http://dx.doi.org/10.1590/1678-4162-13135



*Arq. Bras. Med. Vet. Zootec.*, v.76, n.1, p.84-92, 2024

# Morphological and molecular studies on a nematode parasite, *Syphacia obvelata*, infecting laboratory mice (*Mus musculus*) in Saudi Arabia

[*Estudos morfológicos e moleculares sobre um parasita nematoide, Syphacia obvelata, que infecta camundongos de laboratório (Mus musculus) na Arábia Saudita*]

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## ABSTRACT

During screening laboratory mice (*Mus musculus*) at the Zoology Department of the College of Science, King Saud University, some tiny oxyurid nematodes were detected from the large intestine of these rodents. Worms were identified using morphological and morphometric description. Furthermore, DNA was extracted from worms and subjected to polymerase chain reaction to amplify 18S rDNA and ITS (ITS1, 5.8S and ITS2) regions. Worms detected from caecum and colon of mice were detected in 80% of mice investigated. Morphologically, worms showed elongated body with tapered anterior end and narrow posterior part with cuticular annulations. Male worms measured 0.71-1.12 (0.90) long and 0.01-0.12 (0.11) wide. Female worms measured 3.12-5.011 (4.30) long and 0.13-0.29 (0.17) wide. Esophagus followed by intestine which opens at the posterior end via anal opening in females and via cloacal opening in males. Males have a single spicule with a gubernaculum and an accessory hook. Females' uteri were pack with eggs. Data from 18S rDNA revealed a sequence which was identical to *Syphacia obvelata* in NCBI GenBank. Similarly, sequences from ITS regions grouped with sequences from *S. obvelata* confirming the morphological identity of the worm. However, it showed 3 mutations at the ITS2 region from related sequences from *S. obvelata* at NCBI GenBank.

Keywords: *Syphacia obvelata*, *Mus musculus*, 18S rDNA, ITS region, Saudi Arabia

## RESUMO

*Durante a triagem de camundongos de laboratório (*Mus musculus*) no Departamento de Zoologia da Faculdade de Ciências da Universidade King Saud, alguns minúsculos nematoides oxiurídeos foram detectados no intestino grosso desses roedores. Os vermes foram identificados por meio de descrição morfológica e morfométrica. Além disso, o DNA foi extraído dos vermes e submetido à reação em cadeia da polimerase para amplificar as regiões 18S rDNA e ITS (ITS1, 5.8S e ITS2). Os vermes detectados no ceco e no cólon dos camundongos foram detectados em 80% dos camundongos investigados. Morfologicamente, os vermes apresentaram corpo alongado com extremidade anterior afilada e parte posterior estreita com anulações cuticulares. Os vermes machos mediam 0,71-1,12 (0,90) de comprimento e 0,01-0,12 (0,11) de largura. As fêmeas mediram 3,12-5,011 (4,30) de comprimento e 0,13-0,29 (0,17) de largura. O esôfago é seguido pelo intestino, que se abre na extremidade posterior por uma abertura anal nas fêmeas e por uma abertura cloacal nos machos. Os machos têm uma única espícula com um gubernáculo e um gancho acessório. Os úteros das fêmeas estavam repletos de ovos. Os dados do rDNA 18S revelaram uma sequência idêntica à* Syphacia obvelata *no NCBI GenBank. Da mesma forma, as sequências das regiões ITS foram agrupadas com as sequências de* S. obvelata, *confirmando a identidade morfológica do verme. No entanto, ele apresentou 3 mutações na região ITS2 em relação às sequências relacionadas de S. obvelata no NCBI GenBank.*

*Palavras-chave:* Syphacia obvelata, Mus musculus, *18S rDNA, região ITS, Arábia Saudita*

## INTRODUCTION

The development of many biological assays still depends on the use of living laboratory animal models (Perec-Matysiak *et al*., 2006; Mukherjee *et al*., 2022). Rodents, such as mice and rats, are the most common laboratory animals used in research and testing (Pakdel *et al*., 2013). They have a greater ability than most animal species to harbor parasitic fauna (Gómez-Muñoz *et al*., 2020). Rodents play an important role as reservoir hosts for vector-borne disease agents (Ain-Fatin *et al*., 2021). Pinworm infection is routinely reported in various rodent housing facilities even under strict bio-exclusion

