in rodents; a few species in bats and one in hedgehogs have been reported. Members of this genus have been reported in Africa, Asia, Palearctic, Nearctic, Ethiopia, and Oriental regions (Thompson, 2015). Rodents are the main definitive hosts of both *Hymenolepis nana* and *H. diminuta*, which are zoonotic and known as the dwarf and rat tapeworms, respectively (Steinmann et al., 2012). *H. nana* is the most common cestode infecting humans, whereas *H. diminuta* causes occasional human infections (Soares Magalhães et al., 2013). *H. nana* is the only cestode capable of completing the life cycle in the final host without the need for an intermediate host. Infection is most commonly acquired from eggs in an infected individual's feces, which spread by contaminated food (Smyth & McManus, 1989). Infections with *H. nana* in the primary stage are often asymptomatic. Nevertheless, as the disease progresses to the chronic stage, the host manifests symptoms as diarrhea, abdominal pain, nausea, and dizziness (Huda-Thaher, 2012). *H. nana* infections linked to low intestinal vitamin B12 absorption (Mohammad & Hegazi, 2007).

Hymenolepiasis diagnosis and causative species differentiation require the analysis of the eggs recovered from the host feces to

identify morphological characteristics (Nkouawa et al., 2016). Advanced molecular biology including techniques, such as polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), are simple and rapid methods for parasite identification (Perec-Matysiak et al., 2006; Robles & Navone, 2007). In particular, PCR-RFLP is commonly used to identify and classify helminth parasites accurately including cestodes (Francisco et al., 2010; Rokni et al., 2010; Mahami-Oskouei et al., 2011; Teodoro et al., 2011). However, the phylogenetic relationships of Hymenolepididae at the family and generic levels remain elusive (Czaplinski & Vaucher, 1994). *Hymenolepis* species' taxonomic status and systematics are problematic, primarily because of the presence of cryptic species (Haukisalmi et al., 2010). The current, nuclear rDNA internal transcribed spacer (rDNA-ITS1 and ITS2) sequence data are considered to have the revolutionized phylogenetic analysis as a powerful tool for resolving remarkable taxonomic issues and discriminating closely related genera and species (Coleman, 2003). In addition, the use of rDNA-ITS2 to predict secondary structures from primary sequence data may provide additional information for species identification at a higher taxonomic level (Schultz et al., 2005; Ghatani et al., 2012). The mitochondrial cytochrome *c* oxidase subunit 1 (mtCOX1) marker has also been used successfully at family and genus levels to infer and establish phylogenetic Cyclophyllidea relationships (Sharma et al., 2016).

In this study, natural prevalence and morphological as well as molecular characteristics of the partial mt*COX1* genes of *H. nana* species infecting laboratory mice (*Mus musculus*) were evaluated to determine the exact taxonomic and phylogenetic position of this parasite species. In addition, the study examined the impact of sex differences on the prevalence of parasite infection and the role of laboratory mice as reservoirs of hymenolepidid tapeworms.

Materials and Methods

Experimental Animal Collection

A total of 50 adult male and female laboratory mice *Mus musculus* (family: Muridae) were randomly selected from the Laboratory of Animal Breeding Council (King Saud University of Medical Science, Riyadh, Saudi Arabia). They were housed under controlled temperature (24 ± 2°C), light (12 h light/dark cycle), and relative humidity (40–70%) in a room. A standard diet and water *ad libitum* were given to them. The mice were anesthetized and killed by placing them in a small container with ether in accordance with the ethical standards for handling of experimental animals recommended by the King Saud University Ethics Committee, Riyadh, Saudi Arabia. The animals were tested for any external signs of infection. After dissection, the internal organs were removed and examined for worm infections.

Parasitological Examination

Light Microscopic Studies

The recovered cestode parasites were placed in saline solution, fixed in warm alcohol–formalin–acetic acid solution, preserved in 70% alcohol, stained with Semichon's acetocarmine, dehydrated in ascending grades of alcohol, cleared in clove oil, and then mounted in Canada balsam. With the aid of Yamaguti's identification key (1959), the worms were identified. Parasite prevalence was calculated according to the formula of Bush et al. (1997). Adult specimens were examined and photographed using a microscope Leica DM 2500 (NIS ELEMENTS software, v. 3.8). Measurements are recorded in millimeters and shown as the range followed by mean ± standard deviation in parentheses.

Scanning Electron Microscopic Studies

Specimens were fixed in 3% glutaraldehyde, washed with a buffer of sodium cacodylate, dehydrated in a graded ethanol series, and infiltrated with amyl acetate. They were then passed through an ascending series of Genesolv D, processed in a critical point dryer (LEICA EM CPD300) with Freon 13, and then coated with gold–palladium using an auto-fine coater (JEOL, JEC-3000FC). The samples were then analyzed and photographed at 10 kV in a JEOL scanning electron microscope (JSM-6060LV) at the Central Laboratory, King Saud University, Riyadh, Saudi Arabia.

Histopathological Examination

The mouse intestines were collected and fixed for 24 h in 10% neutral formalin immediately after mice sacrifice, and paraffin blocks were generated and routinely processed for light microscopy. The resulting sections of 4–5 μm were stained with hematoxylin and eosin and then visualized to evaluate pathological changes using a microscope Leica DM 2500 (NIS ELEMENTS software, v. 3.8).

Molecular Analyses

Genomic DNA was extracted using a QIAamp DNA mini Kit (Qiagen, Venlo, Netherlands) from ethanol-preserved samples as recommended by the manufacturer. A partial gene region of mt*COX1* was amplified using primers designed by Nkouawa et al. (2016), including Hym-cox1F (5′-GTT ACT AAT CAT GGT ATT ATT ATG-3′) and Hym-cox1R (5′-CCA AAA TAA TGC ATA GGA AAA-3′). Amplicons were sequenced using a 310 automated DNA sequencer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) with the help of an ABI Prism Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA). A BLAST search was performed to identify related sequences from the NCBI database. The mt*COX1* gene sequences were aligned using the CLUSTAL-X multiple sequence alignment (Thompson et al., 1997). A phylogenetic tree with maximum parsimony [neighbor-interchange (CNI) level 3, random addition trees = 100] was built using MEGA v. 6.0. The bootstrap analysis was conducted to determine the robustness of the tree topologies based on 1,000 replicates.

Results

Of the 50 mice hosts, 20 (40%) were infected naturally. The rate of infection in males (52%; 13/25) was higher than that in females (28%; 7/25). A total of 243 specimens of Hymenolepididae species were recovered from the laboratory mice's small intestines.

Microscopic Examination

The strobila length was 3.74 ± 0.1 (2.53–5.70) mm, with a maximum width at pregravid or gravid proglottids, 0.094 ± 0.02 (0.016–0.270) mm (Figs. 1, 2, Table 1). There were distinct metamerisms, craspedote, serrate margins, and proglottids which were wider than long. The scolex globular length was 0.125 ± 0.01 (0.113–

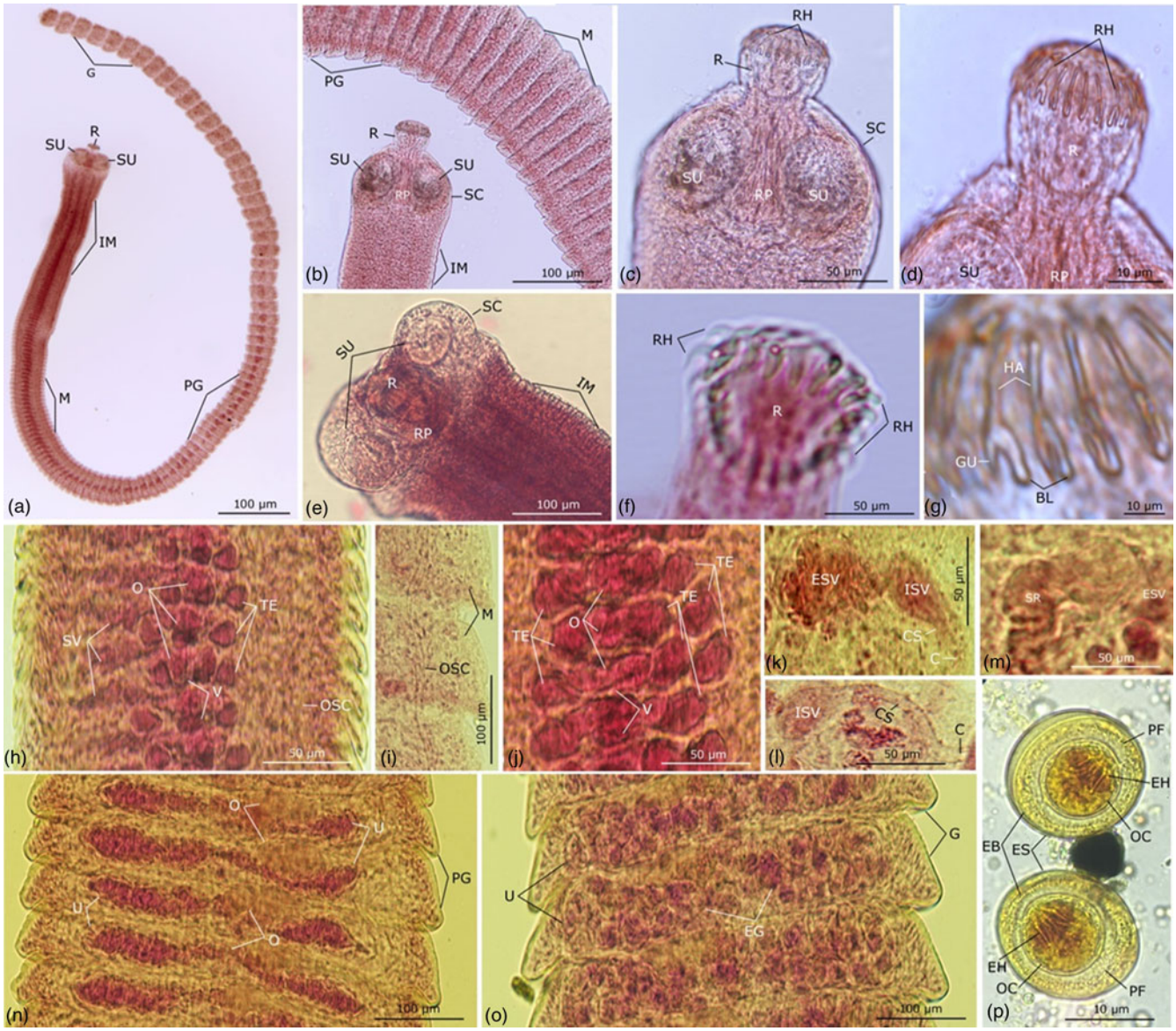

Fig. 1. (**a–p**) Photomicrographs of the adult *H. nana* worm with Semichon's acetocarmine. (**a**) An adult worm with scolex (SC) equipped with suckers (SU) and rostellum (R), armed with numerous hooks (RH), followed by immature (IM), mature (M), and pregravid (PG) proglottids. (**b–g**) High magnification image of the scolex (SC) showing: (**b–d**) a protracted hooked rostellum (R) and rostellar pouch (RP) as well as (**e**) retracted hooked rostellum (R) and a rostellar pouch (RP). (**f**) Rostellum (R) armed with one row of rostellar hooks (RH). (**g**) Rostellar hooks (RH) with handle (HA), guard (GU), and blade (BL). (**h**) Mature proglottids (M) showing testes (TE), ovaries (O), vitelline gland (V), seminal vesicle (SV), and osmoregulatory canals (OSC). (**i–p**) High magnification image of mature proglottids (M) showing: (**i**) osmoregulatory canals (OSC). (**j**) A single set of genitalia in each proglottid consisted of testes (TE) arranged in a transverse row, one poral and two aporal; an ovary (O), and a vitelline gland (V). (**k**) External seminal vesicle (ESV) situated at the anterior end of proglottids, followed by an internal seminal vesicle (ISV), cirrus sac (CS), and cirrus (C). (**l**) Internal seminal vesicle (ISV) followed by cirrus sac (CS) and cirrus (C). (**m**) Seminal receptacle (SR) followed by an external seminal vesicle (ESV). (**n**) Pregravid proglottids (PG) containing ovaries (O), and the uterus (U) filled with eggs (EG). (**o**) Gravid proglottids (G) with uterus (U) completely filled with numerous eggs (EG). (**p**) Eggs (EG) covered with egg shell (ES) enclosing embryophore (EB) with three polar filaments (PF) and oncosphere (OC) with three pairs of embryonic hooks (EH).

