ORIGINAL PAPER



# *Syphacia obvelata* (Nematode, Oxyuridae) infecting laboratory mice *Mus musculus* (Rodentia, Muridae): phylogeny and host-parasite relationship

Rewaida Abdel-Gaber<sup>1</sup>

Received: 13 October 2015 / Accepted: 10 November 2015 / Published online: 19 November 2015 © Springer-Verlag Berlin Heidelberg 2015

Abstract Syphacia obvelata is a pinworm nematode parasite infecting man and laboratory animals in high abundance. This parasitological study was carried out during the period of March 2014-February 2015 to investigate the helminth parasites infecting the laboratory mice Mus musculus in the Animal House at Cairo University, Egypt. The prevalence of S. obvelata in M. musculus was 75.0 %. The extent of infection with S. obvelata is analyzed according to the sex of the host mice. It was shown that the prevalence of male infection was greater than female worms. Morphological characterization revealed that the present Oxyurid species possesses a rounded cephalic end with less developed lips, esophagus divided into cylindrical corpus, and globular bulb supported internally with valvular apparatus; three mamelons are located at the ventral surface with a single chitinized spicule and a gubernaculum provided with an accessory hook in males, and ovijector apparatus opens ventrally by the vulva surrounded by protruded lips in female worms. Body of the male was 0.623-1.130(0.830±0.11)mm long and 0.092- $0.130(0.110\pm0.01)$  mm wide; the esophagus was 0.164- $0.280(0.210\pm0.01)$  mm long; the nerve ring and excretory pore are located at 0.035-0.132 (0.073±0.01) and 0.087- $0.191(0.145\pm0.01)$  mm from the anterior end, respectively, while the female measured  $2.930-4.650(3.540\pm0.1)$  mm long and  $0.120-0.232(0.156\pm0.001)$  mm wide; the esophagus was  $0.213-0.410(0.342\pm0.01)$  mm long; the nerve ring, excretory pore, and vulval opening are located at 0.026-0.157 (0.121± 0.01), 0.134–0.243 (0.195 $\pm$ 0.01), and 0.323–0.632(0.546 $\pm$ 

0.11)mm from the anterior end, respectively; eggs measured 0.120–0.139(0.129 $\pm$ 0.001)mm long and 0.030–0.052(0.045  $\pm$ 0.001)mm wide. It compared morphometrically with other *Syphacia* species described previously and showed little differences in measurements. Molecular characterization based on small subunit ribosomal DNA (rDNA) was done to confirm the obtained morphological and morphometric results. A preliminary genetic comparison between SSU rDNA of the present parasite and other species of Oxyuridae places it as a putative sister taxon to other *S. obvelata*.

**Keywords** Laboratory mice · *Syphacia* spp. · Host-parasite relationship · Phylogenetic relationship

#### Introduction

Rodents have a greater ability than most other animal species to harbor many zoonotic agents (Neifer et al. 1991; Mehlhorn et al. 2005; Pakdel et al. 2013). Given their broad distribution and their close contact with different animals and humans, rodents play an important role as reservoir hosts for vector-borne disease agents (Mahmoud et al. 2009). Pinworms belonging to the family Oxyuridae Cobbold 1864 are the most common helminth parasites infecting laboratory animals (Robles and Navone 2010; Klimpel et al. 2011; Abdel-Gaber and Fol 2015). Species of the genus Syphacia Seurat, 1916, are considered to have generally co-evolved with their hosts (Okamoto et al. 2007). Identification of Syphacia spp. infecting rodents is based on examination of male worms for the location of the mamelons; however, male worms are rarely recovered because they die after mating, while female S. muris and S. obvelata differ only in the location of the vulva being slightly posterior in S. muris than that in S. obvelata (Farrar et al. 1994; Baker 1998; Parel et al. 2008; Khalil et al. 2013, 2014). The fact that these morphological

Rewaida Abdel-Gaber rewaida@sci.cu.edu.eg

<sup>&</sup>lt;sup>1</sup> Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

differences are difficult to determine the urge for needs more diagnostic criteria for the differentiation between these pinworm species (Semenova et al. 1996; Bazzano et al. 2002; Robles and Navone 2007; Parel et al. 2008). Molecular tools have been recently used to discriminate morphologically closely related species and have contributed effectively to taxonomical, phylogenetic, and epidemiological studies of many parasites (Ambroise-Thomas 1990; Mc-Manus and Bowles 1996). Polymerase chain reaction (PCR)-based assays have been used effectively for the fast and simple detection of genomic variations among parasites (Singh 1997; Perec-Matysiak et al. 2006).

In the present study, the natural prevalence and morphological as well as molecular analyses of ribosomal DNA of *S. obvelata* infecting laboratory mice *Mus musculus* were carried out to determine the exact taxonomic and phylogenetic position of this parasite species and, in addition, investigate the effect of host-related sex factor on the prevalence of parasite species and role of laboratory rodents as reservoirs of oxyurids in Egypt.