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Submitted: August 25, 2023. Accepted: October 5, 2023.

procedures (Hickman *et al*., 2008; Meade and Watson, 2014). Parasitic infection occurs through the fecal-oral route (Effler *et al*., 2008). Although pinworms are generally non-pathogenic (Abdel-Gaber *et al*., 2018), and the resulting clinical signs are rare unless there are heavy loads of infection in immunocompetent animals (Chawla *et al*., 2015), pinworms may have deleterious effects on several research implications (Agersborg *et al*., 2001).

Nematodes of the genus *Syphacia* Seurat, 1916 (family Oxyuridae) are parasitic pinworms that display host-specificity in various muroid rodents (Asakawa, 2005; Perec *et al.*, 2006). Infection with these pinworms has been thought to affect the weight gain and the growth rate of rodents (Zenner, 1997; Lytvynets *et al*., 2010). Among 24 species of this genus, *Syphacia obvelata* and *Syphacia muris* commonly infect the caecum and colon of rodents (Mahmoud *et al*. 2009; Plachý *et al*., 2015; Gerwin *et al*., 2017). The primary discriminating tool among these nematodes was based on morphological examination of male worms which include the presence/absence of gubernaculum, shape and size of spicules, number and form of mamelons and cloacal papillae (Landaeta-Aqueveque *et al*., 2007; Dewi *et al*., 2014; Khalil *et al*., 2014; Abdel-Gaber, 2016; Behnke *et al*., 2022). Regarding the insufficient criteria to differentiate between pinworms, molecular advanced tools have been recently used to discriminate closely related oxyurid species (Cao *et al*., 2020; Omer *et al*., 2020). The nuclear small subunit ribosomal DNA (18S) (Abdel-Gaber, 2016; Stewart *et al*., 2018) and the mitochondrial cytochrome c oxidase subunit 1 (cox1) (Okamoto *et al*., 2007; Stewart *et al*., 2018) were used for molecular identification of *Syphacia* species.

No morphological or molecular data regarding *S. obvelata* from Saudi Arabia is available despite the extensive use of laboratory mice in various field research. Therefore, the present study aimed to investigate the natural prevalence of *S. obvelata* infecting laboratory mice *Mus musculus*, as well as identify the parasite at morphological and molecular levels.

## MATERIALS AND METHODS

A total of ten laboratory mice, *Mus musculus*, were randomly collected from the animal house in the Zoology Department, College of Science, King Saud University (Saudi Arabia). Animals were sacrificed with cervical dislocation and then transferred to our lab for further examination. Animals were used in the experiment following the institution's guidelines on the care and use of animals in research (approval no. KSU-SU-22-66).

After dissection, the caecum and colon of each mouse were longitudinally dissected in normal saline and then examined for parasitic infection under a stereomicroscope (Nikon SMZ18, NIS ELEMENTS software). Worms were collected, washed thrice in saline (0.9%), and then preserved in 70% ethanol for morphological studies or 96% ethanol for molecular analysis. Prevalence of infection was calculated according to Bush *et al*. (1997). Fixed worms were cleared in lactophenol and photographed using a Leica DM 2500 microscope (NIS ELEMENTS software, version 3.8). The identification of specimens followed the guidelines of Falcón-Ordaz *et al*. (2010). Drawings were made with the aid of the Lucida camera. Measurements of different body parts for 30 adult worms were taken in millimeters (mm) using ImageJ 1.53e software (Wayne Rasband and contributors, National Institute of Health, USA) and expressed as a range followed by mean in parentheses.

