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Original article

Antihelimentic effect of *Androctonus crassicauda* scorpion venom against *Trichuris arvicolae* isolated from *Psammomys obesus* in Egypt

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ABSTRACT

Trichuridae family has a genetic and morphological variability between species affecting rodents, but it is considerably hard to morphologically diagnose species within the genus of *Trichuris* and the individuals of these species are identified according to their host, as it is known that *Trichuris spp*. is strictly host-specific. However, some species lack host specificity. So, it is necessary to use molecular data in order to well identify the *Trichuris* spp. in Egyptian rodents. The host examined in the current research is *Psammomys obesus* and the molecularly identified species from its cecum is *Trichuris arvicolae*. In addition, *Trichuris arvicolae* was subjected to *in vitro* treatment with *Androctonus crassicauda* Crude Venom as a model of natural alternative treatment for gastrointestinal nematodes that increasingly develop anthelmintic drug resistance. The changes in *Trichuris arvicolae* were monitored using scanning electron microscopy, *Androctonus crassicauda* Crude Venom made a significant ultrastructural surface changes in *Trichuris arvicolae*, including marked cuticular sloughing, disintegrated bacillary glands, bursting of vulva and edema of anal region. This study was done for closer identification of *Trichuris spp*. infecting rodents in Egypt and evaluating the efficacy of *Androctonus crassicauda* Crude Venom *in vitro*.

1. Introduction

Muroidae rodents represent common hosts for various parasites, such as tapeworms, roundworms, and coccidians (Rahdar et al., 2016). One member of *Muridae* family is *Psammomys obesus (P. obesus)* (Schmid et al., 1993; El- Salkh et al., 2008). *P. obesus* which is called sand rat has diurnal and terrestrial characteristics (Fichet-Calvet et al., 1999). It is mainly found in North Africa, from Mauritania to Egypt and Sudan, east across the Arabian Peninsula, Asia, Western Asia, and Jordan (Fichet-Calvet et al., 2000). The *Trichuris* spp. isolated from Murinae species around the world, is called *Trichuris muris (T. muris)* (Schrank, 1788) (Tattersall et al., 1994). *T. muris* is similar to *Trichuris trichura (T. trichura)* in its biology, so it is used as a laboratory model for trichuriasis in humans in pharmacological, immunological, and genetic research (Feliu et al., 2000; Foth et al., 2014; Aravindhan and Anand, 2017).

Genus *Trichuris* is commonly isolated from African rodents, the described species were ten (Ribas et al., 2013), also twenty-seven species are found, recorded from rodents on the continents of the new world and isolated from 10 families (Robles, 2011; Robles et al., 2014). Many *Trichuris* kinds were carefully described on the morphological basis (Feliu et al., 2000), however, some species cannot be reliably differentiated, and the difficulty in the morphological characterization of species is due to the overlapping between the characters, which helps in species' differentiation (Gagarin, 1974).

The employment of molecular data to accurately quantify the biodiversity of *Trichuridae* and describe their inter-relationships have become necessary, as many species of the *Trichuris* are mor-

Abbreviations: ACCV, Androctonus crassicauda Crude Venom; GI, gastrointestinal; SEM, scanning electron microscopy; PCR, Polymerase chain reaction; rDNA, nuclear ribosomal DNA; PBS, phosphate-buffered saline; TE, Tris EDTA.

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phologically comparable, though their priorities for a host are variable (Hughes et al., 2020). Polymerase chain reaction (PCR) has many benefits and it is used for the isolation and amplification of the selected gene even from a mixed DNA sample. One of the most popularly used genes for the identification of organisms is the 18S gene, which is available in eukaryotes. Frequently used PCR primers attach to the genes' highly conserved regions, which leads to the amplification of 18S homolog in the chosen parasite (Floyd et al., 2005). Latest investigations have shown that the internal transcribed spacers (ITS1-5.8S-ITS2) of nuclear ribosomal DNA (rDNA) help to correctly identify the associated nematode kinds as genetic markers (Rocío Callejón et al., 2015).

Currently, anthelmintic drugs are the most widely used prophylaxis in the management of gastrointestinal (GI) nematodes (Marco Albonico et al., 1999). However, there is growing evidence of anthelmintic resistance, particularly among GI nematodes in livestock, in places like New Zealand, Australia, South Africa, and South America, so much so that sheep farming has become near impractical on several zones because threefold reluctance to whole three types of anthelmintic drugs (van Wyk et al., 1997). The problem is more extensively felt in plantation where both sheep and goats are pastured (Varady et al., 1993; Gill and Lacey, 1998). Until now, anthelmintic drug resistance is not noticed in humans, but it may be a problem if no serious steps toward this problem will be taken (Coles, 1995; Geerts and Gryseels, 2000). However, in Africa and Australia, there are some observations about less effective anthelmintic drugs, especially in human GI nematode infection (De Clercq et al., 1997; Albonico et al., 2003). In light of this, there is an imperious requirement to improve a novel anthelmintic remedy, which possesses novel mechanisms of action.

The new approaches in helminths' treatment are more directed to venomous animals such as scorpions, which provide a potentially effective safer source of drugs for the treatment of GI nematodes. Many studies demonstrated that scorpion and snake venoms have major impacts on several deadly epidermal protozoan like *Leishmania*, *Plasmodium*, and *Trypanosoma* (Khaleghi Rostamkolaie et al., 2019). The venoms of scorpions contain a number of pharmacological peptides that are of regard to researchers working on medicine creation (Perumal Samy et al., 2017). According to Olivier (1807), the scorpion species *A. crassicauda*, also known as the puffy-tailpieced Arabic, is related to the family of *Buthidae* and among the most threatening kinds in this family, with medicinal significance in the Midst East and Saudi Arabia. Although *Androctonus crassicauda* crude venom (ACCV) is responsible for several human mortalities, the structure, and job of its ingredients were studied but relatively limited. The analysis of ACCV was first done by (Caliskan et al., 2006). The extracted antimicrobial peptides have a broad-spectrum restrained impact versus bacteria (Du et al., 2014). The ACCV was first demonstrated emperically contra *Echinococcus granulosus* protoscolices by some studies (Al-Malki and Abdelsater, 2020; Al-Malki et al., 2022) reporting that it has powerful anthelminthic effects. The aim of our study is to molecularly determine *Trichuris* spp. found in fat sand rats and to study its phylogenetic tree, and also to study the effect of ACCV as a model of human trichuriasis by SEM.

2. Materials & methods

2.1. Preparation and gathering of scorpion venom

The scorpions were gathered, and venoms were obtained and diluted according to the instructions published previously (Al-Malki and Abdelsater, 2020; Al-Malki et al., 2022).

2.2. Animals

In the current study, 25 adult fat sand rats (*P. obesus*) were caught from saline marsh areas (navigations) in the western part of North Shore, Egypt with the assistance of huntsmans and transported to Al-Azhar University (Assiut Branch), Faculty of Science, Parasitology laboratory. Rats were rested in clean cages and held at ambient temperature with a naturalistic 12 h illumination / tenebrous period. They were given a day to acclimatise before being killed by anesthesia (sodium pentobarbital 9.1 mg/kg diluted in sterile 0.9% NaCl, via IM injection), and they were given their special food plant and tap water throughout the experiment.

2.3. Parasitological examination

Twenty-five fat sand rats (*P. obesus*) were sacrificed, and their intestine was opened. The *Trichuris* spp. adults were collected from rats' caecum by the use of feather-weight forceps and put in a sterile Petri dish, then washed several times with physiological saline solution (Anwar et al., 2022). A total of 31 *Trichuris* spp. adults (25 females and 6 males) were collected, 11 Trichuris adults (5 females & 6 males) were stored in 70% alcohol in a sterile falcon tube for molecular characterization, and the rest of the helminths (20 females) were used to study the anthelmintic effect of ACCV by using SEM.