#### Materials and methods

Experimental animals and parasitological examination Sixty adult laboratory mice M. musculus were randomly chosen during the period of March 2014-February 2015 from the Animal House of Cairo University, Egypt. The selected rodents were transferred to the Laboratory of Parasitology Research in Zoology Department, Faculty of Science, Cairo University, Egypt, where they were anesthetized and sacrificed according to the ethical rules for handling experimental animals. After dissection, internal organs of each rodent's carcass were removed and examined for adult or larval stages of helminths under a stereomicroscope. The prevalence of parasitic infection was evaluated according to Bush et al. (1997). The collected pinworms were removed, counted, and preserved in 70 % ethyl alcohol then cleared in 5 % glycerin for further examination and species identification according to the guidelines of Falcon-Ordaz et al. (2010). Specimens were measured using a calibrated ocular micrometer and photographed using Zeiss Axiovert 135 microscope with Cannon digital camera. For scanning electron microscopy, specimens were fixed in 3 % phosphate buffered glutaraldehyde, post-fixed in 1 % osmium tetra-oxide, critical point dried, coated with gold, examined, and photographed using a JEOL 6100 SEM at an accelerating voltage of 20 kV in the Electron Microscope Unit at Microanalytical Center, Faculty of Science, Beni Suef University, Egypt. All drawings were made with the aid of the camera Lucida. All measurements were taken in millimeter as a range followed by mean±SD in parentheses.

**Determination of phylogenetic relationship** DNA was extracted from ethanol-preserved worms by using a QIAamp<sup>®</sup> DNA Mini Kit (Quiagen, GmbH, Germany) following the manufacturer's protocol. Polymerase chain reaction (PCR) was carried out to amplify the target genomic DNA (gDNA) using the primer pairs SyphaCOIF 5'-TGGTCTGGTTTTGTTGGTAGTT-3' and SyphaCOIR 5'-AACCACCCAACGTAAAC ATAAA-3' according to Okamoto et al. (2007). The PCR reaction condition was occurred as follows: 3 min initial denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C, and 1 min 30 s at 72 °C. Post-PCR extension was carried out for 7 min at 72 °C. PCR products were purified and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with 310 Automated DNA Sequencer (Applied Biosystems, USA) using the same primers for annealing. Data of DNA sequences were aligned using CLUSTAL-X multiple sequence alignment (Thompson et al. 1997) and compared with some of previously recorded data from Genbank to analyze intra-specific differences. Phylogenetic tree was constructed by neighbor-joining method using MEGALIGN (DNASTAR, Window version 3.12e).

**Ethical considerations** Animal use followed a protocol approved and authorized by the Institutional Animal Care and Use Committee (IACUC) in the Faculty of Science, Cairo University, Egypt.

# Results

A total of 325 specimens of adult Oxyurid species were found in the small intestine, caecum, colon, and around the anal opening of the laboratory mice *M. musculus*. A total of 45 out of 60 mice hosts were naturally infected with 75.0 %. The infection increased in male mice to be 93.33 % (28/30) and lowered to be 56.66 % (17/30) in female mice.

**Microscopic examination** (Figures 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, and 28) revealed that the body of the

Figs. 1–10 Photomicrographs of *S. obvelata* infecting laboratory mice ► M. musculus. 1 The adult female worm with cephalic end supplied with three lips (L), labial papillae (LP), and amphids (Am) surrounding mouth opening (MO) followed by pharynx (PH), esophagus of two parts were corpus (C) and bulb (B) with valvular apparatus (VA), intestine (IN) ended with anal opening (AN), and tail (T). Note the presence of excretory pore (EP), excretory bladder (EB), vulva (V) surrounded with vulval lips (VL), ovijector apparatus (OA), uterus (U) filled with eggs (EG). 2 The adult male worm. Note the presence of testis (TE). 3, 4 The anterior end with high magnification in 4. Note the presence of nerve ring (NR). 5-10 High magnifications of 5 Cuticle with transverse annulations (TA). 6 Vulva opening (V) surrounded with vulval lips (VL). 7 Embryonated egg (EG) with juvenile (J). 8, 9 The posterior end of female with rectum (R), rectal gland (RG), anal opening (AN), and tail (T). 10 The posterior end of male with cloacal opening (CO) provided with pre-cloacal papillae (PCP), adcloacal papillae (ACP), post-cloacal papillae (PoCP), spicule (SP), and body ended with tail (T)





**Figs. 11–20** Scanning electron micrographs of *S. obvelata* showing 11-14 The anterior part with three lips (*L*) surrounding mouth opening (*MO*) provided with labial papillae (*LP*) and amphids (*Am*), with high magnifications in 12-14. 15 Cuticle with transverse striations (*TA*). 16–

20 High magnifications of 16 Female with anal opening (AN) ended with tail (T). 17 Eggs (EG). 18 Mamelon striae (S). 19 Male with cloacal opening (CO) surrounded with pre-cloacal papillae (PCP) and Ad-cloacal papillae (ACP). 20 Post-cloacal papillae (PoCP)

recovered Oxyurid species was small, colorless to offwhite with narrow posterior extremity. The cephalic plate is round; the mouth is surrounded by three less developed lips (one dorsal and two ventro-lateral), four sub-median cephalic papillae, and two amphidial pores. **Figs. 21–28** Line drawing with camera Lucida of *S. obvelata. 21* Female, holotype, lateral view. *22* Male, holotype, lateral view. *23, 24* Apical view of cephalic end (3 female, 4 male). *25* Mamelon (*M*), lateral view. *26* Embryonated egg surrounded with egg shell (*ES*), with operculum (*OP*). *27, 28* Posterior end of male (4 lateral view, 5 ventral view) with gubernaculum (*GN*)