0.164) mm, with a maximum width of 0.287 ± 0.01 (0.221–0.295) mm at four suckers and a retractable rostellum. The rostellum, located at the scolex center, 0.027 ± 0.001 (0.022–0.047) mm in length by 0.065 ± 0.001 (0.047–0.081) mm in width, had an irregular surface without microtriches, armed with 20–30 hooks, which were also retractable into a contractile rostellar pouch measuring 0.099 ± 0.002 (0.089–0.125) mm in length by 0.091 ± 0.01 (0.083–0.137) mm in width. The diameter of each hooklet was 0.35 ± 0.01 (0.29–0.42) mm. Suckers were rounded or oval in shape, unarmed, and 0.095 ± 0.002 (0.081–0.167) mm in length by 0.084 ± 0.002 (0.062–0.104) mm in width. The scolex was approximately 0.157 ± 0.01 (0.156–0.234) mm in diameter, followed by a short unsegmented neck region.

There were two pairs of longitudinal canals in the excretory system. Each pair was 0.098–0.127 mm from the lateral proglottide margins. Transverse anastomoses connected the ventral osmoregulatory canals, while the dorsal ones moved bilaterally to the lateral proglottid margins in relation to the ventral canals. Proglottid development was progressive and protandrous, with external segmentation being evident at the premature strobila section.

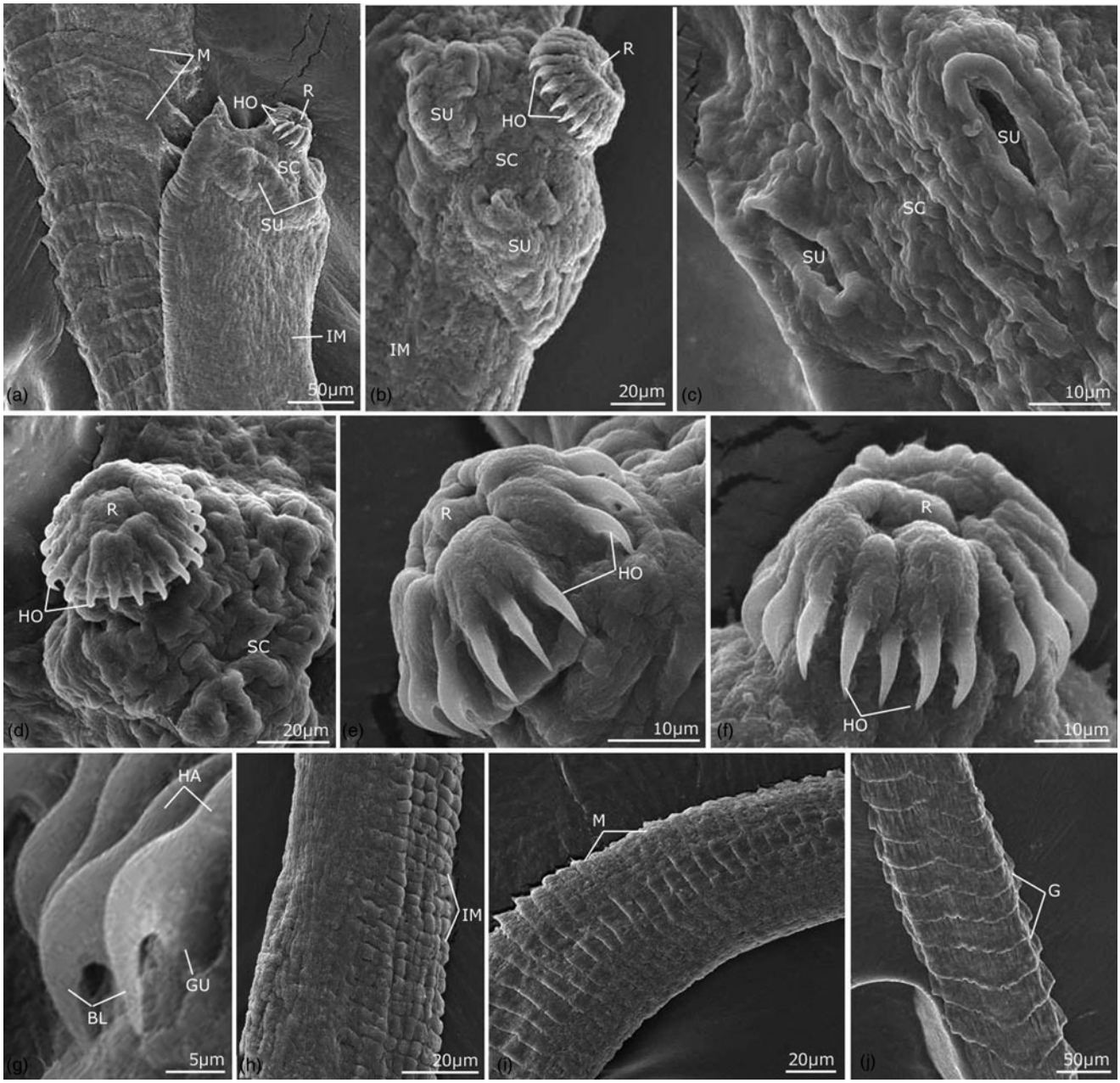

Fig. 2. (**a–j**) Scanning electron micrographs of *H. nana* infecting *M. musculus* showing: (**a**) an adult worm with scolex (SC) equipped with suckers (SU) and hooked rostellum (R), followed by immature (IM) and mature (M) proglottids. (**b–g**) High magnification images of: (**b**) scolex (SC) provided with suckers (SU) and rostellum (R) armed with rostellar hooks (RH), followed by immature (IM) proglottids. (**c**) Scolex (SC) equipped with suckers (SU). (**d–f**) Scolex (SC) provided with hooked rostellum (R) in one row. (**g**) Rostellar hooks (RH) consist of handle (HA), blade (BL), and guard (GU). (**h**) Immature proglottids (IM). (**i**) Mature proglottids (M). (**j**) Gravid proglottids (G).

Mature proglottids had a length of 0.104 ± 0.05 (0.089–0.157) mm by 0.402 ± 0.09 (0.395–0.563) mm in width. Genital pores were unilateral, irregularly alternating, and slightly located anterior to the middle of each proglottid. The genital ducts passed dorsally to the longitudinal osmoregulatory canals, both ventral and dorsal.

Three sub-spherical testes arranged in a transverse row, one poral and two aporal, but not in contact with the longitudinal excretory canals, and 0.068 ± 0.002 (0.047–0.098) mm in length by 0.071 ± 0.001 (0.066–0.102) mm in width. The vas deferens expanded to form an external seminal vesicle of 0.109 ± 0.02 (0.089–0.174) mm in length by 0.062 ± 0.003 (0.045–0.088) mm in width. The cylindrical cirrus sac was 0.054 ± 0.001 (0.042–0.087) mm in length by 0.143 ± 0.04 (0.091–0.280) mm in width and did not extend beyond the longitudinal excretory canals. The internal seminal vesicle was 0.040 ± 0.001 (0.031–0.078) mm in length by 0.086 ± 0.003 (0.068–0.165) mm in width and occupied almost the entire cirrus sac. The slightly elongated external seminal vesicle, 0.073 ± 0.001 (0.060–0.079) mm in length by 0.039 ± 0.001 (0.031–0.042) mm in width, was located at the anterior half of the proglottids.

Initially, after the cirrus sac, the vagina gradually expanded into the voluminous seminal receptacle, measuring 0.201 ± 0.03 (0.185–0.298) mm in length by 0.017 ± 0.001 (0.010–0.020) mm

Table 1. Main morphological features and measurements of *H. nana* compared with those in previous studies.

	Parameters											
			Measurements for different body parts									
Related species	Host species (locality)	Body size	Scolex	Suckers	Rostellum	No. of rostellar hooks	Testes	Ovary	Vitellarium	Eggs	Embryophore	Embryonic hook
H. asymmetrica Janicki (1904)	*Microtus arvalis* (Laposak)	5.0–13.5 × 0.2–0.4	0.220–0.300	0.085–0.120	–	18–21	–	0.200–0.400	–	0.045–0.067	0.020–0.030	0.0085–0.009
H. microstoma Joyeux & Kobozieff (1928)	*Mus musculus* (South-Oran)	3.50 × 0.20	0.200	–	0.100	27	0.120 × 0.170	–	–	0.080 × 0.090	0.030	0.017 × 0.020
H. christensoni Macy (1931)	*Myotis yumanensis* (Alaska)	5.4–6.5 × 0.295–0.323	0.340–0.434	0.104–0.116	0.100	40	0.125 (0.104–0.144) × 0.105 (0.080–0.120)	0.247 (0.060–0.116) × 0.092 (0.220–0.288)	0.099 (0.084–0.132) × 0.069 (0.056–0.084)	0.038 (0.035–0.042) × 0.034 (0.030–0.037)	0.025–0.032 × 0.020–0.025	0.013–0.015
H. citelli McLeod (1933)	*Citellus tridecemlineatus* (Canada)	15 × 0.28	–	0.113 × 0.245	0.038	Unarmed	–	–	–	0.078–0.086 × 0.059–0.065	–	–
H. nagatyi Hilmy (1936)	*Crocidura occidentalis* (Rutshuru)	2.5 × 0.810	0.467–0.548	0.114–0.125	0.195–0.225	100–110	–	–	–	0.040–0.043	0.023	–
H. roudabushi Macy & Rausch (1946)	*Eptesicus fuscus* (Iowa)	3.9–7.4 × 0.270–0.488	0.240–0.325	0.084–0.096	0.120	45	0.138 (0.092–0.140) × 0.122 (0.112–0.164)	0.094 (0.080–0.120) × 0.246 (0.180–0.328)	0.056 (0.036–0.072) × 0.085 (0.068–0.120)	0.041 (0.035–0.045) × 0.036 (0.032–0.045)	0.025–0.030 × 0.022–0.30	0.015–0.020
H. gertschi Macy (1947)	*Eptesicus fuscus* (Iowa)	6.1 × 0.9	0.400	–	0.031–0.035 × 0.096–0.108	50	0.084 (0.072–0.100) × 0.076 (0.06–0.088)	0.099 (0.072–0.125) × 0.163 (0.145–0.180)	0.067 (0.055–0.092) × 0.090 (0.075–0.117)	0.052–0.62 (0.055)	0.025–0.020	0.012–0.015
H. diminuta Wardle & McLeod (1952)	*Ratas ratones* (Cosmopolita)	2.00–3.00 × 0.30–0.40	0.299–0.300	0.10–0.120	Rudiment	Unarmed	–	–	–	0.060–0.070	–	–
H. lasionycteridis Rausch (1975)	*Lasionycteris noctivagans* (Ohio)	6.1 × 0.615–0.734	0.168–0.220	0.070–0.084	0.130	38–40	0.091 (0.068–0.108) × 0.129 (0.100–0.176)	0.080 (0.064–0.092) × 0.328 (0.240–0.468)	0.044 (0.036–0.052) × 0.139 (0.124–0.160)	0.044 (0.037–0.048) × 0.034 (0.030–0.037)	0.025–0.032	0.013–0.015
H. geomydis Gardner & Schmidt (1987)	*Geomys bursarius* (Colorado)	72.26–168.41 × 0.198–0.330	0.189–0.252 × 0.194–0.245	0.092–0.124 × 0.065–0.094	Rudiment	Unarmed	–	–	–	0.060–0.070	–	–
H. rhinopomae Sawada & Mohammad (1989)	*Rhinopoma microphyllum* (Iraq)	2.6–2.9 × 0.11–0.12	0.280–0.385 × 0.190–0.210	0.098–0.112 × 0.091–0.105	0.119–0.140 × 0.063–0.084	Unarmed	0.056–0.70 × 0.028–0.042	0.077	0.049–0.063 × 0.028–0.035	0.049–0.063 × 0.028–0.035	0.032–0.046	0.018
H. dymecodontis Sawada & Harada (1990)	*Dymecodon pilirostris* (Japan)	5.6–7.8 × 0.19–0.21	0.245–0.280 × 0.231–0.315	0.105–0.119 × 0.119–0.154	0.140 × 0.056–0.077	Unarmed	0.091–0.098 × 0.063–0.070	0.280–0.315	0.105–0.126 × 0.077–0.084	0.064–0.077 × 0.063–0.070	0.039 × 0.035	0.014