2.4. Evaluation of in vitro anthelmintic efficacy of scorpion venom

Out of 20 adults female *Trichuris* spp. worm set aside for the ACCV anthelminthic study, 16 were incubated in eight Petri dishes, equal to two female worms per dish, containing two concentrations of Hanks' saline solution: one mixed with 100 and another with 200 μg/mL of ACCV. A control group of four female worms, two female worms per well, were incubated with Hanks' saline solution solo. The nematodes were kept at 37 ^{0}C for two hours. The worms were also removed every 30 min and cleaned with PBS several times and promptly stable with 2.5% glutaraldehyde in 0.15 M phosphate buffer at a pH of 7.2 for one hour. After that, the nematodes were prepared for SEM (Stepek et al., 2007). The scan results of the ACCV-treated *Trichuris* spp. worms were compared with those of the non-treated control group.

2.5. DNA extraction

Extraction of the genomic DNA of nematodes was done by using the QIAamp Tissue Kit (Qiagen). This paves the way for DNA purification of the worms and is in line with the manufacturer's protocols. It was further eluted in 200 μl Tris EDTA (TE) buffer. The genomic DNA quality was examined by electrophoresis of 1.5% agarose gel, stained with ethidium bromide (10 μg/mL) (Lázaro-Silva et al., 2015) and OD260 was determined by Nanodrop.

2.6. Amplification

PCR was conducted through the use of a 25 μl volume of solution that contains 12.5 μl Emerald Amp GT PCR master mix, 1 μl of primer F, 1 μl of primer R (10 pmoles), 5.0 μl of template DNA (100 ng), 1 μl of primer R (10 pmoles), 1 μl of primer F, 5.0 μl of template DNA (100 ng) and topped up with nuclease-free water to the final volume. The PCR reaction was conducted within a thermocycler (Biometra) by using the subsequent protocol; five minutes of initial denaturation at 94 °C and subsequent 35 cycles at 94 °C, 54 °C, and 72 °C for 30 s, 40 s, and 1 min, respectively. The last expansion was conducted for 10 min at 72 °C. PCR-derived amplified outputs were electrophoresis on 1.5% agarose gels and speckled with EtBr. Each gel was loaded with a 100 bp ladder (Jena

Bioscience, GmbH, Germany) and subsequently photographed under UV light, alongside the gel documentation system. The DNA sequences of fore and inverse primers called NC5 (5′-CGCGAATRGCTCATTACAACAGC -3′) and NC2 (5′- GGGCGGTATCT-GATCGCC -3′), respectively. This is in accordance with the 3′-5′ ends of the ITS1-5.8S-ITS2 flanking the 18S and 28S gene sites (Floyd et al., 2005).

2.7. Sequences analysis

Purification of PCR products was completed through the use of a QIAquick PCR Product extraction kit. The sequence reaction was done by using the Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer). Purification was subsequently completed through the use of a Centrisep spin column. An Applied Biosystems3130 genetic analyzer (HITACHI, Japan) was used to obtain DNA sequences. The sequence identity was compared with GenBank through BLAST analysis (Basic Local Alignment Search Tool). The phylogenetic tree was formed through the MegAlign module of Laser gene DNA Star version 12.1 (Thompson et al., 1994). Analyses of phylogenetics were conducted through the use of maximum likelihood, neighbor-joining, and maximum parsimony in MEGA6 (Tamura et al., 2013).

3. Results

The nucleotide sequences of the ITS2 region in individual *Trichuris* were deposited in the GenBank with the accession number (Om 757840 FSA). PCR products of this region consisted of 1000 bp. The alignment of the 18S rDNA showed that the ITS2 sequences of our strain were mostly identical to those of the *Trichuris arvicolae (T. arvicolae),* previously described in Spain with accession number HF 586908, and to those of *T. muris* with accession number AF36637 (Fig. 1). The phylogenetic arbor was formed based on the ultimate eventuality process involving *Trichuris, Capillaria, and Trichinella* families as sister Taxa. The phylogenetic tree consisted of two main clades with the family *Trichuridae* as an independent subclade, which is further clustered into 3 subclades: (1) the first subclade contains the strain of our study, which was clustered with *T. arvicolae* as isolated from the Arvicolid host and *T. muris* an independent cluster and a second cluster consisting of *T. vulpis and T. fossor;* (2) the second subclade includes *T. skrjabini*, *T. leporis*, *T. ovis,* and *T. discolor*; (3) The third subclade comprises *T. suis,* and *T. trichiura.* There is a high identity between *T. ovis and T. discolor, T. suis* and *T. trichiura, and between T. leporis* and T. *skrjabini* with two identical species arising from every independent subclade (Fig. 2).

3.1. SEM examination

3.1.1. T. Arvicolae normal adult female

A SEM scan of the normal adult female *T. arvicolae* showed an intact cuticle with a normal architecture of the esophageal region, vulva, and anus opening. The body of *T. arvicolae* is split into two sections: A thin front portion and a thick rear part, the esophagus–intestinal junction is the transference of the superfine to the stout fraction of the soma, the enlarged part of the anterior region of the body showed the cellular esophagus–whip site and the buccal cavity containing a single rudimentary triangular tooth. A scan on the ventrolateral face of the adult female *T. arvicolae* showed the enlarged part of the front zone of the corpus. The scan revealed the cuticle with fine transversal striations, a line separating cuticular striations on one side of the body, and bacillary glands on the other side of the body. The cuticle was transversally striated with a wide longitudinal bacillary band. The anterior region of the worm was covered with cuticular transverse striations, bacillary glands occupying the proximal portion of the bacillary band, and the vulva which was found in the ventrolateral face of the female, and the posterior end of the female, showing the terminal anus (Fig. 3).

3.1.2. Trichuris arvicolae adult females treated with (Androctonus crassicauda) scorpion venom

SEM imaging was done to notice the ultra-structural surface cuticular changes of *T. arvicolae* females after being incubated with ACCV in two doses (100 and 200 µl) during different times (30, 60, 90, and 120 min.) of treatment, which exhibited a range of cuticular damage when compared with the texture, smooth longitudinal ridges and the bacillary glands of normal *T. arvicolae* individuals**,** these ultra-structural deformities increased and become more intense by increasing the incubation time with the ACCV.

3.1.2.1. Cuticular changes at 100 µg/mL concentration. At 30 min, cuticular wrinkles were first observed along with loss of architecture of the female cuticle that was more evident within the anterior region. The area of cuticular inflation with bacillary glands starts to separate, containing the sloughing of small parts of the cuticle. During the second observation at 60 min of the severe edema in the posterior region, sloughed cuticle was present in the anterior region. Internal organs similar to the uterus have bulged and turned edematous. This could be because of cuticular thinning. The edema was continuing by the posterior region thus having an impact on the anal opening. Observation at 90 min revealed the twisting and curving of the posterior region of the worms. Their soma region has extensive sloughing of the cuticle. It may be dehydrated and loss of osmoregulation has happened as evidenced by the sloughing of bacillary glands with furrowing and bulging of inter bacillary ridges. Observation at 120 min showed that the cuticle of the whip legion of the body has experienced partial loss. The mouth opening was collapsed as evidenced by deformity, which can be seen as wrinkles and body collapse (Fig. 4).