(29)



Fig. 29 Dendrogram based on SSU rDNA gene sequences showing the phylogenetic relationship between S. obvelata and other Oxyuridae species

The cuticle has transverse striations. The mouth is followed by a shallow, tri-radiate buccal cavity lined with three thickened chitinized plates provided distally by sharp tooth-like denticles. The buccal cavity leads to a short esophagus that is divided into an anterior cylindrical part, a corpus, and a globular bulb which is supported internally with tri-radiate valvular apparatus. The esophagus leads to a long intestine which opens exteriorly with an anal opening in females and cloacal opening in males. The excretory pore is located posterior to the esophago-intestinal junction.

Body of the male worm (based on 10 mature specimens) It measured 0.623-1.130(0.830±0.11)mm long and 0.092- $0.130(0.110\pm0.01)$ mm wide. The Lateral alae are large, vesicular, and extending from the esophageal bulb level to the posterior mamelon level. The esophagus (including the pharynx, corpus, and bulb) measured  $0.164-0.280(0.210\pm0.01)$ mm long. The pharynx measured  $0.122-0.150(0.135\pm0.01)$ mm long, the corpus measured  $0.013-0.042(0.030\pm0.01)$ mm long, and the bulb measured 0.049-0.156(0.110±0.01)mm long. The nerve ring and excretory pore are located at 0.035-0.132 (0.073±0.01) and 0.087-0.191(0.145±0.01) mm from the anterior end, respectively. Three mamelons are located at the ventral surface; the anterior mamelon measured  $0.038-0.048(0.042\pm0.01)$  mm long, the middle mamelon measured  $0.039-0.053(0.046\pm0.01)$  mm long, and the posterior mamelon measured 0.030-0.042(0.037±0.01)mm long. Distance from the cephalic end to anterior edges of the anterior, middle, and posterior mamelons was 0.144-0.294 (0.199

 $\pm 0.011$ ), 0.198–0.395 (0.287 $\pm 0.11$ ), and 0.292–0.492 (0.412  $\pm 0.11$ ), respectively. Males possess a single chitinized spicule and a gubernaculum provided with an accessory hook and may appear protruded above the surface of the body cuticle. Three symmetrical pairs of caudal papillae surround the cloacal region: 1st pre-cloacal, 2nd ad-cloacal, and 3rd large and more pronounced post-cloacal lying further posterior to the cloacal opening. The body ended with a tail which measured 0.073–0.142(0.120 $\pm 0.001$ )mm long.

Body of the female is larger than the male worm (based on **10 mature specimens)** It measured  $2.930-4.650(3.540\pm0.1)$ mm long and 0.120-0.232(0.156±0.001)mm wide. Lateral alae are absent. The esophagus measured 0.213- $0.410(0.342\pm0.01)$  mm long. The pharynx measured 0.146- $0.192(0.181\pm0.01)$  mm long, the corpus  $0.018-0.062(0.051\pm$ 0.01)mm long, and the bulb 0.049-0.156(0.110±0.01)mm long. The nerve ring, excretory pore, and vulval opening are located at 0.026–0.157 (0.121 $\pm$ 0.01), 0.134–0.243 (0.195 $\pm$ 0.01), and  $0.323-0.632(0.546\pm0.11)$  mm from the anterior end, respectively. The uterus fills the body and is packed with eggs that obscure the rest of the genitalia. The vagina gives rise to a muscular ovijector that opens ventrally by the vulva, which appears as tetra-radiate and surrounded with two anterior and two posterior fleshy lips that protrude above the surface of body cuticle. Eggs are ellipsoidal, asymmetrical with one side flattened, operculated in the convex side, embryonated in uteri, and measured 0.120-0.139(0.129±0.001)mm long and 0.030-0.052(0.045±0.001)mm wide. The body