Genomic DNA (gDNA) was extracted from ethanol-preserved samples using QIAamp® DNA Mini Kit (Qiagen, Germany) with consideration of the manufacturer's protocol steps. The region spanning both Internal Transcribed Regions 1 and 2 (ITS1 and ITS2 including 5.8S) was amplified by PCR using the primer pair NC5F, 5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′ and NC2R, 5′-TTA GTT TCT TTT CCT CCG CT-3′, following the conditions described by Zhu *et al*. (1999). Moreover, the whole of the 18S rDNA gene was amplified using AP1, 5′-AAC CTG GTT GAT CCT GCC AGT-3′ and AP2, 5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′, with the recommended conditions of Ellis *et al*. (1995). Amplicons were visualized by electrophoresis with stained agarose gel (1.5%) by ethidium bromide. PCR products were sequenced using a Macrogen sequencing facility (Seoul, South Korea). Data of DNA sequences were aligned using CLUSTAL-X software (Thompson *et al*., 1997) and compared with data recorded in GenBank. The dendrogram was constructed by Neighbor-Joining (NJ) and Maximum likelihood

(ML) analyses using MEGA X software (Kumar *et al.*, 2018). These analyses were evaluated using 1000 resampling's for the bootstrap support.

## RESULTS

A total of eight mice were naturally infected with the oxyurid nematode parasite indicating a prevalence of 80%. The infection was recorded in the caecum and colon of the infected mice. This parasite has unique taxonomic criteria of the genus *Syphacia*, especially for *S. obvelata* as mentioned below.

Description (Figure 1). Body elongated with tapered anterior end and narrow posterior one. Cuticle transversely annulated. The mouth is surrounded by three less developed lips (1 dorsal and 2 ventrolateral), four sub-median cephalic papillae, and 2 amphid pores. Mouth followed by a shallow, tri-radiate buccal cavity lined with tooth-like denticles. The buccal cavity leads to the esophagus which is divided into an anterior cylindrical part (corpus) and a globular part (bulb) supported by the valvular apparatus. Esophagus followed by the intestine which opens exteriorly with an anal opening in females and a cloacal opening in males. Excretory pores are located posteriorly to the esophageal-intestinal junction. Male worms characterized by the presence of 3 mamelons located at the ventral surface. A single spicule presents with a gubernaculum associated with an accessory hook. Three pairs of caudal papillae present (1$^{st}$ pre-cloacal, 2$^{nd}$ ad-cloacal, and 3$^{rd}$ post-cloacal papillae). Moreover, females characterized by fully packed uteri with eggs. The vagina gives rise to a muscular ovijector that opens by the vulva with protruded lips. Ellipsoidal and embryonated eggs were present.

Measurements for male worms (Table 1). Body of the male worms measured 0.72-1.112 (0.901) long and 0.01-0.12 (0.11) wide; corpus 0.02-0.05 (0.04) long; bulb 0.03-0.09 (0.07) long; nerve ring at 0.05-0.13 (0.08) from the anterior end; excretory pore at 0.09-0.20 (0.17) from the anterior end; 1$^{st}$ mamelon 0.05–0.07 (0.06) long from the posterior end; 2$^{nd}$ mamelon 0.040–0.08 (0.05) long from the posterior end; 3$^{rd}$ mamelon 0.03–0.05 (0.04) long from the posterior end; spicule 0.05-0.09 (0.07) long; and tail 0.08-0.16 (0.14) long.

Measurements for female worms (Table 2). Body of the female worms measured 3.12-5.01 (4.30) long and 0.13-0.29 (0.17) wide; corpus 0.02-0.06 (0.05) long; bulb 0.05-0.17 (0.12) long; nerve ring at 0.03-0.15 (0.13) from the anterior end; excretory pore at 0.14-0.20 (0.18) from the anterior end; vulval opening at 0.40-0.60 (0.57) from the anterior end; eggs 0.07-0.09 (0.08) long and 0.028-0.047 (0.038) wide; and tail 0.22-0.40 (0.34) long.