3.1.2.2. Cuticular changes at 200 µg/mL concentration.:. At **30 min**, the first observable changes were the bursting of the soma region of the female vulva and the release of numerous eggs, which suggested probable osmoregulatory impairment. At **60 min** of incubation, the cuticle of the whip region of the body showed some wrinkling and cracking. There was also a marked flattening and disorganization of the bacillary glands some of which exhibited pore formation. At **90 min** of incubation, a marked edema and an abnormal twisting of the posterior region can be seen together with some flattened bacillary glands and the cuticle developed several pimples along the soma region. At **120 min** of incubation, only a remnant of the digested cuticle in the anterior region can be seen. The worm's body was extensively wrinkled and cracked, the bacillary gland was flattened and the posterior region was severely inflated (Fig. 5).

4. Discussion

The fat sand rat, *P. obesus* belongs to the largest family of rodents called the Muridae. It is a well-known fact that *Trichuris* spp. is mainly host-specific (Ribas et al., 2013; Swar and Shnawa, 2021). The *Trichuris* species of worms, isolated from the Muridae family of rodents, were known as *T. muris,* while those isolated from the *Arvicolidae* family were known as *T. arvicolae.* These two species of worms are very similar so much so that scientists have decided that the best criterion for recognizing the two kinds is their strict attachment to the host family, which is either *Muridae* or *Arvicolidae*. Previous studies have used materials that were ana-

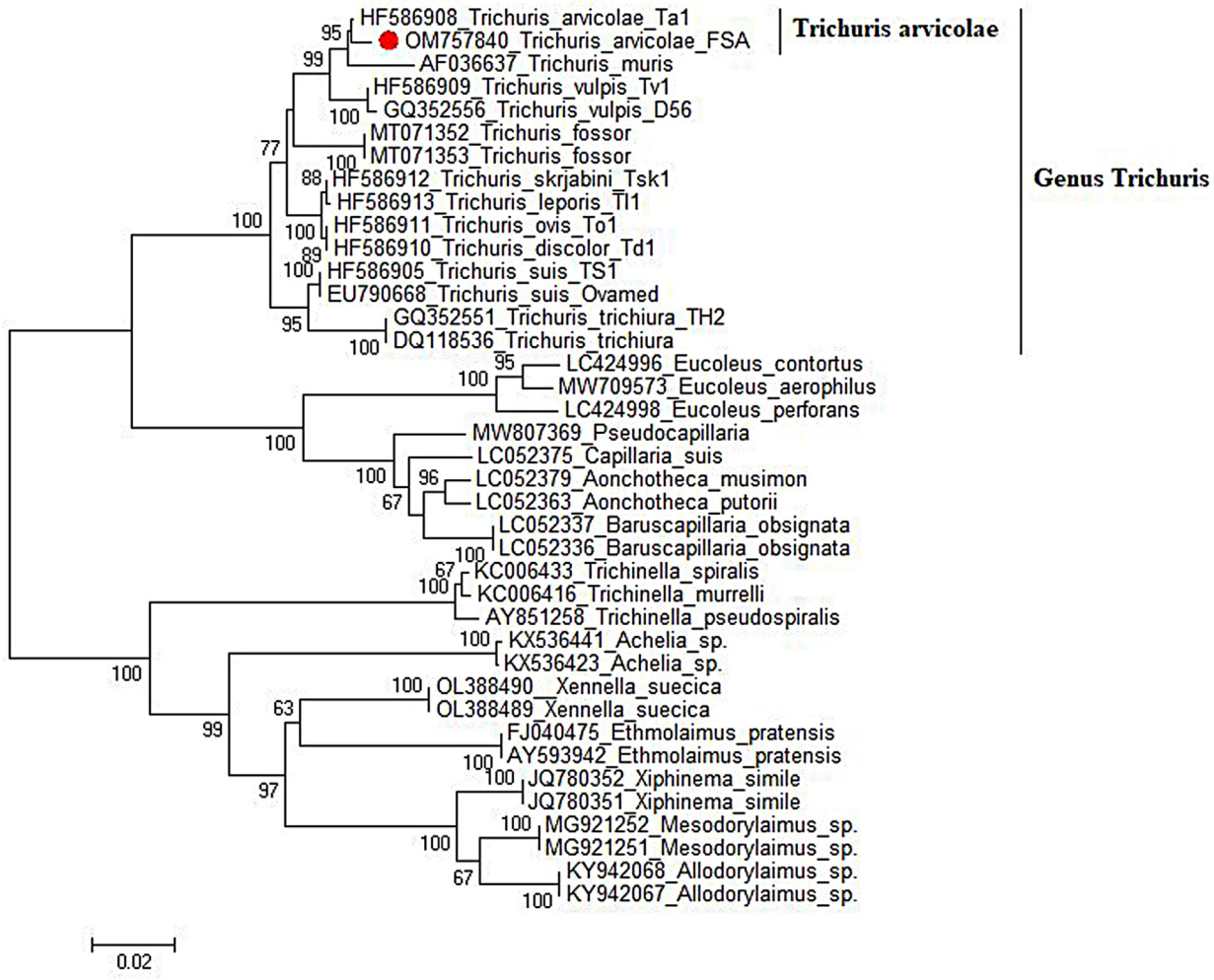

Fig. 1. The phylogenetic tree of *Trichuris arvicolae* isolated from *Psammomys obesus*, the red circle is the diagnosed strain, *Trichuris arvicolae* from Egypt.