| Parasite species                              |                | S. obvelata  | S. obvelata         | S. obvelata            | S. obvelata Landaeta-               | S. obvelata Khalil et al. 2014      | S. obvelata (Present study)         |
|---|----------------|--------------|---------------------|------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Parameters                                    |                | 1061 Kassuri | Oguen 1771          | Maganiacs of al. 1994  | Aqueveque et al. 2007               |                                     |                                     |
| Host  |                | Mus musculus | M. musculus         | M. musculus            | M. musculus                         | M. musculus                         | M. musculus                         |
| Host-locality                                 |                | USA          | Several countries   | Río de Janeiro, Brasil | Santiago, Chile                     | Cairo, Egypt                        | Cairo, Egypt                        |
| Body length                                   |                | 1.334        | 1.13-1.61 (1.35)    | 1-1.1                  | $0.88 - 1.23 \ (1.04 \pm 0.3)$      | $0.432 - 1.09 \ (0.677 \pm 0.234)$  | 0.623-1.130 (0.830±0.11)            |
| Body width (point ant.<br>to foremost mamelon |                | 0.131        | 0.131–0.172 (0.146) | 0.050-0.072            | $0.120-0.190\ (0.150\pm0.04)$       | $0.067 - 0.150 \ (0.108 \pm 0.028)$ | 0.092-0.130 (0.110±0.01)            |
| Distance from anterior                        | Nerve ring     | I            | I                   | 0.090-0.097            | $0.090 - 0.120 \ (0.100 \pm 0.02)$  | $0.040 - 0.126 \ (0.065 \pm 0.028)$ | $0.035-0.132(0.073\pm0.01)$         |
| extremity                                     | Excretory pore | 0.302        | I                   | 0.079                  | $0.170 - 0.220 \ (0.190 \pm 0.028)$ | $0.099 - 0.220 \ (0.134 \pm 0.043)$ | 0.087-0.191 (0.145±0.01)            |
| Total esophagus length                        |                | I            | 0.188-0.249 (0.226) | 0.144-0.158            | $0.180 - 0.260 \ (0.210 \pm 0.030)$ | $0.122 - 0.300 (0.174 \pm 0.065)$   | 0.164-0.280 (0.210±0.01)            |
| Oesophageal bulb lengtl                       | h              | 1            | I                   | 1                      | $0.050 - 0.067 \ (0.060 \pm 0.005)$ | $0.027 - 0.090 (0.047 \pm 0.022)$   | $0.023 - 0.086 \ (0.053 \pm 0.01)$  |
| Oesophageal corpus len                        | gth            | I            | I                   | 1                      | I                                   | $0.016 - 0.045 \ (0.037 \pm 0.010)$ | $0.013 - 0.042 \ (0.030 \pm 0.01)$  |
| Distance from                                 | 1st mamelon    | 0.47         | I                   | I                      | $0.280{-}0.450\ (0.330{\pm}0.070)$  | $0.157 - 0.315 (0.242 \pm 0.064)$   | $0.144 - 0.294 (0.199 \pm 0.011)$   |
| anterior extremity                            | 2nd mamelon    | 0.618        | Ι                   | I                      | $0.330 - 0.520 \ (0.420 \pm 0.090)$ | $0.202 - 0.441 \ (0.300 \pm 0.134)$ | 0.198-0.395 (0.287±0.11)            |
|   | 3rd mamelon    | I            | Ι                   | I                      | 0.5-0.8 (0.57±0.012)                | $0.252 - 0.612 \ (0.393 \pm 0.134)$ | 0.292-0.492 (0.412±0.11)            |
| Distance from cloacal                         | 1st mamelon    | Ι            | Ι                   | I                      | Ι                                   | $0.153 - 0.504 (0.287 \pm 0.115)$   | 0.172-0.483 (0.320±0.11)            |
| opening                                       | 2nd mamelon    | I            | Ι                   | I                      | I                                   | $0.108 - 0.387 (0.215 \pm 0.097)$   | 0.112-0.225 (0.198±0.01)            |
|   | 3rd mamelon    | Ι            | Ι                   | I                      | Ι                                   | $0.058 - 0.207 \ (0.106 \pm 0.059)$ | 0.062-0.196 (0.145±0.01)            |
| Gubernaculum length                           |                | I            | 0.026-0.039 (0.034) | 0.025-0.039            | $0.025 - 0.040 \ (0.032 \pm 0.005)$ | $0.030 - 0.048 \ (0.036 \pm 0.006)$ | $0.028 - 0.036 \ (0.031 \pm 0.001)$ |
| Spicule length                                |                | I            | 0.068-0.089 (0.078) | 0.072-0.082            | $0.055 - 0.085 (0.077 \pm 0.001)$   | $0.058 - 0.097 \ (0.073 \pm 0.015)$ | $0.042 - 0.082 \ (0.054 \pm 0.01)$  |
| Tail length                                   |                | 0.122        | 0.122-0.172 (0.149) | 0.072 - 0.090          | 0.110 - 0.170(0.130 + 0.020)        | 0.094 - 0.117 (0.105 + 0.009)       | 0 073-0 142 (0 120+0 001)           |