(*Continued*)

Table 1. (*Continued.*)

	Parameters											
			Measurements for different body parts									
Related species	Host species (locality)	Body size	Scolex	Suckers	Rostellum	No. of rostellar hooks	Testes	Ovary	Vitellarium	Eggs	Embryophore	Embryonic hook
H. magna Makarikova et al. (2010)	*Rhinolophus affinis* (China)	4.9 (4.3–5.0) × 0.23 (0.22–0.34)	0.330 (0.310–0.330)	0.120 (0.116–0.130) × 0.140 (0.132–0.143)	Absent	Unarmed	0.153–0.176 × 0.116–0.163	0.109–0.120 × 0.068–0.087	–	0.040–0.41 × 0.041–0.042	0.022–0.028 × 0.025–0.30	0.014–0.015
H. microstoma Cummingham and Olson (2010)	*Mus musculus* (London)	4.7 (2.5–8.1) × 0.065 (0.029–0.108)	0.138 (0.116–0.157) × 0.232 (0.204–0.284)	0.102 (0.079–0.129) × 0.096 (0.076–0.113)	0.038 (0.026–0.052) × 0.071 (0.051–0.075)	25 (22–26)	0.072 (0.051–0.114) × 0.078 (0.061–0.115)	0.063 (0.034–0.103) × 0.234 (0.130–0.360)	0.041 (0.038–0.094) × 0.056 (0.032–0.068)	–	–	–
H. asketus Brooks & Mayes (2011)	*Blarina brevicauda* (Nebraska)	1.2–2.0 × –	0.136–0.142	0.052–0.087 × 0.035–0.052	0.040–0.075 × 0.029–0.061	10	0.015–0.058 × 0.023–0.052	–	–	–	–	–
H. bicauda Makarikov et al. (2013)	*Apomys microdon* (Philippines)	2.6–2.9 × 0.99–1.19	0.260–0.288	0.092–0.103 × 0.080–0.095	–	Unarmed	0.070–0.103 × 0.065–0.100	0.108–0.140	0.038–0.055 × 0.050–0.065	0.046–0.054 × 0.050–0.060	0.027–0.033 × 0.031–0.038	0.0175–0.019
H. haukisalmii Makarikov et al. (2013)	*Bullimus luzonicus* (Philippines)	13.2 × 0.24	0.240–0.265	0.083–0.105 × 0.081–0.093	–	Unarmed	0.116–0.160 × 0.085–0.157	0.193–0.208	0.061–0.083 × 0.080–0.125	0.029–0.034 × 0.037–0.046	0.015–0.017 × 0.018–0.020	0.011–0.013
H. alterna Makarikov et al. (2013)	*Rattus everetti* (Philippines)	16.5–17.0 × 0.29–0.38	0.380–0.410	0.154–0.189 × 0.130–0.144	–	Unarmed	0.072–0.111 × 0.065–0.091	0.506–0.525	0.090–0.165 × 0.125–0.205	0.048–0.051 × 0.049–0.053	0.023–0.026 × 0.025–0.027	0.0123–0.014
H. bilaterala Makarikov et al. (2013)	*Apomys datae* (Philippines)	8.6 × 0.15–0.25	0.347–0.400	0.110–0.150 × 0.105–0.120	–	Unarmed	0.092–0.126 × 0.075–0.106	0.190–0.230	0.070–0.085 × 0.080–0.115	0.067–0.090 × 0.071–0.103	0.035–0.045 × 0.037–0.048	0.017–0.0191
H. diminuta Panti-May et al. (2018)	*Rattus everetti* (Xkalakdzonot)	–	0.146 × 0.217	0.090–0.097 × 0.078–0.090	–	Unarmed	–	–	–	0.058–0.073 × 0.045–0.063	–	–
H. nana Present study	*Mus musculus* (Saudi Arabia)	3.74 ± 0.1 (2.53–5.70) × 0.094 ± 0.01 (0.016–0.270)	0.125 ± 0.01 (0.113–0.164) × 0.287 ± 0.01 (0.221–0.295)	0.095 ± 0.001 (0.081–0.167) × 0.084 ± 0.001 (0.062–0.104)	0.027 ± 0.001 (0.022–0.047) × 0.065 ± 0.001 (0.047–0.081)	25 (20–30)	0.068 ± 0.001 (0.047–0.098) × 0.071 ± 0.001 (0.066–0.102)	0.061 ± 0.001 (0.029–0.112) × 0.214 ± 0.01 (0.147–0.302)	0.028 ± 0.001 (0.021–0.088) × 0.051 ± 0.001 (0.030–0.063)	0.049 ± 0.01 (0.042–0.052) × 0.050 ± 0.001 (0.047–0.053)	0.028 ± 0.001 (0.023–0.030) × 0.032 ± 0.001 (0.029–0.037)	0.012 ± 0.001 (0.010–0.014) × 0.016 ± 0.001 (0.015–0.017)

in width. The ovaries were lobulated, 0.061 ± 0.002 (0.029–0.112) mm in length by 0.214 ± 0.01 (0.147–0.302) mm in width. The vitelline gland compact, measuring 0.028 ± 0.001 (0.021–0.088) mm in length by 0.051 ± 0.001 (0.030–0.063) mm in width, was situated posterior to the ovaries. The average length of the seminal receptacle was 0.047 ± 0.001 (0.035–0.087) mm in length by 0.090 ± 0.002 (0.069–0.170) mm in width. The uterus formed as a transversely elongated perforated sac, located dorsally to other organs, and extending laterally beyond the longitudinal osmoregulatory canals. The uterus formed numerous diverticula on the dorsal and ventral sides during the proglottid development. Testes have been shown to persist in mature proglottids, while in gravid proglottids, the cirrus sac and vagina persist. Gravid proglottids measured 0.154 ± 0.04 (0.123–0.243) mm in length by 0.975 ± 0.16 (0.854–1.021) mm in width. A full developed uterus which occupied the entire midpoint and expanded laterally beyond the longitudinal osmoregulatory canals was saccate and had several ventral and dorsal diverticula; the lateral sides of a gravid uterus were usually not perforated. There were many (up to 200) small eggs in the uterus.

Eggs, 0.049 ± 0.01 (0.042–0.052) mm in length and 0.050 ± 0.001 (0.047–0.053) mm in width, were oval or spherical with a thin hyaline shell. The shell enclosed the embryophore, approximately 0.028 ± 0.001 (0.023–0.030) mm in length by 0.032 ± 0.001 (0.029–0.037) mm in width, with three polar filaments, oncospheres 0.013 ± 0.001 (0.011–0.014) mm in length by 0.040 ± 0.001 (0.032–0.075) mm in width, and three pairs of embryonic hooks arranged in parallel. The size of each embryonic hook was 0.012 ± 0.001 (0.010–0.014) mm in length by 0.016 ± 0.001 (0.015–0.017) mm in width.

Developmental Biology of Cysticercoid and Adult H. nana in Mice

During the infection, the infected mice remained asymptomatic (Figs. 3, 4). After *H. nana* eggs were ingested, infection occurred. Oncospheral embryos hatched in fully developed egg shells, emerged from their shells, and penetrated the liver tissue of mice (day 0), where they developed into cysticercoids within 4 days (day 4), through a sequence of developmental stages during which suckers took their final shape and filled the rostrum with hooks. The suckers appeared initially as somewhat refractive rods. Eventually, in the rest of the scolex, the fully developed rostrum was invaginated. The evaginated juveniles were pushed out into the intestinal lumen after eosinophilic infiltration and possible physical pressure (days 4–5), where they matured into adult worms within 7 days (day 12).

Taxonomic Summary

Parasite name: *Hymenolepis nana* (Family Hymenolepididae Ariola, 1899)

Host: Laboratory mice *Mus musculus* Linnaeus, 1758 (Family: Muridae Illiger, 1811)

Site of infection: Small intestine of infected mice

Type-locality: Laboratory of Animal Breeding Council of Medical Science, King Saud University, Riyadh, Saudi Arabia

Prevalence and intensity: 20 out of 50 (40%) specimens were infected, with a total number of 243 cestodes

Remarks: The recovered cestode parasite was compared with those collected from different regions: it most closely resembled *H. nana* previously described by Lapage (1951), Roberts & Janovy (2000), Schantz (2006), Richard (2008), Schmidt et al. (2009), Sadaf et al. (2013), and Kim et al. (2014), since all of them had generic characteristics, except that reported by Mayhew (1925), which indicated that the testes were a compound structure. The parasite is also substantially similar to *H. roudabushi* due to the serrate strobila margins as described by Macy & Rausch (1946). It is also similar to *H. microstoma* described by Joyeux & Kobozieff (1928) because it inhabits the same host species, has the same testes arrangement, as well as lobulated ovaries with an irregular microtrichevoid surface, differing only in the number of rostellar hooks [20–30 versus 25 (22–26)].