Percent Identity

Divergence

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	28	29	
	98.9	97.3	98.1	97.9	96.1	96.0	96.0	95.9	94.8	94.7	93.1	81.2	81.1	79.7	81.1	80.1	79.7	79.2	78.0	78.0	76.6	76.9	76.9	76.2	75.9	75.4	75.3	75.7	1
1.2		96.3	97.9	97.7	95.9	95.8	95.8	95.7	94.4	94.2	92.6	80.6	80.5	79.0	80.5	79.5	79.3	79.0	77.4	77.4	76.3	76.6	76.6	75.8	75.4	75.0	74.7	75.4	2
1.5	2.7		95.5	95.3	93.5	93.4	93.4	93.3	92.3	92.2	90.6	79.6	79.4	78.1	79.4	78.3	78.0	77.5	76.0	76.0	74.9	75.2	75.2	74.0	74.0	73.4	73.3	73.7	3
2.0	2.2	3.6		99.8	95.9	96.0	96.0	95.9	95.0	94.5	92.6	80.6	80.6	79.2	80.6	79.5	79.4	79.1	77.5	77.5	76.7	77.0	77.1	75.9	75.9	75.1	75.2	75.5	4
2.3	2.5	3.8	0.2		95.7	95.8	95.8	95.7	94.8	94.3	92.4	80.4	80.4	79.0	80.4	79.3	79.2	78.9	77.3	77.3	76.5	76.8	76.9	75.7	75.7	74.9	75.0	75.3	5
4.1	4.3	5.6	4.3	4.6		99.2	99.1	99.7	95.3	95.6	94.2	81.5	81.1	80.0	81.2	79.9	79.6	79.4	78.0	78.0	77.0	77.3	77.2	76.1	75.7	75.4	75.5	75.8	6
4.2	4.5	5.7	4.2	4.5	0.6		99.8	99.3	95.5	95.8	94.3	81.7	81.1	80.2	81.4	79.8	79.6	79.4	77.7	77.7	76.5	76.9	77.0	75.8	75.3	75.0	75.1	75.4	7
4.2	4.5	5.8	4.2	4.5	0.7	0.2		99.2	95.4	95.8	94.3	81.8	81.2	80.2	81.6	79.8	79.6	79.4	77.7	77.7	76.5	76.8	76.9	75.6	75.3	75.0	75.1	75.4	8
4.5	4.7	6.0	4.5	4.7	0.2	0.6	0.7		95.2	95.5	94.1	81.5	80.9	79.8	81.2	79.8	79.6	79.5	77.8	77.8	76.9	77.2	77.1	75.9	75.5	75.2	75.3	75.6	9
5.7	6.2	7.1	5.5	5.7	4.9	4.7	4.8	5.1		94.4	92.4	80.1	80.2	79.1	80.3	78.7	79.1	78.6	77.2	77.2	76.3	76.3	76.4	75.7	75.4	74.8	74.2	75.2	10
5.2	5.8	6.7	5.5	5.7	4.2	4.2	4.2	4.5	5.5		97.4	80.9	80.6	79.4	80.6	80.2	80.3	79.5	76.7	76.7	76.2	76.3	76.4	75.1	75.0	74.6	74.7	75.0	11
7.1	7.7	8.6	7.7	7.9	5.8	5.9	5.9	6.0	7.8	2.8		80.7	80.2	79.0	80.7	80.2	80.2	79.5	76.3	76.3	75.5	75.6	75.7	74.6	74.7	74.1	74.2	74.7	12
18.0	18.8	18.8	18.8	19.1	17.6	17.6	17.4	17.7	19.4	19.1	19.3		97.6	91.5	95.4	86.4	86.0	86.0	72.0	72.0	70.6	70.6	70.7	69.9	70.4	69.4	68.3	69.0	13
17.6	18.4	18.5	18.2	18.5	17.6	17.8	17.7	17.9	18.9	18.9	19.4	2.0		92.3	95.7	86.8	86.8	86.4	72.2	72.2	71.1	70.9	71.0	70.3	70.9	69.8	68.8	69.4	14
17.3	18.3	18.3	18.0	18.3	16.9	16.9	16.9	17.3	18.3	18.4	19.0	6.0	5.6		91.1	84.4	84.4	84.5	71.8	71.8	70.0	70.3	70.5	69.2	70.5	69.6	68.8	69.2	15
17.4	18.2	18.4	18.1	18.4	17.3	17.3	17.0	17.4	18.4	18.8	18.6	3.7	3.4	5.7		85.9	85.9	85.3	73.0	73.0	70.4	70.4	70.5	70.2	71.7	70.8	69.5	70.9	16
19.6	20.5	20.8	20.5	20.8	19.9	20.3	20.3	20.2	21.8	20.1	20.1	13.1	12.8	13.6	13.8		96.2	94.3	71.9	71.9	71.4	71.3	71.7	71.5	70.1	69.0	68.6	69.1	17
20.1	20.7	21.3	20.6	20.9	20.3	20.6	20.6	20.5	21.2	20.0	20.1	13.6	12.7	13.5	13.7	4.0		96.1	71.4	71.4	71.1	71.1	71.5	70.6	69.9	68.9	68.5	69.1	18
20.9	21.2	22.1	21.1	21.4	20.7	21.0	21.0	20.7	22.0	21.2	21.2	14.0	13.6	14.3	13.7	4.8	3.0		70.9	70.9	70.5	70.8	71.0	70.4	69.1	68.4	67.8	68.7	19
21.4	22.3	22.9	22.1	22.4	21.4	21.6	21.6	21.6	22.5	23.0	23.6	27.2	27.4	27.4	27.5	29.5	30.3	30.6		100.0	82.8	82.6	82.8	84.7	91.5	89.7	88.9	89.0	20
21.4	22.3	22.9	22.1	22.4	21.4	21.6	21.6	21.6	22.5	23.0	23.6	27.2	27.4	27.4	27.5	29.5	30.3	30.6	0.0		82.8	82.6	82.8	84.7	91.5	89.7	88.9	89.0	21
25.0	25.4	26.1	24.8	25.1	24.4	24.9	24.9	24.4	25.6	25.2	26.4	31.3	30.3	30.5	31.2	31.3	31.8	32.5	17.1	17.1		98.1	98.3	78.6	82.2	81.5	81.0	81.7	22
24.3	24.7	25.4	24.1	24.4	23.7	24.0	24.2	23.7	25.4	24.8	26.0	31.0	30.4	30.0	30.9	31.5	31.8	32.0	17.4	17.4	1.8		99.4	78.7	82.3	81.5	81.0	81.7	23
24.3	24.7	25.4	24.0	24.3	23.9	23.9	24.0	23.9	25.2	24.7	25.8	30.8	30.2	29.7	30.7	30.8	31.1	31.7	17.1	17.1	1.6	0.6		78.7	82.4	81.4	80.9	81.6	24
26.1	26.7	28.0	26.5	26.8	26.2	26.3	26.6	26.3	26.9	27.3	28.1	32.5	32.0	32.5	31.9	31.4	32.6	32.7	14.0	14.0	22.4	22.0	22.0		81.8	82.4	81.5	82.1	25
24.4	25.1	25.8	24.3	24.6	24.7	25.0	25.0	24.8	25.0	25.4	25.8	29.8	29.5	29.0	29.6	32.3	32.6	33.4	9.1	9.1	17.6	17.5	17.3	17.3		88.3	87.6	87.6	26
25.4	26.0	27.1	25.9	26.2	25.5	25.8	25.8	25.6	26.3	26.4	27.2	31.9	31.7	31.2	31.4	34.7	34.8	35.1	11.6	11.6	19.2	19.2	19.3	17.0	12.6		96.6	96.0	27
25.6	26.5	27.3	25.7	26.0	25.3	25.6	25.6	25.4	27.3	26.2	27.0	33.9	33.5	32.6	33.8	35.4	35.5	36.2	12.7	12.7	19.9	19.9	20.0	18.2	13.5	3.9		94.2	28
25.5	25.9	27.2	25.8	26.1	25.4	25.7	25.7	25.5	26.2	26.3	26.7	32.9	32.6	32.1	31.5	35.0	34.9	35.1	12.1	12.1	19.2	19.2	19.3	17.9	13.1	4.0	6.1		29
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	28	29	

1 HF586908 Trichuris arvicolae Ta1
2 OM757840 Trichuris arvicolae FSA
3 AF036637 Trichuris muris
4 HF586909 Trichuris vulpis Tv1
5 GQ352556 Trichuris vulpis D56
6 HF586912 Trichuris skrjabini Tsk1
7 HF586911 Trichuris ovis To1
8 HF586910 Trichuris discolor Td1
9 HF586913 Trichuris leporis Tl1
10 MT071352 Trichuris fossor
11 HF586905 Trichuris suis TS1
12 DQ118536 Trichuris trichiura
13 LC052379 Aonchotheca musimon
14 LC052363 Aonchotheca putorii
15 MW807369 Pseudocapillaria
16 LC052375 Capillaria suis
17 LC424998 Eucoleus perforans
18 LC424996 Eucoleus contortus
19 MW709573 Eucoleus aerophilus
20 OL388490 Xennella suecica
21 OL388489 Xennella suecica
22 AY851258 Trichinella pseudospiralis
23 KC006433 Trichinella spiralis
24 KC006416 Trichinella murrelli
25 KX536441 Achelia sp.
26 FJ040475 Ethmolaimus pratensis
27 MG921252 Mesodorylaimus sp.
28 KY942068 Allodorylaimus sp.
29 JQ780352 Xiphinema simile

Fig. 2. Percent identity between Trichuris spp.