| lable 2 Comparative measurements          | of the present lema | ale S. obvelata with previou | isly described species   |                                     |                                     |                                     |
|---|---------------------|------------------------------|--------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Parasite species                          | S. obvelata         | S. obvelata Ogden 1971       | S. obvelata              | S. obvelata Landaeta-               | S. obvelata Khalil et al. 2014      | S. obvelata (Present study)         |
| Parameters                                | 1061 Kassenti       |                              | Ivlagalilacs cl al. 1774 | 1002 is is in the second            |                                     |                                     |
| Host                                      | Mus musculus        | M. musculus                  | M. musculus              | M. musculus                         | M. musculus                         | M. musculus                         |
| Host-locality                             | USA                 | Several countries            | Río de Janeiro, Brasil   | Santiago, Chile                     | Cairo, Egypt                        | Cairo, Egypt                        |
| Body length                               | 5.203               | 3.72-5.61 (4.69)             | 4.5-5                    | $3.7 - 4.8$ $(4.28 \pm 0.48)$       | $1.443 - 3.14(2.12 \pm 0.596)$      | $2.930 - 4.650 (3.540 \pm 0.1)$     |
| Body width (at the level<br>of the vulva) | 0.312               | 0.234-0.372 (0.316)          | 0.245-0.350              | $0.290 - 0.400 \ (0.320 \pm 0.065)$ | 0.081-0.252 (0.037±0.007)           | $0.120 - 0.232 \ (0.156 \pm 0.001)$ |
| Distance from anterior Nerve ring         | I                   | I                            | 0.082 - 0.090            | $0.115 - 0.125(0.120 \pm 0.005)$    | $0.0405 - 0.14 \ (0.081 \pm 0.024)$ | $0.026 - 0.157 (0.121 \pm 0.01)$    |
| extremity Excretory pon                   | e 0.523             | I                            | 0.086                    | $0.250 - 0.510 \ (0.350 \pm 0.110)$ | $0.175 - 0.369 \ (0.260 \pm 0.056)$ | 0.134-0.243 (0.195±0.01)            |
| Vulval openin                             | lg 0.874            | $0.450 - 0.740 \ (0.590)$    | 0.662 - 0.806            | $0.430 - 0.790 \ (0.550 \pm 0.17)$  | $0.279 - 0.585 (0.401 \pm 0.105)$   | 0.323-0.632 (0.546±0.11)            |
| Total esophagus length                    | I                   | 0.329-0.431 (0.362)          | 0.316-0.324              | $0.320 - 0.400 \ (0.360 \pm 0.030)$ | $0.162 - 0.29 (0.233 \pm 0.036)$    | 0.213-0.410 (0.342±0.01)            |
| Oesophageal bulb length                   | I                   | Ι                            | I                        | $0.085 - 0.126(0.111 \pm 0.016)$    | $0.0405 - 0.118 \ (0.065 \pm 0.02)$ | $0.049 - 0.156 (0.110 \pm 0.01)$    |
| Oesophageal corpus length                 | I                   | Ι                            | I                        | I                                   | $0.023 - 0.094 \ (0.063 \pm 0.017)$ | $0.018 - 0.062 \ (0.051 \pm 0.01)$  |
| Tail length                               | 0.762               | 0.680-0.890 (0.780)          | 0.518                    | $0.600 - 0.770 \ (0.670 \pm 0.070)$ | $0.3195 - 0.576 (0.432 \pm 0.103)$  | $0.220 - 0.410 \ (0.361 \pm 0.10)$  |
| Egg length                                | 0.134               | 0.099-0.118                  | 0.118-0.126              | $0.132 - 0.140 \ (0.136 \pm 0.003)$ | 1                                   | 0.120-0.139 (0.129±0.001)           |
| Egg width                                 | 0.036               | 0.036-0.041                  | 0.043 - 0.054            | $0.031 - 0.050 \ (0.040 \pm 0.009)$ | I                                   | $0.030 - 0.052 (0.045 \pm 0.001)$   |
|   |                     |                              |                          |                                     |                                     |                                     |

ended with a conical tail which measured 0.220-0.410(0.361  $\pm 0.10$ )mm long.

Molecular analysis A total of 560 bp was deposited in the GenBank under accession no. KT823411 with 30.12 % GC content for SSU rDNA gene sequences of the present Oxyurid species. Pairwise comparison of the isolated gDNA sequence of the present parasite species with a range of other Oxyuridae species and genotypes revealed a unique sequence. Calculating the percentage of identity between this novel sequences with the other retrieved from Genbank demonstrated a high degree of similarity (>81 %). Comparison of the nucleotide sequences and divergence showed that SSU rDNA of the present Oxyurid species revealed the highest blast scores with a small number of nucleotide differences with other S. obvelata species under the following accession numbers: KF738581, KF738574, KF738575, KF738595, KF738587, KF738573, KF738572, KF738571, KF738568, KF738589, KF738595, KF738585, and KF738582. Phylogenetic analysis produced a neighbor-joining tree constructed with partial sequences and showed that Oxyurid consistently formed two major clades for the most related sister families in the order Oxyurida (Fig. 29). The first one representing the family Heteroxynematidae by EF464551 is Aspiculurs tetraptera while the other representing the family Oxyuridae by three genera were Enterobius, Oxyuris, and Syphacia with sequence similarity ranging between 97 and 91 %. This sequence in conjunction with existing data investigates the placement of this Oxyurid species within the family Oxyuridae. It was shown that the present species is deeply embedded in the genus Syphacia with close relationships to other Syphacia species especially to S. obvelata as putative sister taxon.

### **Taxonomic summary**

Parasite-Syphacia obvelata Rudolphi, 1802 (F: Oxyuridae Cobbold, 1864)

Type-Host—Laboratory mice *Mus musculus* Linnaeus (1758) (F: Muridae)

Site of infection-Small intestine, caecum, colon, and around the anal opening of infected host

Type-locality-Animal House at Cairo University, Egypt Prevalence—75.0 % (45 out of 60)

Specimen deposition-Specimens were deposited in the Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