Molecular Analyses

A total of 699 bp of the mt*COX1* gene sequence were deposited in GenBank for the cestode parasite being studied (gb| MK133141.1), and the GC content was determined (32%). A unique genetic sequence has been discovered by comparisons of the genomic sequence of the isolated parasite species with different alternative group species and genotypes. The comparison between this novel genetic sequence and others obtained from GenBank showed up to 80% similarity (Table 2). Comparing the nucleotide sequences and divergence, the *COX1* gene sequence of this cestode species showed the highest BLAST scores with the lowest divergence values for *H. nana* (gb| AP017666.1, GU433104.1, KT951722.1, LC063187.1, AB033412.1, GU433103.1), *H. diminuta* (gb| GU433102.1, AF314223.1, AP017664.1, KC990401.1), and *H. microstoma* (gb| AP017665.1) (Fig. 5). Analyses involved 29 nucleotide sequences (Fig. 6). In order to estimate the maximum likelihood values, a topology for the tree was automatically computed. The constructed dendrogram revealed two orders in the Eucestoda subclass; Pseudophyllidea and Cyclophyllidea, with the former represented by *Spirometra erinaceieuropaei* (gb| AB015754.1) in the Diphyllobothriidae family, with 80% sequence similarity. Four families represent the latter: Taeniidae, Anoplocephalidae, Mesocestoididae, and Hymenolepididae. Such analyses showed that Taeniidae formed the sister group with strong nodal support for Hymenolepididae. In addition, a clade, including paraphyletic Hymenolepididae, was recovered from the current analysis. Our phylogenetic analysis, which combined new and existing data, showed that the hymenolepidid species analyzed was within the Cyclophyllidea order. It has been found that the present species is deeply embedded in the genus *Hymenolepis*, with close relationships to the previously described *H. nana* (gb| AP017666.1), as a putative sister taxon.

DISCUSSION

Parasitic laboratory animal infections, even in the absence of clinical signs, can act as an important variable during experimental testing and can potentially infect staff and researchers (Rehbinder et al., 1996; Rosas, 1997; Gonçalves et al., 1998; Bazzano et al., 2002). During experiments, rodent colonies are often contaminated with helminths in conventional animal facilities or may become infected in research laboratories (Rehbinder et al., 1996).

In this study, adult *Hymenolepis* species were recovered from the small intestine of 40% of the laboratory mice, representing a moderate prevalence value. This rate is considerably similar to that reported in the Belgrade area of Serbia (37%; Kataranovski et al., 2010) and the Tarai region of Uttarakhand (40–44%; Sharma et al., 2013). Conversely, it is higher than that reported in Korea (5%), the Philippines (19%; Fedorko, 1999), Grenada

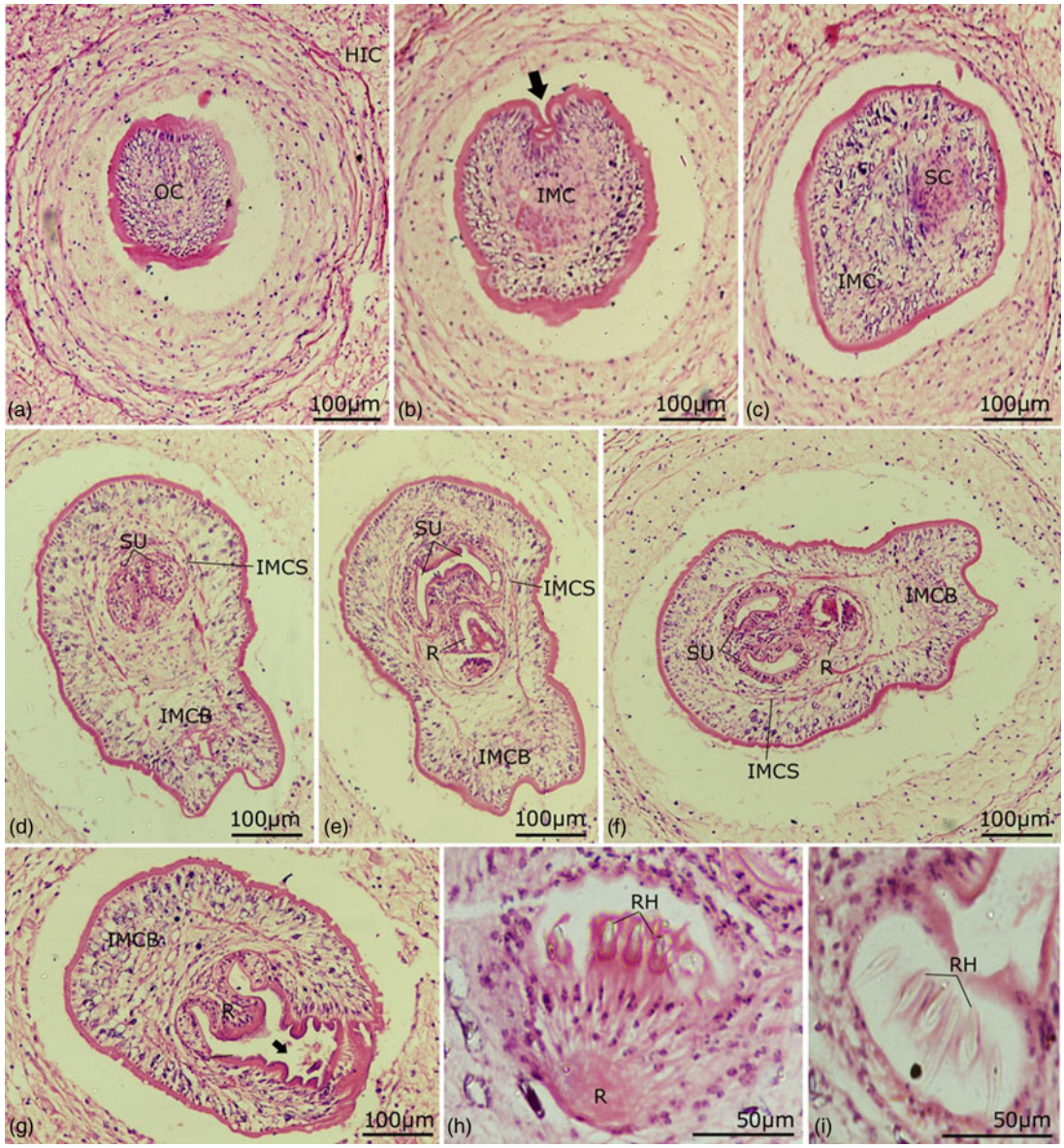

Fig. 3. (**a–i**) Photomicrographs of eosinophil infiltration surrounding a cysticercoid of *H. nana* developed in the intestinal villus of *M. musculus*. (**a**) Oncosphere (OC) escapes from the egg shell and penetrates into intestinal cells. (**b**) Immature cysticercoid (IMC) with clear demarcation of its anterior pole (arrow). (**c**) The beginning of the formation of a scolex (SC) in the immature stage of cysticercoid (IMC). (**d–g**) Sucker (SU) formation in the scolex of an immature cysticercoid (IMCS) with elongation of the cysticercoid body (IMCB) and the appearance of rostellum (R) in (**e,f**), as well as the direction of rostellum evagination (arrow). (**h,i**) High magnification image of the scolex showing: (**h**) Rostellum (R) armed with rostellar hooks (RH). (**i**) Rostellar hooks (RH).

(16%; Coomansingh et al., 2009), Dhaka (27%; Gofur et al., 2010), Addis Ababa (7–27%; Gudissa et al., 2011), the Tabriz of Iran (4%; Garedaghi & Khaki, 2014), the suburban area of Hamadan City of Western Iran (17%; Yoisefi et al., 2014), Italy (29%; D'Ovidio et al., 2015), and the Heilongjiang Province of China (6–15%; Yang et al., 2017). It is also lower than the infection rate reported in Manila (64%; Tubangui, 1931), Japan (53%; Perec-Matysiak et al., 2006), Shiraz of Southern Iran (67%; Tanideh et al., 2010), the City of Aracaju in the Sergipe State of Brazil (67%; Guimarães et al., 2014), and Ahvaz of South-West Iran (63%; Rahdar et al., 2017).

In this study, the prevalence of cestode infections in males was higher than in females, in line with the findings of Folstad & Karter (1992) and Gofur et al. (2010), whom indicated that the typical male bias in parasitism could be due to the evolutionary mechanisms of endocrinological and behavioral sex differences produced to maximize the reproductive production of each sex. Klein (2004) showed that sex differences in the parasite burden are usually attributed to pleiotropic effects of the steroid hormone, especially the possible immunosuppression associated with increased testosterone levels. Hayward (2013) and Hämäläinen et al. (2015) reported that the sexual bias in infection rates could be attributed to different behavioral repertoires, which in turn could be mediated by hormonal conditions. By contrast, Bhuiyan et al. (1996) reported in the following three rodent's species: *Bandicota bengalensis*, *Rattus rattus*, and *M. musculus* that the prevalence of infection in females was higher than that in males.

Morphological variability associated with cestode taxonomy has received comparatively little attention. Although several

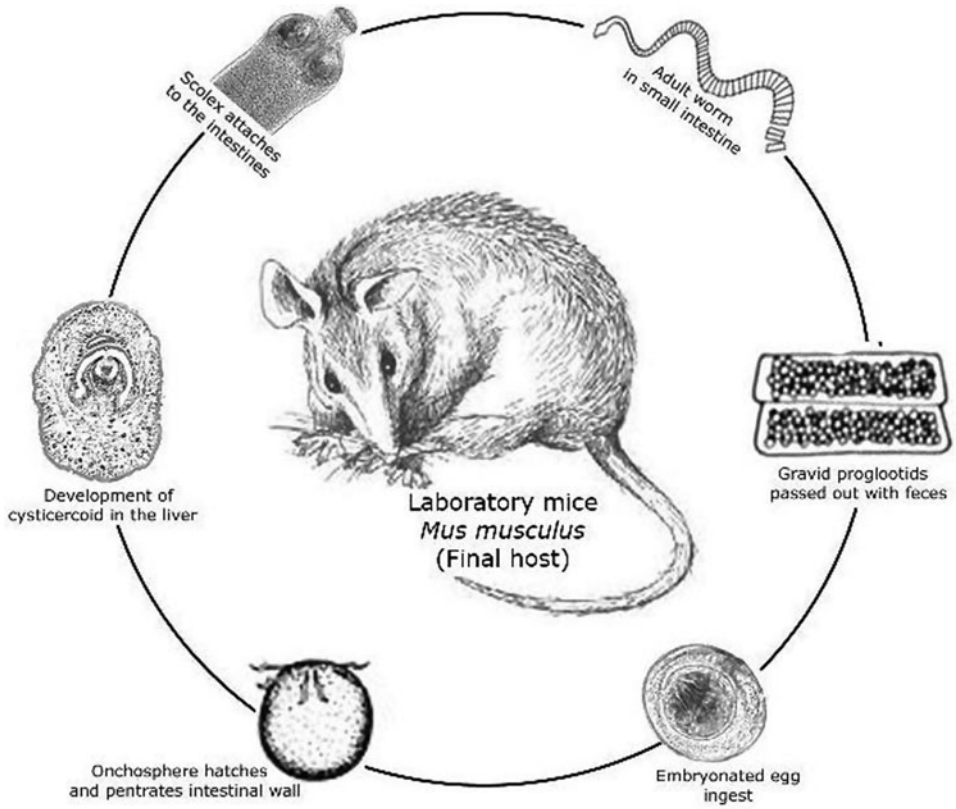

Fig. 4. Direct life cycle of *H. nana* inside the laboratory mouse *Mus musculus* (by own production).

studies have identified variants of character by Mayhew (1925),), and Schiller (1959), few researchers have attempted to determine their rate of occurrence in the host species. The study of the Hymenolepididae family members was carried out using samples from naturally infected hosts. Of the several variant characters known in hymenolepidid cestodes, (i) the scolex shape and size, (ii) sucker shape and size, (iii) number, shape, size, and location of rostellar hooks, (iv) number, size, shape, and relative position of the testes, (v) irregularities in the number and branching of the vasa efferentia, (vi) ovary and vitellarium shape, (vii) position of the genital pores, and (viii) the size of egg structures constitute important taxonomic characters in this family and have thus been the most satisfactorily described.