Molecular analysis. Amplification of both the 18S rDNA and internal transcribed spacers 1 and 2 regions was successful. A fragment of ~1700 bp was obtained for the 18S rDNA and ~800 bp was obtained for ITS1 and ITS2 including 5.8S. Sequences from three strains of the 18S rDNA were identical and 2 sequences from the ITS regions (ITS1, 5.8S, and ITS2) were also identical and representative sequences from each region were deposited in GenBank under the accession numbers OQ875250 and OQ874775, respectively. 18S rDNA sequences from *Syphacia* species (6 sequences) together with sequences from related nematodes on NCBI GenBank (13 sequences) were used to construct the phylogenetic trees from a 1229-bp long alignment, using *Clonorchis sinensis* (MK450527) as an outgroup. On both analyses (Neighbor Joining {NJ} and Maximum Likelihood {ML}) the sequence from the present study OQ875250 grouped with sequences from the paraphyletic *Syphacia* species with a strong bootstrap support (95 NJ and 70 ML) grouping it with *Syphacia obvelata* (Figure 2). There were only a few sequences from *S. obvelata* in GenBank and most of them are short sequences. However, two sequences of *S. obvelata* with equivalent lengths were included in the analysis (KY462826 from South Africa and OK138907 from the UK). The 18S rDNA sequence obtained in the present study (OQ875250) was identical to that obtained from the UK identified as Nottingham isolate (OK138907). It showed 4 differences to the *S. obvelata* sequence from South Africa (KY462826). Those mutations were all transversions at positions 112, 201, and 649 of the alignment, in addition, at position 173 there was an insertion of a T base on sequence OQ875250 whereas it was missing from the sequence KY462826.

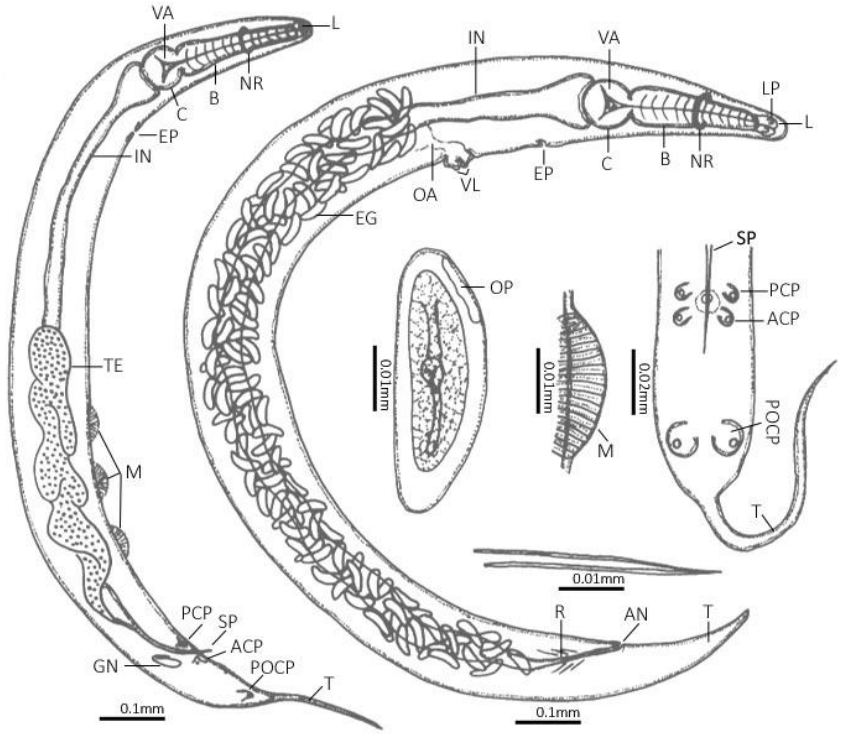


Figure 1. Line drawing of *Syphacia obvelata* infecting laboratory mice. (A) Lateral view of male, holotype. (B) Lateral view of female, holotype. (C) Lateral view of embryonated egg. (D) Lateral view of mamelon. (E) Ventral view of the posterior end of male. (F) Ventral view of the spicule. Note: ACP, ad-cloacal papillae; AN, anal opening; B, bulb; C, corpus; CO, cloacal opening; EG, eggs; EP, excretory pore; GN, gubernaculum; IN, intestine; L, lips; LP, lateral papillae; M, mamelon; NR, nerve ring; OA, ovijector apparatus; OP, operculum; PCP, pre-cloacal papillae; POCP, post-cloacal papillae; R, rectum; SP, spicule; T, tail; TE, testis; VA, valvular apparatus; VL, vulval lips.