# Discussion

Oxyurids are cosmopolitan nematode parasites of public health importance (Khalil et al. 2014). Nematodes from the

genus Svphacia (Oxvuridae) are common parasitic pinworms of rodents all over the world (Adamson 1994; Robles and Navone 2007; Milazzo et al. 2010; Sotillo et al. 2012; Verma et al. 2013). Based on morphological characters, the present Oxyurid species have all characteristic features of the genus Syphacia such as less developed lips provided with cephalic papillae and amphidial pores, protruding vulva in females, and three mamelons in males. According to all the above-mentioned characters, the present species identified as S. obvelata and recorded with 75.0 % in the laboratory mice M. musculus represented a high value for prevalence. This results coincided with data obtained by Bluszcz et al. (1987) who stated that mice was found to be naturally infected with Oxyurid species with 11.5-53.6 %, followed by Bazzano et al. (2002) who reported the frequency of S. obvelata in laboratory animals ranged between 9 and 74 %, Izdebska and Rolbiecki (2006) who reported 41.2 % as a level of infection of Syphacia spp. in house mice M. musculus, and Baird et al. (2012) who showed that S. obvelata is a common gastro-intestinal parasite of the house mouse M. musculus with high prevalence in wild mice populations as well as in laboratory animals from breeding facilities. There are higher recorded values of individual prevalence in males (93.33 %) than in females (56.66 %). A higher level of invasiveness in male hosts was also recorded by Klimpel et al. (2007) and Kataranovski et al. (2008). Previous laboratory studies by Behnke (1975) indicated that female M. musculus resist infection more effectively than do males and those older animals are less susceptible to infection.

It was compared morphologically and morphometrically with other Syphacia species as shown in Tables 1 and 2 and showed great extent to other S. obvelata reported in other studies by Hussey (1957), Ogden (1971), Magalhaes et al. (1994), Landaeta-Aqueveque et al. (2007), and Khalil et al. (2014) with few differences in measurements of the different body parts while it differs from other Syphacia species by the structure of the head, shape and size of the spicule and gubernaculum, pronounced post-cloacal papillae, length of tail and number and form of mamelons in males, length of esophagus, position of vulva, and size of eggs in females as stated by Khalil et al. (2014). As it differs from S. australasiensis Smales (2004), S. millardiae Hugot (2005) and S. muris Yamaguti (1935) possess a quadrangular cephalic plate; from S. abertoni Weaver and Smales (2006), S. brevicaudata Weaver and Smales (2008), S. darwini Hugot and Quentin (1985), S. longaecauda Smales (2001), S. ohtaorum Hasegawa (1991), S. pseudomyos Weaver and Smales (2008) possess a laterally elongated cephalic plate; from S. sulawesiensis by longer body length and presence of distinct cervical alae and absence of lateral alae; from S. muris by excretory opening and vulva lies anteriorly, tetra-radiate ovijector protrude above the surface of the cuticle and opens by vulva guarded by four fleshy lips, middle mamelon lies at the middle of body length, pedunculated cloacal papillae; and from S. rifaii and S. sulawesiensis by larger egg length and smaller tail length in male. Molecular biological tools have contributed effectively in association with traditional morphological techniques to be reliable methods for the unequivocal identification and differentiation between closely Oxyurid nematodes (Vermund and Wilson 2000; Chang et al. 2009). In the present study, a nuclear rDNA region of the recovered species was amplified by using SyphaCOIF/SyphaCOIR primers mentioned in previous studies by Okamoto et al. (2007). ITS-1 and ITS-2 regions representing the Oxyurid species identified herein had the sequence similar to homologous regions within the nuclear ribosomal sequence of other S. obvelata described previously. Apparently, the phylogenetic tree estimated in this study strongly supported the higher taxonomic groups of order Oxyurida were family Oxyuridae and Heteroxynematidae. These results coincided with data obtained by Khalil et al. (2014) who stated that order Oxyurida incorporates three families namely: Oxyuridae Cobbold 1864; Pharyngodonidae Travassos 1919; and Heteroxynematidae Skrjabin and Schikhobalova 1948. In addition, Petter and Quentin (2009) included S. obvelata and S. muris with 22 genera in the family Oxyuridae and included Aspiculuris, together with seven genera in the subfamily Heteroxynematinae of the family Heteroxynematidae, this sentence agreed with the present results which assured that Oxyuridae species represented by the genus Svphacia is monophyletic in origin and supported the taxonomic position of the present Syphacia species which is deeply embedded in the genus Syphacia with a close relationship with S. obvelata as a more related sister taxon.

#### Conclusion

The present parasite species was an Oxyurid nematode, termed as *S. obvelata*, with a unique genetic sequence and Laboratory mice *M. musculus* represent as a specific host for it. Rodents are usually infected with a number of zoonotic parasites; hence control of these animals has an important role in safeguarding public health.

Acknowledgments This work is supported by Faculty of Science, Cairo University, Cairo, Egypt. Also, author extends her appreciations to members of Molecular Biology and Genetic Engineering Unit in the Holding Company for Biological Products and Vaccines (VACSERA) in completing this work.

#### Compliance with ethical standards

Conflict of interest I declare that we have no conflict of interest.