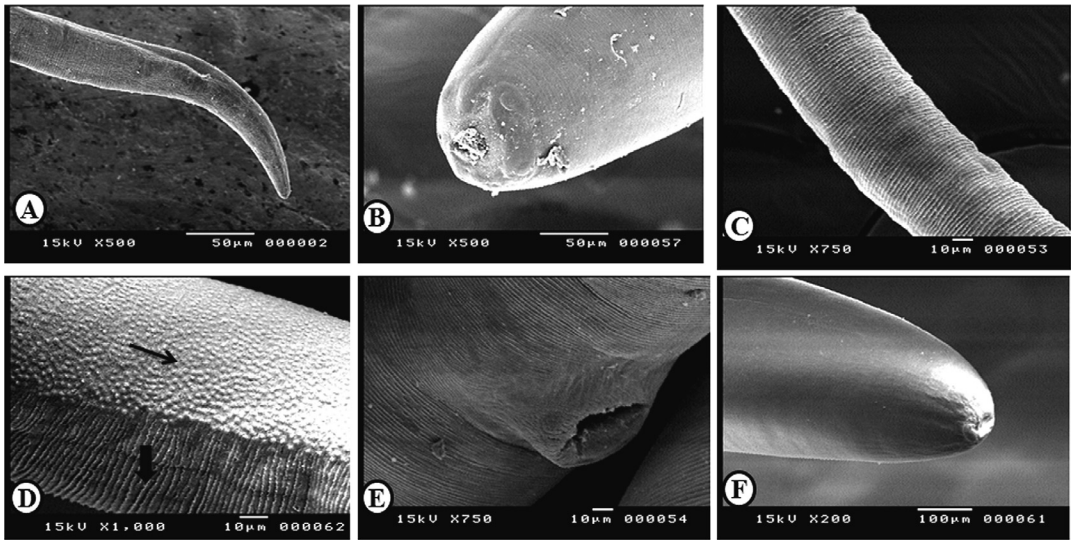

Fig. 3. Photomicrograph of scan electron microscopy of normal female *Trichuris arvicolae* showing: (A) Thin anterior portion of the body (B) anterior region of the worm, note mouth opening is smooth but contains sensory papillae. (C) Anterior region of the body showing the cellular esophagus-whip region, note the cuticle with fine transversal striations. (D) A middle region showing the cuticle's fine transversal striations (thick arrow) and the bacillary glands on the other side of the body (thin arrow). (E) The ventrolateral face of the female shows the vulva and the detail of the vulva opening with an absent spine. (F) The posterior end of the female *Trichuris arvicolae* shows the terminal anus.

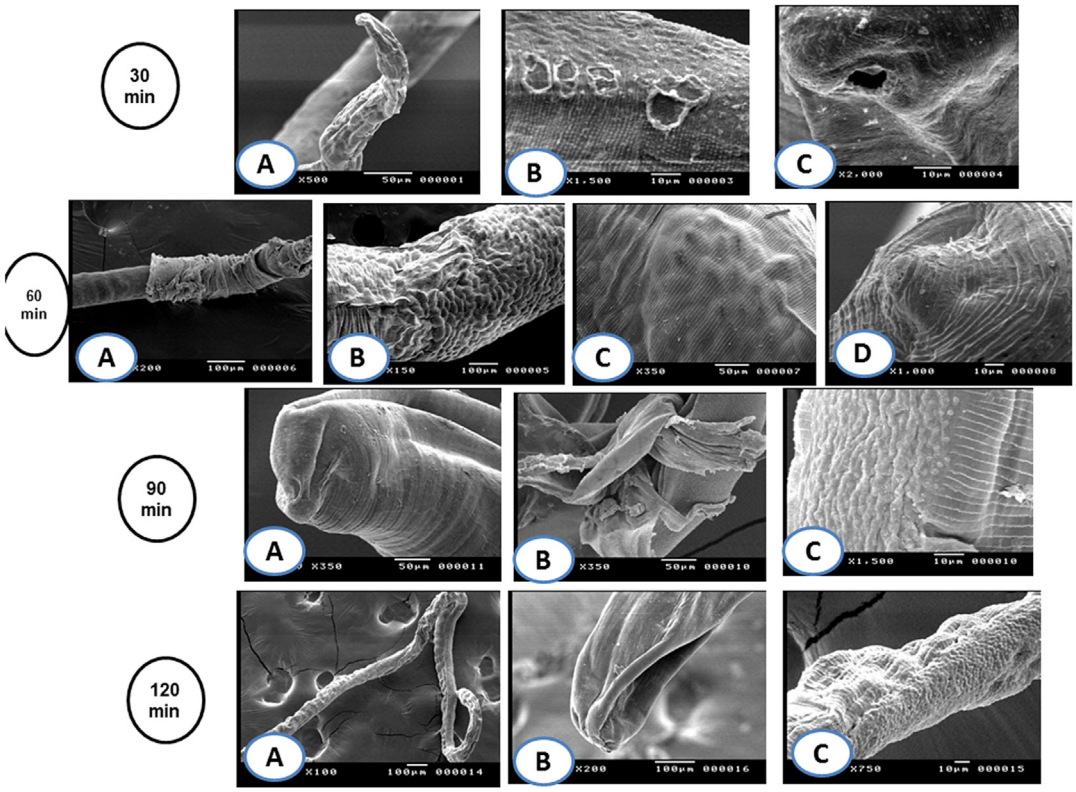

Fig. 4. The EM imaging of adult females of *Trichuris arvicolae* changes at 100 µg/mL conc. **At 30 min:** (A) wrinkles, furrowing and shrinking of the cuticle of the anterior whip region of the body, (B) bacillary glands were sloughed (C) severe edematous region at the posterior end of the worm. At **60 min**: (A) Note cuticle digestion at the anterior part, (B) marked deformity in the female uterus, (C) cuticle damage and thinning so the uterus has bulged (D) Anus opening damage and edema. At **90 min:** (A) twisted mouth, (B) sloughing of the cuticle, (C) flattened bacillary glands, and furrowing of interpapillary ridges. At **120 min:** (A) partial loss of cuticle of whip region of the body, (B) collapsed mouth opening, (C) deformities in the anterior part of the body.

lyzed using electrophoresis and shown that there was no host switching between these two species (Feliu et al., 2000; Rocio Callejón et al., 2010). Phylogenetic reconstructions of *T. muris* worms from the various *Muridae* rodent hosts have shown that they were not distinguished based on the type of hosts they parasitize. In conclusion, the *T. muris* worms exhibited no host particularity where they did not have breeds that disconnect through host kinds. Hence, the *Trichuris* spp. obtained from *P. obesus* in Egypt