Table 1. Morphological characteristics of male worms for *Syphacia obvelata* from laboratory mice

| Source of *Syphacia* | Body | | Esophagus | | Distance from anterior end | | Spicule length | Tail length | Locality |
|---|---|---|---|---|---|---|---|---|---|
| | Length | Width | Corpus | bulb | Nerve ring | Excretory pore | | | |
| Hussey, 1957 | 1.334 | 0.131 | -- | -- | -- | 0.302 | -- | 0.122 | USA |
| Ogden, 1971 | 1.13-1.61 (1.35) | 0.131-0.172 (0.146) | -- | -- | -- | -- | 0.068-0.089 (0.078) | 0.122-0.172 (0.149) | Several countries |
| Magalhaes *et al*., 1994 | 1-1.1 | 0.050-0.072 | -- | -- | 0.090-0.097 | 0.079 | 0.072-0.082 | 0.072-0.090 | Brazil |
| Landaeta-Aqueveque *et al*., 2007 | 0.88-1.23 (1.04) | 0.120-0.190 (0.150) | -- | 0.050-0.067 (0.060) | 0.090-0.120 (0.100) | 0.170-0.220 (0.190) | 0.055-0.085 (0.077) | 0.110-0.170 (0.130) | Chile |
| Khalil *et al*., 2014 | 0.432-1.09 (0.677) | 0.067-0.150 (0.108) | 0.016-0.045 (0.037) | 0.027-0.090 (0.047) | 0.040-0.126 (0.065) | 0.099-0.220 (0.134) | 0.058-0.097 (0.073) | 0.094-0.117 (0.105) | Egypt |
| Abdel-Gaber, 2016 | 0.623-1.130 (0.830) | 0.092-0.130 (0.110) | 0.013-0.042 (0.030) | 0.023-0.086 (0.053) | 0.035-0.132 (0.073) | 0.087-0.191 (0.145) | 0.042-0.082 (0.054) | 0.073-0.142 (0.120) | Egypt |
| Present study | 0.72-1.11 (0.90) | 0.10-0.12 (0.11) | 0.02-0.05 (0.04) | 0.03-0.10 (0.07) | 0.05-0.13 (0.08) | 0.09-0.20 (0.17) | 0.05-0.09 (0.07) | 0.08-0.16 (0.14) | Saudi Arabia |

Table 2. Morphological characteristics of female worms for *Syphacia obvelata* from laboratory mice

| Source of *Syphacia* | Body | | Esophagus | | Distance from anterior end | | | Eggs | | Tail length | Locality |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Length | Width | Corpus | bulb | Nerve ring | Excretory pore | Vulva opening | Length | Width | | |
| Hussey, 1957 | 5.203 | 0.312 | -- | -- | -- | 0.523 | 0.874 | 0.134 | 0.036 | 0.762 | USA |
| Ogden, 1971 | 3.72-5.61 (4.69) | 0.234-0.372 (0.316) | -- | -- | -- | -- | 0.450-0.740 (0.590) | 0.099-0.118 | 0.036-0.041 | 0.680-0.890 (0.780) | Several countries |
| Magalhaes *et al*., 1994 | 4.5-5 | 0.245-0.350 | -- | -- | 0.082-0.090 | 0.086 | 0.662-0.806 | 0.118-0.126 | 0.043-0.054 | 0.518 | Brazil |
| Landaeta-Aqueveque *et al*., 2007 | 3.7-4.8 (4.28) | 0.290-0.400 (0.320) | -- | 0.085-0.126 (0.111) | 0.115-0.125 (0.120) | 0.250-0.510 (0.350) | 0.430-0.790 (0.550) | 0.132-0.140 (0.136) | 0.031-0.050 (0.040) | 0.600-0.770 (0.670) | Chile |
| Khalil *et al*., 2014 | 1.443-3.08 (1.96) | 0.13-0.252 (0.166) | 0.054-0.081 (0.066) | 0.045-0.118 (0.069) | 0.063-0.14 (0.082) | 0.175-0.369 (0.251) | 0.279-0.585 (0.401) | 0.087-0.11 (0.103) | 0.022-0.027 (0.024) | 0.319-0.576 (0.455) | Egypt |
| Abdel-Gaber, 2016 | 2.930-4.650 (3.540) | 0.120-0.232 (0.156) | 0.018-0.062 (0.051) | 0.049-0.156 (0.110) | 0.026-0.157 (0.121) | 0.134-0.243 (0.195) | 0.323-0.632 (0.546) | 0.120-0.139 (0.129) | 0.030-0.052 (0.045) | 0.220-0.410 (0.361) | Egypt |
| Present study | 3.12-5.01 (4.30) | 0.13-0.29 (0.17) | 0.02-0.06 (0.05) | 0.05-0.17 (0.12) | 0.03-0.15 (0.13) | 0.14-0.20 (0.18) | 0.40-0.60 (0.58) | 0.07-0.09 (0.08) | 0.03-0.05 (0.04) | 0.22-0.40 (0.34) | Saudi Arabia |