# References

- Abdel-Gaber R, Fol M (2015) *Aspicularis tetrapetra* (Nematode, Heteroxynematidae) of laboratory mice *Mus musculus* (Rodentia, Muridae): a potential risk of zoonotic infection for researchers. Cien Tec Viti 30(8):125–136
- Adamson ML (1994) Evolutionary factors influencing the nature of parasite specificity. Parasitology 109:S85–S95
- Ambroise-Thomas P (1990) Molecular probes in the study and diagnosis of parasitic diseases. Ann Parasitol Hum Comp 65(I):83–88
- Baird SJE, Ribas A, Macholan M, Albrecht T, Pialek J, Gouy de Bellocq J (2012) Where are the wormy mice? A re-examination of hybrid parasitism in the European house mouse hybrid zone. Evolution 66(9):2757–2772
- Baker D (1998) Naturel pathogens of laboratory mice, rats and rabbits and their effects on research. Clin Microbiol Rev 11:231–266
- Bazzano T, Restel TI, Pinto RM, Gomes DC (2002) Patterns of infection with nematodes Syphacia obvelata and Aspicularis tetraptera in conventionally maintained laboratory mice. Mem I Oswaldo Cruz 97:847–853
- Behnke JM (1975) Immune expulsion of the nematode Aspiculuris tetraptera from mice given primary and challenge infections. Int J Parasitol 5:511–515
- Bluszcz A, Blaski M, Sabesta R, Szilman P (1987) Helmintofauna drobnych gryzoni (Rodentia) kilku miejscowosci okrêgu katowickiego [Helminth fauna of small rodents (Rodentia) of several locality from the environment of Katowice]. Acta Biol Siles Zool 6:127–129
- Bush AO, Lafferty KD, Lotz J, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revised. J Parasitol 83(4): 575–583
- Chang TK, Liao CW, Haung YC (2009) Prevalence of *Enterobius* vermicularis infection among preschool children in kinder gardens of Taipei City, Taiwan in 2008. Korean J Parasitol 47:185–187
- Cobbold TS (1864) Entozoa: an introduction to the study of helminthology, more particularly to the internal parasites of man. pp 508. Quoted from Petter and Quentin (1976)
- Falcón-Ordaz J, Pulido-Flores G, Monks S (2010) New species of Aspiculuris (Nematoda: Heteroxynematidae), parasite of Mus musculus (Rodentia: Muridae), from Hidalgo, Mexico. Rev Mex Biodiv 81:669–676
- Farrar PL, Wanger JE, Kagiyama N (1994) Syphacia spp. In: Waggie K, Kagiyama N, Allen A, Nomura T (eds) Manual of microbiologic monitoring of laboratory animals, 2nd edn. Natl I Health, USA, pp 219–224
- Hasegawa H (1991) Syphacia (Syphacia) ohtaorum n. sp. (Nematoda: Oxyuridae) from Mus caroli on Okinawa Island, Japan. Syst Parasitol 18:221–226
- Hugot JP (2005) *Syphacia (Syphacia) millardiae* n. sp. (Nematoda: Oxyurida: *Syphaciinae*) from the Soft-Furred Field Rat, *Millardia meltada*, in India. Comp Parasitol 72(1):1–5
- Hugot JP, Quentin JC (1985) Etude morphologique de six espe'ces nouvelles ou peu connues appartenant au genre *Syphacia* (Oxyuridae, Nematoda), parasites de rongeurs Cricetides et Murides. Bull Mus Nat Hist Nat 4 Serie 7:383–400
- Hussey K (1957) *Syphacia muris* vs. *Syphacia obvelata* in laboratory rats and mice. J Parasitol 43:555–559
- Izdebska JN, Rolbiecki L (2006) Correlation between the occurrence of mites (*Demodex* spp.) and nematodes in house mice (*Mus musculus* Linnaeus, 1758) in the Gdañsk urban agglomeration. Biol Lett 43(2):175–178
- Kataranovski D, Vukićević-Radić OD, Kataranovski M, Radović DL, Mirkov II (2008) Helminth fauna of *Mus musculus* Linnaeus 1758 from the suburban area of Belgrade, Serbia. Arch Biol Sci 60(4):609–617