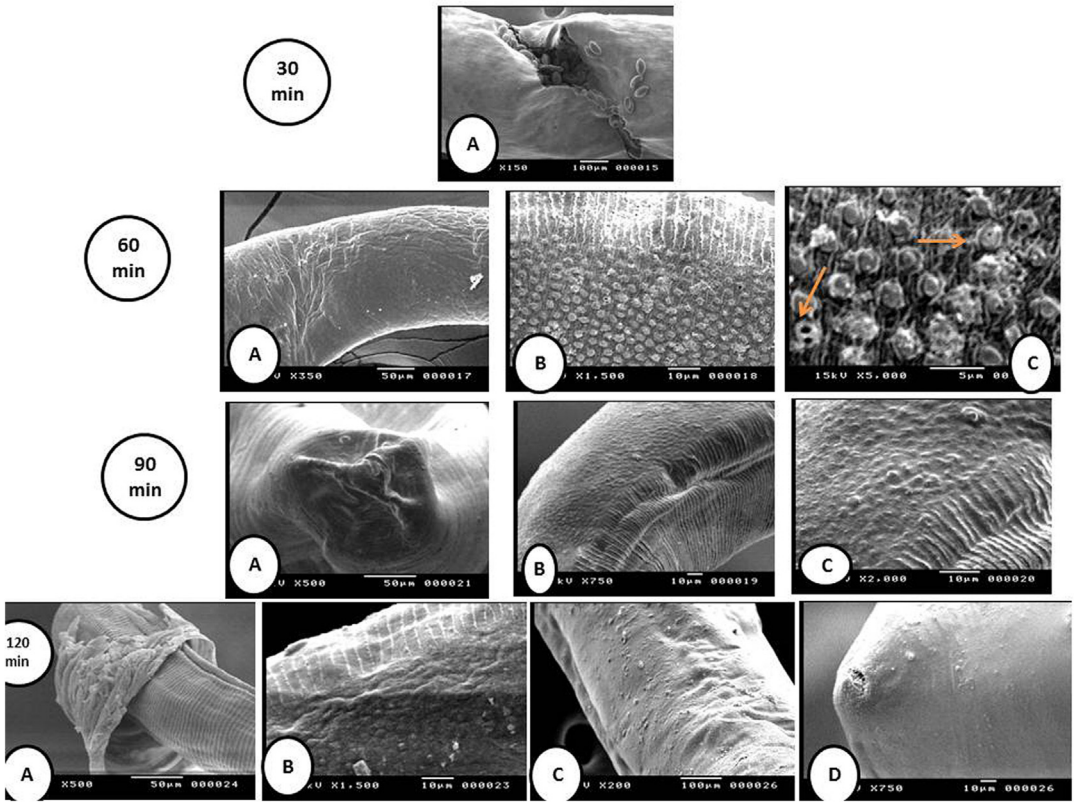

Fig. 5. The SEM imaging of adult females of *Trichuris arvicolae* changes at 200 µg/mL conc. At 30 min. (A) bursting of the vulva and release of eggs. At **60 min:** (A) shrinking and furrowing surface at the anterior region, (B) flattened bacillary glands, (C) perforation & disintegration of some bacillary glands (arrows). At **90 min**: (A) twisted and edematous mouth, (B, and C) sloughed and flattened bacillary gland. At **120 min:** (A) cuticle digestion in the anterior part, (B) the longitudinal ridges of the cuticle became transverse with severe flattening and deformation of the bacillary gland (arrow), (C) enlarged part of the body's anterior region folds on the tegument, (D) edema and malformation of the posterior end of the worm.

was described as *T. muris* (Banaja et al., 2012; Anwar et al., 2022) but there is no data about the host, specificity in the case of *T. arvicolae* as it was considered as a novel race till 2000 (Feliu et al., 2000)**,** although the host in this study belonging to the family of *Muridae,* the recovered species identified molecularly as *T. arvicolae*, which is the first report of in Egypt rodents. This may conflict with a less host specificity in *T. arvicolae* than *T. muris*, or the high genetic similarity between the two species. Nuclear rDNA, especially the 18S rDNA, which is widely used for nematode phylogenetic analysis (Meldal et al., 2007; Van Megen et al., 2009)**,** even among the primary clades in the nematode phylogenetic tree (Fitch, 1997; Nadler et al., 2007)**,** now it is known that they are the preferable molecular indications for studying and resolving the genetic phylogenetic tree, and relevances between *Trichuris species* is the rDNA segments in ITS1 and ITS2 regions. The extension of the ITSs of 7 diverse *Trichuris* spp. shows similarities with sequences of *Trichuris leporis* **Froelich 1789**, *T. ovis*, *T. muris*, *T. arvicolae*, *T. suis*, *T. discolor*, and *T. trichiura*, which allows precise classification. Moreover, the comparative phylogenetic tree study of ITS1 and ITS2 series from different *Trichuris* spp. parasitizing several hosts manifested the presence of 3 various genetic origins related to host collections (Rocío Callejón et al., 2016).

The phylogenetic tree in the present study showed that *T. Trichinella*, and *Capillariid spp*. represented monophyletic groups with 100% support. The *Capillariidae* and *Trichuridae* clades represented two sister taxa (100% support). These data are compatible with the findings of earlier investigations (Hughes et al., 2020).

Our new sequence found within the *Trichuris spp*. clade and the similarity percentage between described *Trichuris* spp. ranged from 92.6% to 98.9%. Three subclades were present within *Trichuris* family: (1) the first subclade where our strain was clustered with *T. arvicolae* isolated from *Myodes glareolus* (*Arvicolidae*) from Spain with the highest identity (98.9%) (Rocío Callejón et al., 2013), and *T. muris* with 96.3% identity (Blaxter et al., 1998) in an independent cluster, the following cluster includes *T. vulpis* isolated from *Canis lupus familiaris* from Spain with 97.9% identity (Rocío Callejón et al., 2013)**,** *T. vulpis* from dogs in Thailand with 97.7% (Areekul et al., 2010) and *T. fossor* isolated from *Thamomyes* spp. in the United States (94.3%) (Hughes et al., 2020); (2) the second subclade including *T. skrjabini*, isolated from *Capra hircus* in Spain (95.9%) (Rocío Callejón et al., 2013)**,** *T. leporis* from *Lepus europaeus* from Spain (95.7%) (Rocío Callejón et al., 2013)**,** *T. ovis* from *Capra hercus* from Spain (95.8%) (Rocío Callejón et al., 2013) and *T. discolor* secluded from *Bos Taurus* from Spain (95.8%) (Rocío Callejón et al., 2013); (3) the third subclade includes *T. suis* secluded from *Sus scrofa domestica* in Spain and Netherlands (94.2%) (Holterman et al., 2006; Rocío Callejón et al., 2013)**,** respectively, and *T. trichura* isolated from Thailand and Edinburg (Will, 2005; Areekul et al., 2010)**,** respectively with 92.6% identity. The highest resemblance scores for personated kinds were found among *T. ovis* and *T. discolor* (99.8%). These data are similar to previous data (Rocío Callejón et al., 2013)**,** also proving that there is a high similarity between *T. muris* and *T. arvicolae*. The present study revealed that our strain is very close to strains in Spain, owing to the hypothesis of the sole driver of speciation, which is not dependent on the geographic distance alone (Ribas et al., 2020).

Alternative therapies, involving plant extracts and natural toxins with their derivatives are increasingly being applied to cure a variety of human illnesses, including carcinoma and germ contagions (Ortiz et al., 2020). The broad and indiscriminate use of drugs

has led to a growing resistance against numerous medications, including many anthelmintic drugs. This has also resulted in many adverse side effects that harm both human and animal populations. All of these factors have driven the world to search for more potent biological products for the cure of diseases without hostile side effects. New developments in drug research have turned to materials extracted from venomous creatures, such as scorpions as potential nominees for forthcoming medications, which offer innovative approaches to treatment (Perumal Samy et al., 2017). The scorpion venoms contain several pharmacological peptides, which attracted the interest of investigators who are in search of developing new remedies (Perumal Samy et al., 2017). The candidate venom in this study is the ACCV. It has been proven that the natural venom of a scorpion contains molecules, with antibacterial, antifungal, and antiparasitic properties (Yacoub et al., 2020). However, Up till now it didn't tested to be used invivo. *Androctonus crassicauda*, its LD50 is very low which may need many precautions before being applied. These molecules are considered to be a lot safer than synthetic drugs and also have the ability to avoid drug resistance (Khaleghi Rostamkolaie et al., 2019).

Trichuris spp. isolated from rodents in Egypt were classified as *T. muris* and no further molecular study was performed on this species in Egypt, as it is well established as *T. muris,* according to its morphological characterization (Banaja et al., 2012; Anwar et al., 2022)**,** which become now a popular model for different researches as a model for human and animal trichuriasis (Klementowicz et al., 2012; Mair et al., 2021).

The current survey was designed to define the anthelmintic influence of ACCV on *T. arvicolae,* as a candidate antiparasitic treatment.

In the present study, SEM imaging revealed several ultrastructural damage changes, related linearly with increased concentration and duration, with the venom incubation.

A previous research was performed to assess the effectiveness of nitazoxanide against *A. ceylanicum* and *T. muris* as a well-known parasite-rodent model, mirrored human helminthiases which are soil-transmitted. There were no SEM cuticular changes by (200 μg/mL) on the *T. muris* when contrast to controls (Tritten et al., 2012). From the previous data, we concluded that ACCV may be more powerful than nitazoxanide, another study used SEM imaging on *T. muris* incubated with a range of naturalist vegetation cysteine proteinases in lab. Transverse wrinkling was found in 30 min, followed by damage to the cuticle at 60 min, then signs of cuticle digestion at 120 min (Stepek et al., 2006)**,** these data closely agree with our SEM results, hence, the effect of these plant enzymes may be as powerful as the ACCV effect, as an antiparasitic candidate.