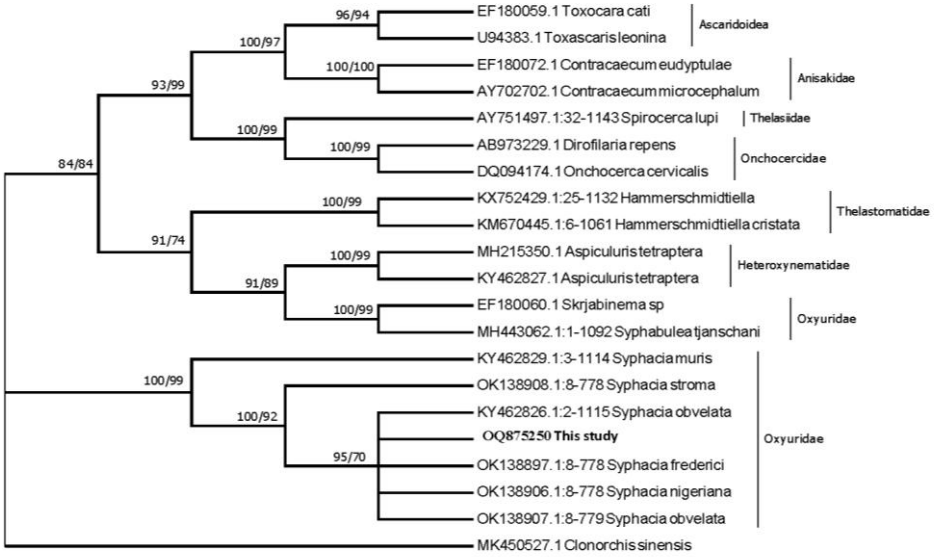


Figure 2. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor Joining (NJ) methods, showing phylogenetic relationships among *Syphacia obvelata* and other *Syphacia* species and related taxa in NCBI GenBank with *Clonorchis sinensis* as an outgroup. The ML and NJ trees are inferred from the 18S rDNA sequence data generated from the *S. obvelata* recovered from *Mus musculus* (OQ875250 given in bold) and related taxa from GenBank. Numbers indicated at branch nodes are bootstrap values (ML/NJ). Only bootstraps > 70% are shown.

Sequences from the ITS 1 and ITS 2 including 5.8S from *Syphacia* species (22 sequences including 12 from *S. obvelata*) together with sequences from related nematodes on NCBI GenBank (10 sequences) were used to construct the phylogenetic trees from an 879-bp long alignment, using *Clonorchis sinensis* (MF319655) as an outgroup. On both analyses (Neighbor Joining {NJ} and Maximum Likelihood {ML}) the sequence from the present study OQ874775 grouped with sequences from the paraphyletic *Syphacia* species with a strong bootstrap support (100 NJ and 95 ML) grouping it with *Syphacia obvelata* (Figure 3). There were

also three mutations; two transversions (T to A) at positions 658 and 788 of the alignment and one transition (C to T) at position 848 of the sequence OQ874775 compared with other sequences from *S. obvelata*.