- Khalil AI, Morsy GH, Lashein GH, Abd El-Mottaleb DI, Sharaf AA (2013) Molecular variations based on RAPD among Oxyurids in Egypt. PUJ 6(1):39–50
- Khalil AI, Lashein GH, Morsy GH, Abd El-Mottaleb DI (2014) Oxyurids of wild and laboratory rodents from Egypt. Life Sci J 11(3):94–107
- Klimpel S, Förster M, Günter S (2007) Parasite fauna of the bank vole *Chletrionomys glareolus* in an urban region of Germany: reservoir of zoonotic metazoan parasites? Parasitol Res 102:69–75
- Klimpel S, Abdel-Ghaffar F, Al-Rasheid KA, Aksu G, Fischer K, Strassen B, Mehlhorn H (2011) The effects of different plant extracts on nematodes. Parasitol Res 108(4):1047–1054
- Landaeta-Aqueveque CA, Robles MDR, Cattan PE (2007) The community of gastrointestinal helminths in the house mouse, *Mus musculus*, in Santiago, Chile. Parasitol Latinoam 62:165–169
- Linnaeus C (1758) Systema Naturae, Ed. X. (Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata). Holmiae. Sys Nat, Ed. X. v. 1: i-ii+ 1–824
- Magalhaes R, Vicente J, Noroña D et al (1994) Helminth parasites of conventionally maintained laboratory mice. Mem I Oswaldo Cruz 89:33–40
- Mahmoud AE, Attia RAH, Eldeek HEM, Abdel Baki L, Oshaish HA (2009) Oxyurid nematodes detected by colonoscopy in patients with unexplained abdominal pain. Parasitol Unit J 2(2):93–102
- Mc-Manus DP, Bowles J (1996) Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. Int J Parasitol 26:687–704
- 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(1):S97–S101
- Millazzo C, Ribasa A, Casanova JC et al (2010) Helminths of the brown rat (*Rattus norvegicus*) (Berkenhout, 1769) in the city of Palermo, Italy. Helminthology 47(4):238–240
- Neifer S, Kremsner PG, Weinig M, Harms G, Sahlmüller G, Bienzle U, Heitmann M, Breuer F, Mehlhorn H (1991) Interferon-gamma treatment in mice experimentally infected with Trichinella spiralis. Parasitol Res 77(5):437–442
- Ogden C (1971) Observations in the sistematic of nematodes belonging to the genus *Syphacia* Seurat, 1976. Bull Brit Mus 20:255–291
- Okamoto M, Urushima H, Iwasa M, Hasegawa H (2007) Phylogenetic relationships of rodent pinworms (genus *Syphacia*) in Japan inferred from mitochondrial CO1 gene sequences. J Vet Med Sci 69(5):545– 547
- 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(2):105–109
- Parel JDC, Galula JU, Ooi HK (2008) Characterization of rDNA sequences from *Syphacia obvelata*, *Syphacia muris*, and *Aspiculuris tetraptera* and development of a PCR-based method for identification. Vet Parasitol 153:379–383
- Perec-Matysiak A, Okulewicz A, Hildebrand J, Zalesny G (2006) Helminth parasites of laboratory mice and rats. Wiad Parazytol 52: 99–102
- Petter AJ, Quentin JC (2009) Keys to the genera of the Oxyuroidea. In: Anderson RC, Chabaud AG, Willmott S (eds) CIH keys to the nematode parasites of vertebrates. Com W Agri Bur, England, pp 1-30
- Robles MR, Navone GT (2007) A new species of *Syphacia* (Nematoda: Oxyuridae) from *Oligoryzomys nigripes* (Rodentia: Cricetidae) in Argentina. Parasitol Res 101(4):1069–1075
- Robles MR, Navone GT (2010) Redescription of Syphacia venteli Travassos 1937 (Nematoda: Oxyuridae) from Nectomys squamipes in Argentina and Brazil and description of a new Syphacia from Melanomys caliginosus in Colombia. Parasitol Res 106(5):1117–1126

- Rudolphi CA (1802) Forsetung der Beobachtungen uber die Eingeweide Wurmer. Archiv Zool Zoot 2:1–67
- Semenova SK, Romanova EA, Pyskov AP (1996) Genetic differentiation of helminthes on the basis of data of polymerase chain reaction using random primers. Genetics 32(2):304–309
- Seurat LG (1916) Sur les oxyures des Mammiféres. Can Cream Soc Biol 79:64–68
- Singh B (1997) Molecular methods for diagnosis and epidemiological studies of parasitic infections. Int J Parasitol 27(10):1135–1145
- Skrjabin KI, Schikhobalova ND (1948) Quoted from Petter and Quentin (1976)
- Smales LR (2001) Syphacia longaecauda n. sp. (Nematoda: Oxyuridae) Syphacinea from Melomys spp. (Muridae: Hydromyinae) from Papua New Guinea and Irian Jaya, Indonesia. Parasitology 8:39–43
- Smales LR (2004) Syphacia (Syphacia) australasiensis sp. nov. (Nematoda: Oxyuridae) from Rattus leucopus (muridae) from Papua New Guiena and Australia. Trans R Soc S Aust 128:47–51
- Sotillo J, Trelis M, Cortés A, Luz Valero M, Sánchez del Pino M, Esteban JG, Marcilla A, Toledo R (2012) Proteomic analysis of the pinworm *Syphacia muris* (Nematoda: Oxyuridae), a parasite of laboratory rats. Parasitol Int 61(4):561–564

- 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
- Travassos L (1919) Quoted from Petter and Quentin (2009)
- Verma S, Gaherwal S, Prakash MM, Kanhere RR (2013) Anthelmintic efficacy of Ocimum sanctum against Syphacia muris in mice. Acta Parasitol Glob 4(1):24–28
- Vermund SH, Wilson CM (2000) Pinworm (*Enterobius vermicularis*). Semin Pediatr Infect Dis 11:252–256
- Weaver HJ, Smales LR (2006) Syphacia (Syphacia) abertoni n. sp. (Nematoda: Oxyuridae) from Zyzomys argurus (Thomas) (Rodentia: Muridae) from Northern Australia. Trans R Soc S Aust 131(2):260–210
- Weaver HJ, Smales LR (2008) New species of *Syphacia* (Syphacia) Seurat (Nematoda: Oxyuridae) from Pseudamys species (Rodentia: Muridae) from central Australia. Zootaxa 1775:39–50
- Yamaguti S (1935) Studies on the helminth fauna of Japan. Part 13. Mammalian nematodes. Jpn J Zool 6:433–457