The present *Hymenolepis* species can be separated from all comparable species by its smaller scolex size. However, it differs from other hymenolepidid species, owing to its rudimentary rostellum as that in *H. citelli* and *H. diminuta* (Wardle & McLeod, 1952), *H. geomydis* (Gardner & Schmidt, 1987), *H. rhinopomae* (Sawada & Mohammad, 1989), *H. dymecodontis,* and *H. magna* (Makarikova et al., 2010), *H. bicauda, H. haukisalmii, H. alterna,* and *H. bilaterala*. It also differs from other hymenolepidid species, owing to its 20–30 rostellar hooks, as opposed to 18–21 in *H. asymmetrica* (Janicki, 1904), 40 in *H. christensoni* (Macy, 1931), 100–110 in *H. nagatyi* (Hilmy, 1936), 45 in *H. roudabushi*, 50 in *H. gertschi* (Macy, 1947), and 38–40 in *H. lasionycteridis* (Rausch, 1975). It differs from *H. christensoni*, owing to its serrate strobila margins, and from *H. asketus* (Brooks & Mayes, 2011) because of its 20–30 rather than 10 rostellar hooks, which are longer (0.089–0.125 versus 0.0013–0.0018), its linear arrangement of testes, and lobulated, as opposed to dumbbell-shaped ovaries. Our *Hymenolepis* species also differs from *H. asymmetrica*, *H. christensoni*, *H. citelli*, *H. gertschi*, *H. lasionycteridis*, *H. geomydis*, *H. dymecodontis*, *H. magna*, *H. microstoma*, *H. haukisalmii*, *H. alterna*, and *H. bilaterala*, owing to its smaller strobila size and the shape of its lobate ovary. Its egg sizes are also smaller, compared to those of *H. citelli* (McLeod, 1933), *H. gertschi* (Macy, 1947), *H. dymecodontis* (Sawada & Harada, 1990), and *H. bilaterala*.

The immuno- and developmental biology of *H. nana* are unique in mice, due to the parasite's ability to complete its entire life cycle; from the egg to the adult worm (direct life cycle) in a single mouse (Ito, 2015). It occurs when *H. nana* eggs are ingested by mice. Ito (2003) and Okamoto (2003) reported that this cycle can only occur once, as egg-infected mice develop resistance to reinfection within a few days. Okamoto (2003) reported this acquired immunity to be thymus dependent. In addition, a single oncosphere invasion of the intestinal tissue is enough to elicit rapid immunity from egg reinfection (Baylis, 1924; Joyeux & Kobozieff, 1928; Ito & Yamamoto, 1976). The subsequent development and maturation of parasites depend on eosinophilic infiltration and physical pressure that pushes the invaginated juveniles into the intestinal lumen for adult worms to fully mature; this is consistent with the findings of Moniez (1880), Nicoll & Minchin (1910), and Niwa et al. (1998).

Based on their morphology, transmission patterns, or pathological effects on hosts, intestinal cestode parasites are frequently identified and distinguished. These criteria, however, are often insufficient to identify specifically (Lichtenfels et al., 1997; Andrews & Chilton, 1999). Increased knowledge of the genus *Hymenolepis* has contributed to the possibility of using molecular approaches for cestode identification. The mt*COX1* gene was useful in the identification of genetic differences in *H. nana* that

Table 2. Cestoda species used in the phylogenetic analysis of *H. nana* specimens obtained from this study.

Parasite species	Order/family	Host/host group (origin)	Accession no.	GC content (%)	Percent identity (%)
Echinococcus granulosus	Cyclophyllidea/Taeniidae	Wild animals (Iran)	KX269862.1	36.2	81.0
Spirometra erinaceieuropaei	Diphyllobothriidea/ Diphyllobothriidae	*Homo sapiens* (Japan)	AB015754.1	34.2	80.0
Moniezia benedeni	Cyclophyllidea/ Anoplocephalidae	Cattle intestine (Iraq)	MH259797.1	33.9	80.0
Moniezia expansa	Cyclophyllidea/ Anoplocephalidae	Sheep Intestine (Iraq)	MH259795.1	33.2	80.0
Paranoplocephala kalelai	Cyclophyllidea/ Anoplocephalidae	*Microtus voles* (Europe)	AY189959.1	32.6	81.0
Paranoplocephala omphalodes	Cyclophyllidea/ Anoplocephalidae	*Microtus voles* (Europe)	AY189954.1	36.7	81.0
Paranoplocephala sp.	Cyclophyllidea/ Anoplocephalidae	*Microtus voles* (Europe)	AY181537.1	36.5	81.0
Mesocestoides litteratus	Cyclophyllidea/ Mesocestoididae	*Vulpes vulpes* (Central Europe)	JF268525.1	30.3	80.0
Anoplocephala manubriata	Cyclophyllidea/ Anoplocephalidae	Wild elephant (Sri Lankan)	KU903287.1	31.0	81.0
Anoplocephala magna	Cyclophyllidea/ Anoplocephalidae	*Microtus miurus* (Japan)	AB099691.1	32.3	81.0
Anoplocephala perfoliata	Cyclophyllidea/ Anoplocephalidae	*Microtus miurus* (Japan)	AB099690.1	33.3	81.0
Hymenolepis microstoma	Cyclophyllidea; Hymenolepididae	*Microtus miurus* (Japan)	AP017665.1	28.0	83.0
Pseudanoplocephala crawfordi	Cyclophyllidea/ Hymenolepididae	Unidentified host (China)	KR611041.1	30.3	84.0
Taenia saginata	Cyclophyllidea/Taeniidae	*Homo sapiens* (Thailand)	AB533173.1	30.9	83.0
Taenia asiatica	Cyclophyllidea/Taeniidae	*Homo sapiens* (Japan)	AB608739.1	31.1	82.0
Taenia serialis	Cyclophyllidea/Taeniidae	Wild Rabbits (China)	KY007158.1	30.4	83.0
Hymenolepis diminuta	Cyclophyllidea/ Hymenolepididae	*Rattus norvegius* (Egypt)	GU433102.1	31.7	96.0
H. nana	Cyclophyllidea/ Hymenolepididae	*Rattus norvegius* (Egypt)	GU433103.1	31.6	97.0
Rodentolepis fraterna	Cyclophyllidea/ Hymenolepididae	*Rattus norvegicu* (Spain)	JN258053.1	31.6	84.0
H. nana	Cyclophyllidea/ Hymenolepididae	*Homo sapiens* (Egypt)	GU433104.1	32.0	96.0
H. nana	Cyclophyllidea/ Hymenolepididae	*Mus musculus* (Spain)	AP017666.1	27.1	96.0
H. nana	Cyclophyllidea/ Hymenolepididae	*Homo sapiens* (Japan)	KT951722.1	27.0	95.0
H. nana	Cyclophyllidea/ Hymenolepididae	*Mus musculus* (China)	LC063187.1	31.3	95.0
H. nana	Cyclophyllidea/ Hymenolepididae	*Homo sapiens* (Japan)	AB033412.1	31.9	95.0
Hymenolepis diminuta	Cyclophyllidea/ Hymenolepididae	*Rattus rattus* (India)	AF314223.1	29.0	82.0
Hymenolepis diminuta	Cyclophyllidea/ Hymenolepididae	*Tribolium castaneum* (Unidentified origin)	KC990401.1	31.3	82.0
Hymenolepididae gen. sp.	Cyclophyllidea/ Hymenolepididae	*Cygnus cygnus* (China)	KU980902.1	35.5	83.0

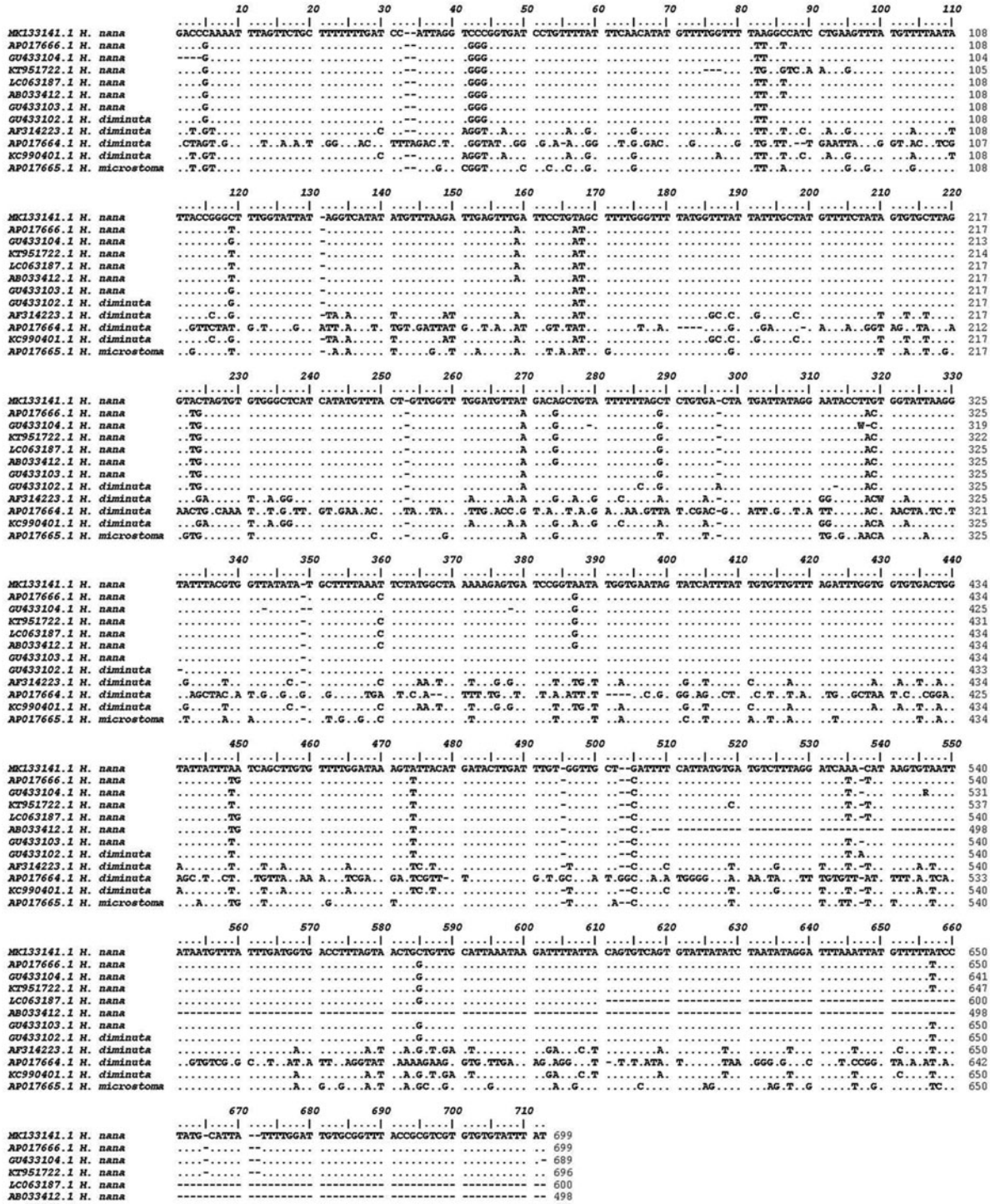

Fig. 5. Sequence alignment of the mt*COX1* gene of *H. nana* with the most closely related hymenolepidid species. (Only variable sites are shown. Dots represent bases identical to those of the first sequences, and dashes indicate gaps).