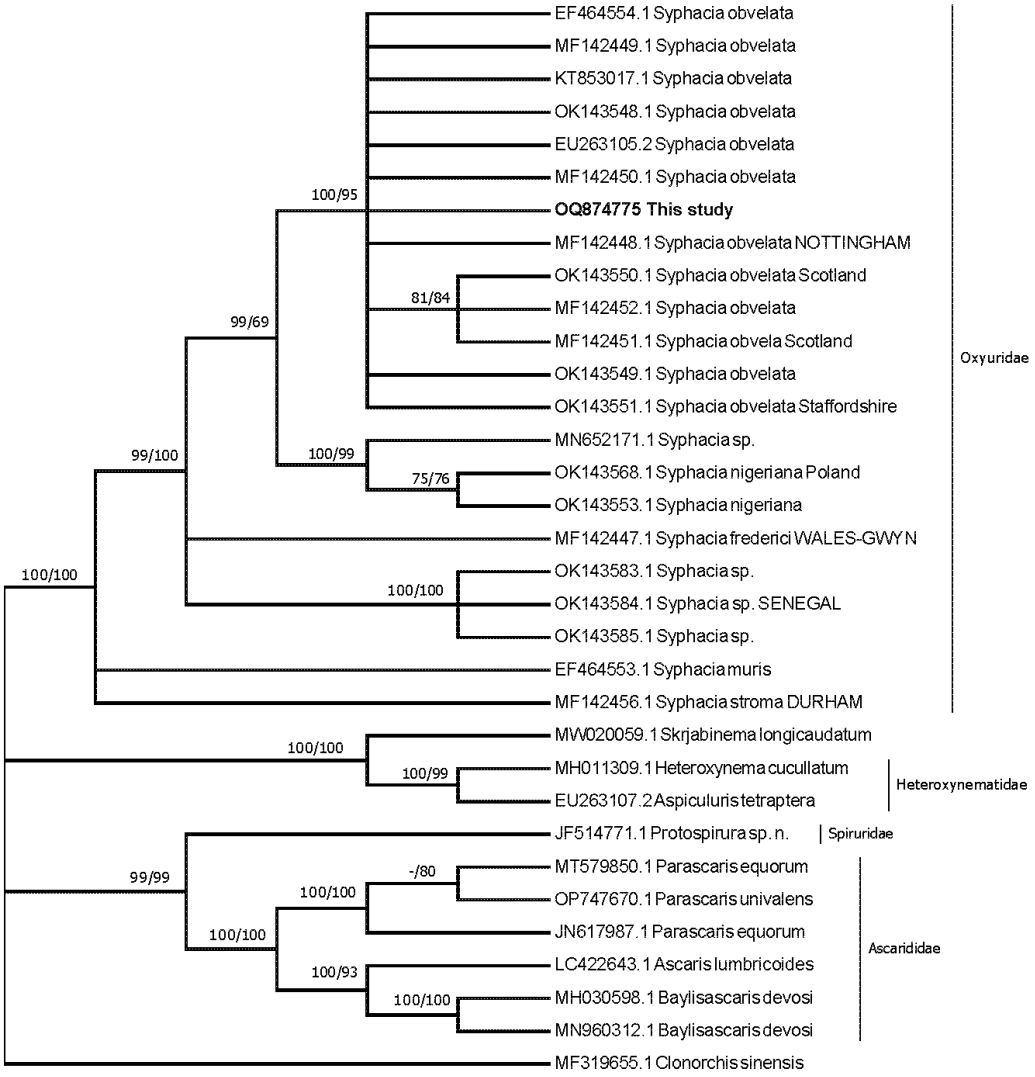


Figure 3. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor Joining (NJ) methods, showing phylogenetic relationships among *Syphacia obvelata* and other *Syphacia* species and related taxa in NCBI GenBank with *Clonorchis sinensis* as an outgroup. The ML and NJ trees are inferred from the ITS spacer regions (ITS1, 5.8S, and ITS2) rDNA sequence data generated from the *S. obvelata* recovered from *Mus musculus* (OQ874775 given in bold) and related taxa from GenBank. Numbers indicated at branch nodes are bootstrap values (NJ /ML). Only bootstraps > 60% are shown.