The scorpion venom's anthelmintic properties effectively extend to cestodes, its effect was studied by (Al-Malki and Abdelsater, 2020)**,** reporting that ACCV is as powerful as it could kill all the protoscolices of hydatid cysts during 240 min, while (Al-Malki et al., 2022) demonstrated that the protoscolices brood at 100 μg/mL, expressed caspase-3 that was noticeably elevated. than those brood with 50 μg/mL of ACCV. Also, ACCV give rise to distinguished ultrastructural and morphological variations in protoscolices, which are detected by TEM, light, and SEM imaging, these changes include tegumental damage, contraction of soma region, disorganization of the rostellum, loss of hooks and shedding of microtriches in scolex regions. Regarding nematodes, a previous study concluded that scorpion venom has a cytotoxic impact on larvae of *Ancylostoma caninum* (Xu et al., 2008). Scorpion venom is also effective as an antiprotozoal candidate, a previous study proved that *Tityus discrepans* scorpion venom led to reduced growth of *Leishmania mexicana* promastigotes in the culture media, it also gave rise to morphological changes as the promastigotes became completely round and vacuolated and lost their elongated shape, with disturbed flagellar movement (Borges et al., 2006). Another *in vitro* study on *Leishmania tropica* stated that ACCV had *in vitro* lethal effect on *L. tropica* promastigotes (Yavuzcan Yildiz et al., 2019). In another study, it was proven that scorpion venom had anti-malarial properties. It decreased *P. berghei* growth and eliminated intra-RBCs *P. falciparum* at low concentricity. It was also shown that even at 50 mM condensation, scorpion venom had no impact on the viability of human GC-2 cells, and at 100 mM condensation, it had no hemolytic influence on rat RBCs (Adade and Souto-Padrón, 2015).

5. Conclusion

This study provides a re-description of *T. muris* molecularly and identifies that it is *T. arvicolae*, which reveals the importance of molecular identification of Trichuris spp. in Egypt, also this research provided a clear evaluation of the effect of black scorpion venom on these prevalent nematodes. This is the first time that the anthelmintic impact of ACCV has been researched versus GI nematodes, to the best of the authors' knowing. Further investigations are necessary to determine the active components by purifying the crude venom before being applied in vivo, because of the scorpion *Androtonus crassicauda* is a deadly scorpion and has a very low LD50 value. If so, the venom from scorpions would be suitable for the development of essential new treatment, and the help in the management of GI helminths of humans and livestock.

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None.

Ethics statement

All of the techniques used in this study have been agreeable and licensed by the ethics committee for scientific research at the faculty of science at Al-Azhar University (Assiut Branch), Egypt. It complies with the moral standards set forth in pertinent national regulations governing the handling and use of experimental animals. AZHAR 6/2022 is the certificate reference number.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Adade, C.M., Souto-Padrón, T., 2015. Venoms as sources of novel anti-parasitic agents. In: Toxins and Drug Discovery (Issue April). 10.1007/978-94-007-6726-3_4-1.

Albonico, M., Crompton, D.W.T., Savioli, L., 1999. Control strategies for human intestinal nematode infections. Adv. Parasitol. 42, 277–341.

Albonico, M., Bickle, Q., Ramsan, M., Montresor, A., Savioli, L., Taylor, M., 2003. Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. Bull. World Health Organ. 81 (5), 343–352.

Al-Malki, E.S., Abdelsater, N., 2020. In vitro Scolicidal effects of *Androctonus crassicauda* (Olivier, 1807) venom against the protoscolices of *Echinococcus granulosus*. Saudi J. Biol. Sci. 27 (7), 1760–1765. https://doi.org/10.1016/j.sjbs.2020.05.014.

Al-Malki, E.S., Aljedaie, M.M., Amer, O.S.O., Abdelsater, N., Badry, A., 2022. Scorpion crude venom induced apoptosis and structural changes of *Echinococcus granulosus* protoscolices. J. King Saud Univ. – Sci. 34, (4). https://doi.org/10.1016/j.jksus.2022.101937 101937.

Anwar, F.A.S., Tolba, M., Abdel-gahfar, S.K., Omar, H.E.M., 2022. Intestinal helminth infestation of sand rats (*Psammomys obesus*) collected from north coast egypt ecological morphological and histopathological approach 31 (xxx), 1–11.

Aravindhan, V., Anand, G., 2017. Review article: Cell type-specific immunomodulation induced by helminthes: effect on metainflammation, insulin resistance and type-2 diabetes. Am. J. Trop. Med. Hyg. 97 (6), 1650–1661. https://doi.org/10.4269/ajtmh.17-0236.

Areekul, P., Putaporntip, C., Pattanawong, U., Sitthicharoenchai, P., Jongwutiwes, S., 2010. *Trichuris vulpis* and *T. trichiura* infections among schoolchildren of a rural community in northwestern Thailand: the possible role of dogs in disease transmission. Asian Biomed. 4 (1), 49–60. https://doi.org/10.2478/abm-2010-0006.

Banaja, A.E.A., Soliman, M.I., Taha, H.A., Ashour, A.A., 2012. SEM studies on *Trichurus muris* from *Psammomys obessus* from Sinai Peninsula, Egypt. J. Egypt. Soc. Parasitol. 42 (2), 453–460. https://doi.org/10.12816/0006331.

Blaxter, M.L., Dorris, M., Frisse, L.M., Vida, J.T., Thomas, W.K., 1998. A molecular evolutionary framework for the phylum Nematoda Mark. 392(March).

Borges, A., Silva, S., Op Den Camp, H.J.M., Velasco, E., Alvarez, M., Alfonzo, M.J.M., Jorquera, A., De Sousa, L., Delgado, O., 2006. In vitro leishmanicidal activity of *Tityus discrepans* scorpion venom. Parasitol. Res. 99 (2), 167–173. https://doi.org/10.1007/s00436-006-0133-z.

Caliskan, F., García, B.I., Coronas, F.I.V., Batista, C.V.F., Zamudio, F.Z., Possani, L.D., 2006. Characterization of venom components from the scorpion *Androctonus crassicauda* of Turkey: peptides and genes. Toxicon: Off. J. Int. Soc. Toxinol. 48 (1), 12–22. https://doi.org/10.1016/j.toxicon.2006.04.003.

Callejón, R., Nadler, S., De Rojas, M., Zurita, A., Petrášová, J., Cutillas, C., 2013. Molecular characterization and phylogeny of whipworm nematodes inferred from DNA sequences of cox1 mtDNA and 18S rDNA. Parasitol. Res. 112 (11), 3933–3949. https://doi.org/10.1007/s00436-013-3584-z.

Callejón, R., Cutillas, C., Nadler, S.A., 2015. Nuclear and mitochondrial genes for inferring Trichuris phylogeny. Parasitol. Res. 114 (12), 4591–4599. https://doi.org/10.1007/s00436-015-4705-7.

Callejón, R., Del, M., Robles, R., Panei, C.J., Cutillas, C., 2016. Molecular diversification of *Trichuris* spp. from Sigmodontinae (Cricetidae) rodents from Argentina based on mitochondrial DNA sequences. Parasitol. Res., 2933–2945 https://doi.org/10.1007/s00436-016-5045-y.

Callejón, Rocio, Rojas, M. De, Nieberding, C., Foronda, P., Feliú, C., Guevara, D., Cutillas, C., 2010. Molecular evolution of *Trichuris muris* isolated from different Muridae hosts in Europe. 631–641. 10.1007/s00436-010-1908-9.