could not be solved using nuclear loci (Okamoto et al., 1995; Zhang & Hewitt, 1996; Awwad et al., 2001; Macnish et al., 2002). In this study, mtCOXI was used to identify the recovered cestode parasite phylogenetically, in agreement with the findings of Lecanidou et al. (1994) who used mt*COX* subunits genes (I and II) to determine the phylogenetic relationships between related organisms with rapid evolutionary rates. Similar to Guo's (2016) method, this study used the maximum likelihood method to build the phylogenetic tree along with representatives of Pseudophyllidea (Diphyllobothriidae) and Cyclophyllidea

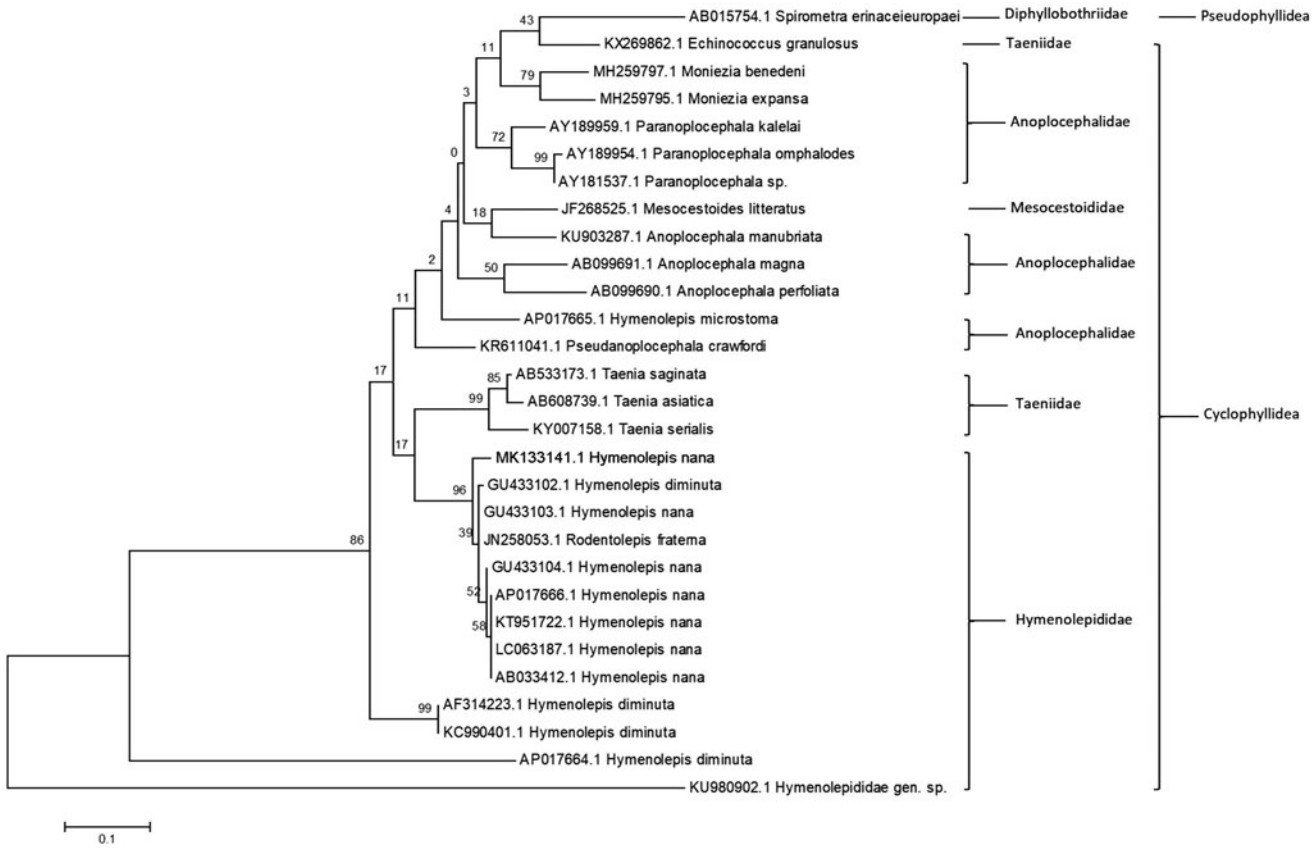

Fig. 6. Molecular phylogenetic analysis carried out using the Tamura–Nei model based on the maximum likelihood method. The percentage of trees shown above the branches that clustered together the related taxa. Initial tree(s) for heuristic search was automatically obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances calculated using the maximum composite likelihood (MCL) approach, then selecting the topology with the higher log-likelihood value. The tree is drawn to the scale, comparing branch lengths as the number of substitutions per site.

(Anoplocephalidae, Hymenolepididae, and Taeniidae) with strongly supported independent clades. Hymenolepididae exhibited a sister-group relationship with Anoplocephalidae, consistent with the findings of studies based on 18S rDNA (Mariaux, 1998; Von Nickisch-Rosenegk et al., 1999), 12S rDNA (Von Nickisch-Rosenegk et al., 1999), and COX genes (Haukisalmi et al., 1998). This was also consistent with the cladistic analysis results of Hoberg et al. (1999) based on morphological characters. In addition, our data support the findings of Al Quraishy et al. (2019), which showed the closely related arrangement of Mesocestodides in Cyclophyllidea with Taeniidae, Hymenolepididae, and Anoplocephalidae.

Our results confirm the paraphyletic origin of the genus *Hymenolepis*, consistent with the findings of Haukisalmi et al. (2010) and Kandil et al. (2010), indicating that both Taeniidae and Hymenolepididae are paraphyletic. Our results, however, contradict Macnish et al. (2002), who reported the *Hymenolepis* genus' monophyly. In our analysis and comparison of mtCOXI with the known sequences of other cyclophyllidean cestodes, the sequences of the query showed close similarity to that of the present and previously described *H. nana* (gb| AP017666.1), with a high bootstrap value of 96%. These results are consistent with the hypothesis of Hillis & Bull (1993), Campbell & Beveridge (1994), and Palm (2004) who found the general rule in the phylogenetic analysis; where the bootstrap value for a given interior branch of a phylogenetic tree is 70% or higher, the topology for that branch is considered reliable or correct. In this study, *H. nana* was found to be more closely related to *H. diminuta* than *H. microstoma*; this contradicted the data obtained by Nkouawa et al. (2016), who built phylogeny using 28S rDNA genomic regions and mt*COX1* marker regions, and demonstrated the genetic relationship between *H. nana* and *H. microstoma*. In contrast, the appearance of both *H. diminuta* and *Pseudacteon crawfordi* in the same clade substantiates the phylogenetic relationship between the two taxa, as clarified by Cheng et al. (2016). The current phylogenetic analyses revealed that the relationship between *Hymenolepis* species and *Rodentolepis fraterna* (gb| JN258053.1) and *Hymenolepididae* gen. sp. (gb| KU980902.1) was unexpected, as they represent different rostellar forms. As described by Haukisalmi et al. (2010), this is due to the loss of rostellar hooks and functional rostellum by *Hymenolepis* species, after colonization from arvicoline rodents. This study further supports the taxonomic position of the recovered hymenolepidid species, with a unique genetic sequence deeply embedded in a genus including the species described of *H. nana*, as a putative sister taxon.

Conclusion

Our recent field study provides new insights into the rapid detection of rodent-infecting hymenolepidids and systematic and phylogenetic analyses. The mitochondrial gene of *H. nana* has also been found as a unique sequence to confirm its taxonomic position in the Hymenolepididae family. In addition, our findings

indicated that laboratory mice could be considered as possible natural reservoirs of different cestode parasites. Further investigation should focus on analyzing different genes that can assist in clarifying the phylogenetic relationships between Hymenolepididae.

Acknowledgments. This study was supported by Researchers Supporting Project (No. RSP-2019/111), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest. The authors declare that they have no conflict of interest.

References

Al Quraishy S, Abdel-Gaber R, Alajmi R, Dkhil MA, Al Jawher M & Morsy K (2019). Morphological and molecular appraisal of cyclophyllidean cestoda parasite *Raillietina saudiae* sp. Nov. infecting the domestic pigeon *Columba livia domestica* and its role as a bio-indicator for environmental quality. *Parasitol Int* **71**, 59–72. https://doi.org/10.1016/j.parint.2019.03.002

Andrews RH & Chilton NB (1999). Multilocus enzyme electrophoresis: A valuable technique for providing answers to problems in parasite systematics. *Int J Parasitol* **29**, 213–253. https://doi.org/10.1016/s0020-7519(98)00168-4

Ariola V (1899). II gen. Scyphocephalus Rigg. E proposta di una nuova classificazione dei cestodi. *Atti Soc Ligust Sci Nat Geogr* **10**, 160–167.

Awwad MH, Lashien GH, Abou E & Kheir SM (2001). Using restriction-site variation of PCR-amplified 18S ribosomal RNA gene for phylogenetic analysis of *Hymenolepis* spp. *Egypt J Hosp Med* **5**, 38–48. https://doi.org/10.12816/EJHM.2001.18861

Baylis HA (1924) The range of variation of *Hymenolepis nana* in rats and mice. *Parasitology* **XVI**, 415–418. https://doi.org/10.1017/S0031182000020308

Bazzano T, Restel TI, Pinto RM & Gomes DC (2002). Patterns of infection with the nematodes *Syphacia obvelata* and *Aspiculuris tetraptera* in conventionally maintained laboratory mice. *Mem Inst Oswaldo Cruz* **97**, 847–853. https://doi.org/10.1590/s0074-02762002000600017

Bhuiyan AI, Ahmed ATA & Khanum H (1996). Endoparasitic helminths in *Rattus* Linnaeus and *Bendicota bengalensis* Gray. *J Asiat Soc Bangladesh Sci* **22**, 189–194.

Brooks DR & Mayes MA (2011). *Hymenolepis asketus* sp. n. (Cestoidea: Hymenolepididae) from the short-tailed shrew, *Blarina brevicauda* Say, from Nebraska. *Proc Helminthol Soc Wash* **44**, 60–62.

Bush AO, Lafferty KD, Lotz J & Shostak AW (1997). Parasitology meets ecology on its own terms: Margolis et al. revised. *J Parasitol* **83**, 575–583.

Campbell RA & Beveridge I (1994) Order trypanorhyncha diesing, 1863. In *Keys to the Cestode Parasites of Vertebrates*, Khalil LF, Jones A & Bray RA (Eds.), pp. 51–148. Wallingford: CAB International.