## DISCUSSION

Rodents are a mammalian group that has become increasingly important in the transmission of pathogens to researchers in the laboratories (Dahmana *et al*., 2020). This study revealed that oxyurids are widely distributed in rodents raised at the animal facility, especially those belonging to the genus *Syphacia*. A high prevalence of 80% can be attributed to the fact that several mice are kept in one cage which makes it more conducive for the transmission of this nematode which has a direct lifecycle. According to Ain Fatin *et al*. (2021), pinworms are opportunistic pathogens

that frequently infect laboratory animals in conventional, semi-open animal facilities. Dewi *et al*. (2014) reported that pinworms of the genus *Syphacia* seem to have rather strict host-specificity and are believed to have a co-evolutionary relationship with their hosts. The present oxyurid species has a high prevalent value of 80%, which agrees with other previous data of Bazzano *et al*. (2002), Klimpel *et al*. (2007), Kataranovski *et al*. (2008), and Abdel-Gaber (2016). This data indicated that the presence of oxyurids is very common, but their influence should not be neglected as infected animals are unsuitable for scientific research such as feed intake and blood parameters as experimental results that may be affected by the parasitic infection. Moreover, the site specificity for infection in this study was observed in the caecum and colon which represents a highly nutritive niche for oxyurids, which agreed with Lytvynets *et al*. (2010).

Due to less availability of morphological studies for oxyurid species, the present study focused on the description of parasite species infecting laboratory mice. Based on morphological criteria, the recovered parasite species has all the taxonomic features of the genus *Syphacia* with special reference to (i) the structure of anterior extremity including lips and its papillae and amphids, (ii) in females, shape and uniformity of eggs and position of the vulva with lips shape, (iii) in males, shape of mamelons, number and distribution of cloacal papillae, size and shape of the spicule, and presence/absence of gubernaculum. It showed great similarity in morphology and morphometrics to *S. obvelata* reported in previous studies (Hussey, 1957; Ogden, 1971; Magalhaes *et al*., 1994; Landaeta-Aqueveque *et al*., 2007; Khalil *et al*., 2014; Abdel-Gaber, 2016) with few differences in measurements of the different body parts. The current study represented the first record in Saudi Arabia focused on the taxonomic description of the *Syphacia* species infecting laboratory mice.

Both sequences obtained in the present study from the 18S rDNA and the internal transcribed spacer (ITS1, 5.8S, and ITS2) regions suggested the organism under investigation is *S. obvelata* confirming morphological characteristics. The sequence obtained from the spacer regions of OQ874775 showed three mutations at the ITS2, whereas ITS1 as well 5.8S were identical to sequences from *S. obvelata* deposited in NCBI GenBank. It has been well documented that *Syphacia* species demonstrate co-evolution with their hosts and host specificity (Hugot, 1988, 1999; Adamson, 1989; Dewi *et al*., 2014; Garcia *et al*., 2018). So far, all isolates of *S. obvelata* for which sequences are available in NCBI GenBank were from *Mus musculus.* Only two 18S rDNA sequences of *S. obvelata* (KY462826 from South Africa and OK138907 from the UK) with the same lengths as the sequences reported in the present study (OQ875250). The 18S rDNA sequence obtained in this study (OQ875250) was identical to that obtained from the UK identified as Nottingham isolate (OK138907) whereas it showed 4 mutations compared with KY462826 from South Africa. Although the sequence (KY462826) from South Africa was labeled as *S. obvelata* that obtained from the Southern multi-mammate mouse (*Mastomys coucha*), it has shown sequence variation on 3 sites, and it differed from other sequences reported from *S. obvelata*. Interestingly, the obtained sequence (KY462826) was identical to that of *S. nigeriana* except for a single mutation suggesting that it could well be related to *S. nigeriana*. Other *Syphacia* species such as *S. muris* occur in different rat species (*Rattus* spp.) and *S. stroma* infects the Eurasian field *Apodemus* mice (*Apodemus agrarius*) while *S. obvelata* prefers *Mus musculus* as a host (Stewart *et al*., 2018). The results of the present study where *S. obevelata* was detected from *Mus musculus* confirm the host specificity of this species as demonstrated in previous studies (Stewart *et al*., 2018). Furthermore, it is the first molecular study confirming the presence of *S. obvelata* in Saudi Arabia.

## ACKNOWLEDGMENTS

This study was supported by the Researchers Supporting Project (RSP2023R94), King Saud University, Riyadh, Saudi Arabia.

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