Cheng T, Liu GH, Song HQ, Lin RQ & Zhu XQ (2016). The complete mitochondrial genome of the dwarf tapeworm *Hymenolepis nana* — A neglected zoonotic helminth. *Parasitol Res* **115**, 1253–1262. https://doi.org/10.1007/s00436-015-4862-8

Coleman AW (2003). ITS-2 is a double-edged tool for eukaryotic evolutionary comparisons. *Trends Genet* **19**, 370–375. https://doi.org/10.1016/S0168-9525(03)00118-5

Coomansingh C, Pinckney RD, Bhaiyat MI, Chikweto S, Bitner A, Baffa A & Sharma R (2009). Prevalence of endoparasites in wild rats in Grenada. *West Indian Vet J* **9**, 17–21.

Cummingham LJ & Olson PD (2010). Description of Hymenolepis microstoma (Nottingham: strain): a classical tapeworm model for research in the genomic era. *Parasites and Vectors* **3**, 123.

Czaplinski B & Vaucher C (1994) Family hymenolepididae ariola, 1899. In *Keys to the Cestode Parasites of Vertebrates*, Khalil LF, Jones A & Bray RA (Eds), pp. 595–663. Wallingford: CAB International.

D'Ovidio D, Noviello E, Pepe P, Del Prete L, Cringoli G & Rinaldi L (2015). Survey of *Hymenolepis* spp. in pet rodents in Italy. *Parasitol Res* **114**, 4381–4384. https://doi.org/10.1007/s00436-015-4675-9

Fedorko JM (1999). *Schistosoma japonicum* in the black rat, *Rattus mindanensis*, from Leyte, Philippines, in relation to Oncomelania snail colonies with reference to other endoparasites. *Southeast Asian J Trop Med Public Health* **30**, 343–349.

Folstad I & Karter AJ (1992). Parasites, bright males, and the immunocompetence handicap. *Am Nat* **139**, 603–622.

Francisco CJ, Almeida A, Castro AM & Santos MJ (2010). Development of a PCR RFLP marker to genetically distinguish *Prosorhynchus crucibulum* and *Prosorhynchus aculeatus*. *Parasitol Int* **59**, 40–43. https://doi.org/10.1016/j.parint.2009.09.004

Gardner SL & Schmidt GD (1987). Cestodes of the genus *Hymenolepis* Weinland, 1858 sensu stricto from pocket gophers *Geomys* and *Thomomys* spp. (Rodentia: Geomyidae) in Colorado and Oregon, with a discriminant analysis of four species of *Hymenolepis*. *Can J Zool* **66**, 896–903. https://doi.org/10.1139/z88-132

Garedaghi Y & Khaki A (2014). Prevalence of gastrointestinal and blood parasites of rodents in Tabriz, Iran, with emphasis on parasitic zoonoses. *Crescent J Med Biol Sci* **1**, 9–12.

Ghatani S, Shylla JA, Tandon V, Chatterjee A & Roy B (2012). Molecular characterization of pouched amphistome parasites (Trematoda: Gastrothylacidae) using ribosomal ITS-2 sequence and secondary structures. *J Helminthol* **86**, 117–124. https://doi.org/10.1017/S0022149X11000125

Gofur MA, Khanum H, Podder MP & Nessa Z (2010). Parasitic infestation in laboratory rat strain, Long-Evans (*Rattus norvegicus* Berkenhout, 1769). *Univ J Zool Rajshahi* **29**, 41–46. https://doi.org/10.3329/ujzru.v29i1.9464

Gonçalves L, Pinto RM, Vicente JJ, Noronha D & Gomes DC (1998). Helminth parasites of conventionally maintained laboratory mice — II. Inbred strains with an adaptation of the anal swab technique. *Mem Inst Oswaldo Cruz* **93**, 121–126. https://doi.org/10.1590/s0074-02761998000100023

Gudissa T, Mazengia H, Alemu S & Nigussie H (2011). Prevalence of gastrointestinal parasites of laboratory animals at Ethiopian Health and Nutrition Research Institute (EHNRI). *Addis Ababa J Infect Dis Immun* **3**, 1–5.

Guimarães AO, Valenca FM, Sousa JBS, Souza SA, Madi RR & de Melo CM (2014). Parasitic and fungal infections in synanthropic rodents in an area of urban expansion, Aracaju, Sergipe state, Brazil. *Acta Sci Biol Sci* **36**, 113–120.

Guo A (2016). The complete mitochondrial genome of the tapeworm *Cladotaenia vulturi* (Cestoda: Paruterinidae): Gene arrangement and phylogenetic relationships with other cestodes. *Parasit Vectors* **9**, 475–484. https://doi.org/10.1186/s13071-016-1769-x

Hämäläinen A, Heistermann M & Kraus C (2015). The stress of growing old: Sex-and season-specific effects of age on allostatic load in wild grey mouse lemurs. *Oecologia* **178**, 1063–1075. https://doi.org/10.1007/s00442-015-3297-3

Haukisalmi V, Hardman LM, Foronda P, Feliu C, Laakkonen J, Niemimaa J, Lehtonen JT & Henttonen H (2010). Systematic relationships of hymenolepidid cestodes of rodents and shrews inferred from sequences of 28S ribosomal RNA. *Zool Scr* **39**, 631–641. https://doi.org/10.1111/j.1463-6409.2010.00444.x

Haukisalmi V, Heino M & Kaitala V (1998). Body size variation in tapeworms (Cestoda): Adaptation to intestinal gradients? *OIKOS* **83**, 152–160. https://doi.org/10.2307/3546556

Hayward AD (2013). Causes and consequences of intra- and inter-host heterogeneity in defence against nematodes. *Parasite Immunol* **35**, 362–373. https://doi.org/10.1111/pim.12054

Hillis DM & Bull JJ (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol* **42**, 182–192. https://doi.org/10.1093/sysbio/42.2.182

Hilmy IS (1936). *Parasites from Liberia and French Guinea. Part 3: Cestodes from Liberia*, 72 p. Le Caire: The Egyptian University.

Hoberg EP, Jones A & Bray RA (1999). Phylogenetic analysis among the families of the Cyclophyllidea (Eucestoda) based on comparative morphology, with new hypotheses for co-evolution in vertebrates. *Syst Parasitol* **42**, 51–73. https://doi.org/10.1023/a:1006100629059

Huda-Thaher A (2012). Prevalence of *Hymenolepis nana* infections in Abu Ghraib City (Baghdad/Iraq). *Iraqi Postgraduate Med J* **11**, 581–584.

Illiger JK (1811). Prodromus Systematis Mammalium et Avium additis terminis zoographicis utriudque classis. C. Salfeld, Berlin, i-xviii, 1-301.

Ito A (2003). Immunology in cestode infection (2). Immunity to the larval cestodes. In *Progress of Parasitology Research in Japan, Tokyo*, Otsuru M, Kamegai S & Hayashi S (Eds), p. 327–337. Tokyo: Meguro Parasitological Museum.

Ito A (2015). Basic and applied problems in developmental biology and immunobiology of cestode infections: *Hymenolepis*, *Taenia* and *Echinococcus*. *Parasite Immunol* **37**, 53–69. https://doi.org/10.1111/pim.12167

Ito A & Yamamoto M (1976). The mode of active protection against *Hymenolepis nana* reinfection in mice inoculated with different doses of shell-free eggs. *Jpn J Parasitol* **25**, 247–253.

Janicki C (1904). Zur Kenntniss einiger Saügetiercestoden. *Zool Anzeig* **XXVII**, 770–782.

Joyeux PC & Kobozieff NI (1928). Recherches sur L' *Hymenolepis microstoma* (Dujardin, 1845). *Annales de Parasitologie* **6**, 59–79.

Kandil OM, Mahmoud MS, Allam NAT & El Namaky AH (2010). Mitochonderial cytochrome c oxidase subunit 1 (cox 1) gene sequence of the *Hymenolepis* species. *J Am Sci* **6**, 1346–1353.

Kataranovski M, Zolotarevski L, Belij S, Mirkov I, Stošić J, Popov A & Kataranovski D (2010). First record of *Calodium hepaticum* and *Taenia taeniaeformis* liver infection in wild Norway rats (*Rattus norvegicus*) in Serbia. *Arch Biol Sci* **62**, 431–440. https://doi.org/10.2298/ABS1002431K

Khatoon N, Bilqees FM, Shahwar D & Rizwana AG (2004). Histopathological alterations associated with *Syphacia* spp. (Nematode) in the intestine of *Nesokia indica*. *Turk J Zool* **28**, 345–351.

Kim BJ, Song KS, Kong HH, Cha HJ & Ock M (2014). Heavy *Hymenolepis nana* infection possibly through organic foods: Report of a case. *Korean J Parasitol* **52**, 85–87. https://doi.org/10.3347/kjp.2014.52.1.85

Klein SL (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunol* **26**, 247–264. https://doi.org/10.1111/j.0141-9838.2004.00710.x

Lapage G (1951). *Parasitic Animals*. UK: The University Press.

Lecanidou R, Douris V & Rodakis GC (1994). Novel features of metazoan mtDNA revealed from sequence analysis of three mitochondrial DNA segments of the land snail *Albinaria turrita* (Gastropoda: Clausiliidae). *J Mol Evol* **38**, 369–382.

Lichtenfels JR, Hoberg EP & Zarlenga DS (1997). Systematics of gastrointestinal nematodes of domestic ruminants: Advances between 1992 and 1995 and proposals for future research. *Nucleic Acids Res* **18**, 4123–4130. https://doi.org/10.1016/s0304-4017(97)00099-x

Linnaeus C (1758). *Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*, vol. **1**, 10th ed, pp. 1–4, 1–824. Stockholm: Laurentius Salvius.

Macnish MG, Morgan-Ryan UM, Monis PT, Behnke JM & Thompson RCA (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

Macy RW (1931). A key to the species of *Hymenolepis* found in bats and the description of a new species, *H. christensoni*, from *Myotis lucifugus*. *Trans Am Microsc Soc* **50**, 344–347. https://doi.org/10.2307/3222075

Macy RW (1947). Parasites found in certain Oregan bats with the description of a new cestode, *Hymenolepis genschi*. *Am Midl Nat* **37**, 375–378.

Macy RW & Rausch RL (1946). Morphology of a new species of bat cestode, *Hymenolepis roudabushi*, and a note on *Hymenolepis christensoni* Macy. *Trans Am Microsc Soc* **65**, 173–175. https://doi.org/10.2307/3223178

Mahami-Oskouei M, Dalimi A, Forouzandeh-Moghadam M & Rokni M (2011). Molecular identification and differentiation of *Fasciola* isolates using PCR-RFLP method based on internal transcribed spacer ITS-1 58S rDNA ITS-2. *Iran J Parasitol* **6**, 35–42.

Makarikov AA, Tkach VV & Bush SE (2013). Two new species of Hymenolepis (Cestoda: Hymenolepididae) from murid rodents (Rodentia: Muridae) in the Philippines. *Journal of Parasitology* **99**, 847–855.

Makarikova TA, Gulyaev VD, Tiunov MP & Feng J (2010). Cestodes *Paramilina nishidai* (Sawada 1982) gen. n., comb. n. and *Hymenolepis magna* sp. n. (Cyclophyllidea: Hymenolepididae) from Chiroptera in China. *Zoolgicheskii Zhurnal* **89**, 131–139.

Mariaux J (1998). A molecular phylogeny of the Eucestoda. *J Parasitol* **84**, 114–124. https://doi.org/10.2307/3284540

Mayhew RL (1925). Studies on the avian species of the cestode family Hymenolepididae. *Illinois Biol Monogr* **10**, 125.

McLeod JA (1933). A parasitological survey of the genus *Citellus* in Manitoba. *Can J Res* **9**, 108–127. https://doi.org/10.1139/cjr33-072

Mehlhorn H, Schmahl G & Mevissen I (2005). Efficacy of a combination of imidacloprid and moxidectin against parasites of reptiles and rodents: Case reports. *Parasitol Res* **97**, S97–101. https://doi.org/10.1007/s00436-005-1451-2

Mohammad MA & Hegazi MA (2007). Intestinal permeability in *Hymenolepis nana* as reflected by non-invasive lactulosa/manitol dual permeability test and its impaction on nutritional parameters of patients. *J Egypt Soc Parasitol* **37**, 877–891.

Mohd Zain SNM, Behnke JM & Lewis JW (2012). Helminth communities from two urban rat populations in Kuala Lumpur, Malaysia. *Parasit Vectors* **5**, 47. https://doi.org/10.1186/1756-3305-5-47.

Moniez R (1880). Essai monographique sur les cysticerques (Thèse Méd. Lille). In-4° de 190 pl. Travaux de l'Institut Zoologique de Lille, III; p. 3.

Nicoll W & Minchin EA (1910). On two species of cysticercoides from the rat flea (*Ceratophyllus fasciatus*). *Proc Zool Soc London* 10–13.

Niwa A, Asano K & Ito A (1998). Eosinophil chemotactic factors from cysticercoids of *Hymenolepis nana*. *J Helminthol* **72**, 273–275. https://doi.org/10.1017/s0022149(00016552

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

Okamoto K (2003). Immunology in cestode infection (1). Adult worm parasitism in the intestine. In *Progress of Parasitology Research in Japan*, vol. **8**, Otsuru M, Kamegai S & Hayashi S (Eds), pp. 319–325. Tokyo: Meguro Parasitological Museum.

Okamoto M, Bessho Y, Kamiya M, Kurosawa T & Horii T (1995). Phylogenetic relationships within *Taenia taniaeformis* variants and other Taeniid cestodes inferred from the nucleotide sequences of the cytochrome C oxidase subunit I gene. *Parasitol Res* **81**, 451–458. https://doi.org/10.1007/bf00931785

Okoye CI & Obiezue RNN (2008). A survey of the gut parasites of rodents in Nsukka ecological zone. *Anim Res Inter* **5**, 846–847. https://doi.org/10.4314/ari.v5i2.48744

Pakdel N, Naem S, Rezaei F & Chalehchaleh AA (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.

Palm HW (2004). *The Trypanorhyncha Diesing, 1863*, 710 p. Bogor: IPB-PKSPL Press.

Panti-May JA, Digiani MC, Palomo-Arjona EE, Gurubel-gonzÁlez YM, Navone GT, Williams CM, HernÁndez-Betancourt SF & Robles MDR (2018). A checklist of the helminthparasites of sympatric rodents from two Mayan villages in Yucatán, México. *Zootaxa* **4403**(3), 495–512.

Perec-Matysiak A, Okulewicz A, Hildebrand J & Zalesny G (2006). Helminth parasites of laboratory mice and rats. *Wiad Parazytol* **52**, 99–102.

Rahdar M, Roointan EAS, Vazirianzadeh B & Alborzi A (2017). Study of internal parasites of rodents in Ahvaz, South-West of Iran. *Jundishapur J Health Sci* **9**, e29067.

Rausch RL (1975). Cestodes of the genus *Hymenolepis* Weinland, 1858 (sensu lato) from bats in North America and Hawaii. *Can J Zool* **53**, 1537–1551. https://doi.org/10.1139/z75-189

Rehbinder C, Baneux P, Forbes D & Winker GC (1996). FELASA recommendations for the health monitoring of mouse, rat, hamster, gerbil, guinea pig and rabbit experimental units. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health accepted by the FELASA Board of Management, November 1995. *Lab Anim* **30**, 193–208. https://doi.org/10.1258/002367796780684881

Richard FO (2008). Diphyllobothrium, dipylidium, and hymenolepsis species. In *Principles and Practice of Pediatric Infectious Diseases*, 3rd ed., Long SS, Pickering LK and Prober CG (Eds), chapter 279. Philadelphia: Churchill Livingstone Elsevier.

Roberts L & Janovy J (2000). *Foundations of Parasitology*, 6th ed. Boston, MA: Mcgraw-Hill Higher Education.

Robles MR & Navone GT (2007). A new species of *Syphacia* (Nematoda: Oxyuridae) from *Oligoryzomys nigripes* (Rodentia: Cricetidae) in Argentina. *Parasitol Res* **101**, 1069–1075.

Rokni MB, Mirhendi H, Mizani A, Mohebali M, Sharbatkhori M, Kia EB, Abdoli H & Izadi S (2010). Identification and differentiation of *Fasciola*

hepatica and *Fasciola gigantica* using a simple PCR restriction enzyme method. *Exp Parasitol* **124**, 209–213. https://doi.org/10.1016/j.exppara.2009.09.015

Rosas GA (1997). Diagnóstico: Parasitosis intestinal por *Aspiculuris tetraptera*. *Anim Experim* **2**, 9–11.

Sadaf HS, Khan SS, Kanwal N, Tasawer BM & Ajmal SM (2013). A review of diarrhoea causing *Hymenolepis nana*-Dwarf tapeworm. *Int Res J Pharmacy* **4**, 32–35.

Sawada I & Harada M (1990). A new *Himenolepis* species (Cestoda: Hymenolepididae) from Lesser Japanese Shrew-mole *Dymecodon pilirostris* of Nagano Prefecture, Japan. *Proc Jap Soc Systemat Zool* **42**, 10–13.

Sawada I & Mohammad KM (1989). Two new species of Hymenolepidid cestodes from Iraqi Bats, with record of a species of Davainidid cestode. *Proc Jap Soc Syst Zool* **39**, 1–7.

Schantz PM (2006). Tapeworms (Cestodiasis). *Gastroenterol Clin North Am* **25**, 637–653.

Schiller EL (1959). Experimental studies on morphological variation in the cestode genus *Hymenolepis*. III. X-irradiation as a mechanism for facilitating analyses in *H. nana*. *Exp Parasitol* **8**, 427–470. https://doi.org/10.1016/0014-4894(59)90033-5

Schmidt GD, Janovy J & Roberts LS (2009). *Foundations of Parasitology*, 8th ed. Boston, MA: McGraw-Hill.

Schultz J, Maisel S, Gerlach D, Müller T & Wolf M (2005). A common core of secondary structure of the internal transcribed spacer 2 (ITS-2) throughout the Eukaryota. *RNA* **11**, 361–364. https://doi.org/10.1261/rna.7204505

Sharma D, Joshi S, Vatsya S & Yadav CL (2013). Prevalence of gastrointestinal helminth infections in rodents of Tarai region of Uttarakhand. *J Parasit Dis* **37**, 181–184. https://doi.org/10.1007/s12639-012-0158-4

Sharma S, Lyngdoh D, Roy B & Tandon V (2016). Differential diagnosis and molecular characterization of *Hymenolepis nana* and *Hymenolepis diminuta* (Cestoda: Cyclophyllidea: Hymenolepididae) based on nuclear rDNA ITS-2 gene marker. *Parasitol Res* **115**, 4293–4298. https://doi.org/10.1007/s00436-016-5210-3

Smyth JD & McManus DP (1989). *The Physiology and Biochemistry of Cestodes*. Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511525841

Soares Magalhães JR, Fançony C, Gamboa D, Langa AJ, Sousa-Figueiredo JC, Clements ACA & Vaz Nery S (2013). Extanding helminths control beyond STH and schistosomiaisis: The case of human hymenolepiasis. *PLoS Negl Trop Dis* **7**, e2321. https://doi.org/10.1371/journal.pntd.0002321

Steinmann P, Cringoli G, Bruschi F, Matthys B, Lohourignon LK, Castagna B, Maurelli MP, Morgoglione ME, Utzinger J & Rinaldi L (2012). FLOTAC for the diagnosis of *Hymenolepis* spp. infection: Proof-of-concept and comparing diagnostic accuracy with other methods. *Parasitol Res* **111**, 749–754. https://doi.org/10.1007/s00436-012-2895-9

Stojcevic D, Mihaljevic Z & Marinculic A (2004). Parasitological survey of rats in rural regions of Croatia. *Vet Med* **49**, 70–74. https://doi.org/10.17221/5679-VETMED

Tanideh N, Sadiiadi SM, Mohammadzadeh T & Mehrabani D (2010). Helminthic infections of laboratory animals in animal house of Shiraz University of Medical Sciences and the potential risks of Zoonotic infections for researches. *Iran Red Crescent Med J* **12**, 151–157.

Teodoro TM, Jannotti-Passos LK, Carvalho-Odos S, Grijalva MJ, Baús EG & Caldeira RL (2011). Hybridism between *Biomphalaria cousin* and *Biomphalaria amazonica* and its susceptibility to *Schistosoma mansoni*. *Mem Inst Oswaldo Cruz* **106**, 851–855. https://doi.org/1590/s0074-02762011000700011

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F & Higgins DG (1997). The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882. https://doi.org/10.1093/nar/25.24.4876.

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

Tubangui MA (1931). Worm parasites of the brown rat (*Mus norvegicus*) in the Philippine Islands, with special reference to those forms that may be transmitted to human beings. *Philippine J Sci* **46**, 537–591.

Von Nickisch-Rosenegk M, Lucius R & Loos-Frank B (1999). Contributions to the phylogeny of the Cyclophyllidea (Cestoda) inferred from mitochondrial 12S rDNA. *J Mol Evol* **48**, 586–596. https://doi.org/10.1007/pl00006501

Wardle MA & McLeod JA (1952). *The Zoology of Tapeworms*, 780 p. Minneapolis, MN: University of Minnesota Press.

Weinland DF (1858). *Human Cestoides. An Essay on the Tapeworms of Man*, p. 93. Cambridge: Metcalf & Co.

Yamaguti S (1959). *"Systema Helminth". Cestodes of Vertebrates*, vol. **II**. 860 p. New York, NY: Interscience Science Publisher Inc.

Yang D, Zhao W, Zhang Y & Liu A (2017). Prevalence of *Hymenolepis nana* and *H. diminuta* from Brown Rats (*Rattus norvegicus*) in Heilongiiang Province, China. *Korean J Parasitol* **55**, 351–355. https://doi.org/10.3347/kjp.2017.55.3.351

Yoisefi A, Eslami A, Mobedi I, Rahbari S & Ronaghi H (2014). Helminth infections of house mouse (*Mus musculus*) and wood mouse (*Apodemus sylvaticus*) from the suburban areas of Hamadan City, Western Iran. *Iran J Parasitol* **9**, 511–518.

Zhang DX & Hewitt GM (1996). Nuclear integrations: Challenges for mitochondrial DNA markers. *Trends Eco Evol* **11**, 247–251. https://doi.org/10.1016/0169-5347(96)10031-8