Coles, G.C., 1995. Chemotherapy of human nematodes: learning from the problems in sheep. J. Roy. Soc. Med. 88 (11), 649P–651P. https://doi.org/10.1177/014107689508801112.

De Clercq, D., Sacko, M., Behnke, J., Gilbert, F., Dorny, P., Vercruysse, J., 1997. Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. Am. J. Trop. Med. Hyg. 57 (1), 25–30. https://doi.org/10.4269/ajtmh.1997.57.25.

Du, Q., Hou, X., Ge, L., Li, R., Zhou, M., Wang, H., Wang, L., Wei, M., Chen, T., Shaw, C., 2014. Cationicity-enhanced analogues of the antimicrobial peptides, AcrAP1 and AcrAP2, from the venom of the scorpion, *Androctonus crassicauda*, display potent growth modulation effects on human cancer cell lines. Int. J. Biol. Sci. 10 (10), 1097–1107. https://doi.org/10.7150/ijbs.9859.

El-Salkh, B.A., Khidr, H.A., Zaki, Z.T., Basuony, M.I., 2008. Anatomical, histological and histochemical studies on some organs of true desert rodents in the Egyptian habitats. Egypt. J. Hospital Med. 33(1), 578–306. 10.21608/ejhm.2008.17622.

Feliu, C., Spakulová, M., Casanova, J.C., Renaud, F., Morand, S., Hugot, J.P., Santalla, F., Durand, P., 2000. Genetic and morphological heterogeneity in small rodent whipworms in southwestern Europe: characterization of *Trichuris muris* and description of *Trichuris arvicolae* n. *Sp. (Nematoda: Trichuridae*). J. Parasitol. 86 (3), 442–449. https://doi.org/10.1645/0022-3395(2000)086[0442:gamhis]2.0.co;2.

Fichet-Calvet, E., Jomâa, I., Ben Ismail, R., Ashford, R.W., 1999. Reproduction and abundance of the fat sand rat (Psammomys obesus) in relation to weather conditions in Tunisia. J. Zool. 248 (1), 15–26. https://doi.org/10.1017/S0952836999005026.

Fichet-Calvet, E., Jomâa, I., Ben Ismail, R., Ashford, R.W., 2000. Patterns of infection of haemoparasites in the fat sand rat, Psammomys obesus, in Tunisia, and effect on the host. Ann. Trop. Med. Parasitol. 94 (1), 55–68. https://doi.org/10.1080/00034983.2000.11813513.

Fitch, D.H.A., 1997. Evolution of male tail development in rhabditid nematodes related to *Caenorhabditis elegans*. Syst. Biol. 46 (1), 145–179. https://doi.org/10.1093/sysbio/46.1.145.

Floyd, R.M., Rogers, A.D., Lambshead, P.J.D., Smith, C.R., 2005. Nematode-specific PCR primers for the 18S small subunit rRNA gene. Mol. Ecol. Notes 5 (3), 611–612. https://doi.org/10.1111/j.1471-8286.2005.01009.x.

Foth, B.J., Tsai, I.J., Reid, A.J., Bancroft, A.J., Nichol, S., Tracey, A., Holroyd, N., Cotton, J. A., Stanley, E.J., Zarowiecki, M., Liu, J.Z., Huckvale, T., Cooper, P.J., Grencis, R.K., Berriman, M., 2014. Whipworm genome and dual-species transcriptome analyses provide molecular insights into an intimate host-parasite interaction. Nat. Genet. 46 (7), 693–700. https://doi.org/10.1038/ng.3010.

Gagarin, V.G., 1974. Critical evaluation of measured characters in the differentiation of helminth species (on the model of trichocephalids). Fauna Gelmintov Zivotnych y Rastenij Kirgizii, 3–9.

Geerts, S., Gryseels, B., 2000. Drug resistance in human helminths: current situation and lessons from livestock. Clin. Microbiol. Rev. 13 (2), 207–222. https://doi.org/10.1128/CMR.13.2.207.

Gill, J.H., Lacey, E., 1998. Avermectin\milbemycin resistance in trichostrongyloid nematodes. Int. J. Parasitol. 28 (6), 863–877. https://doi.org/10.1016/S0020-7519(98)00068-X.

Holterman, M., Van Der Wurff, A., Van Den Elsen, S., Van Megen, H., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol. Biol. Evol. 23 (9), 1792–1800. https://doi.org/10.1093/molbev/msl044.

Hughes, M.R., Duffield, D.A., Howe, D.K., Denver, D.R., 2020. First report of molecular characterization and phylogeny of Trichuris fossor Hall, 1916 (Nematoda: Trichuridae). J. Nematol. 52, 1–6. https://doi.org/10.21307/JOFNEM-2020-036.

Khaleghi Rostamkolaie, L., Hamidinejat, H., Razi Jalali, M.H., Jafari, H., Najafzadeh Varzi, H., Seifi Abadshapouri, M.R., 2019. In vitro therapeutic effect of Hemiscorpius lepturus venom on tachyzoites of *Toxoplasma gondii*. J. Parasit. Dis. 43 (3), 472–478. https://doi.org/10.1007/s12639-019-01113-7.

Klementowicz, J.E., Travis, M.A., Grencis, R.K., 2012. Trichuris muris: a model of gastrointestinal parasite infection. Semin. Immunopathol. 34 (6), 815–828. https://doi.org/10.1007/s00281-012-0348-2.

Lázaro-Silva, D.N., De Mattos, J.C.P., Castro, H.C., Alves, G.G., Amorim, L.M.F., 2015. The use of DNA extraction for molecular biology and biotechnology training: a practical and alternative approach. Creat. Educ. 06 (08), 762–772. https://doi.org/10.4236/ce.2015.68079.

Mair, I., Else, K.J., Forman, R., 2021. *Trichuris muris* as a tool for holistic discovery research: from translational research to environmental bio-Tagging. Parasitology 148 (14), 1722–1734. https://doi.org/10.1017/S003118202100069X.

Meldal, B.H.M., Debenham, N.J., De Ley, P., De Ley, I.T., Vanfleteren, J.R., Vierstraete, A.R., Bert, W., Borgonie, G., Moens, T., Tyler, P.A., Austen, M.C., Blaxter, M.L., Rogers, A.D., Lambshead, P.J.D., 2007. An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Mol. Phylogenet. Evol. 42 (3), 622–636. https://doi.org/10.1016/j.ympev.2006.08.025.

Nadler, S.A., Carreno, R.A., Mejía-Madrid, H., Ullberg, J., Pagan, C., Houston, R., Hugot, J.-P., 2007. Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. Parasitology 134 (Pt 10), 1421–1442. https://doi.org/10.1017/S0031182007002880.

Ortiz, E., Gurrola, G.B., Ferroni, E., Possani, L.D., 2020. Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19 . The COVID-19 resource centre is hosted on Elsevier Connect , the company ' s public news and information . January.

Perumal Samy, R., Stiles, B.G., Franco, O.L., Sethi, G., Lim, L.H.K., 2017. Animal venoms as antimicrobial agents. Biochem. Pharmacol. 134, 127–138. https://doi.org/10.1016/j.bcp.2017.03.005.

Rahdar, M., Sadat Roointan, E.-A., Vazirianzadeh, B., Alborzi, A., 2016. Study of internal parasites of rodents in Ahvaz, South-West of Iran. Jundishapur J. Health Sci. 9 (1). https://doi.org/10.17795/jjhs-29067.

Ribas, A., López, S., Makundi, R.H., Leirs, H., De Bellocq, J.G., 2013. Trichuris spp. (Nematoda: Trichuridae) from two rodents, Mastomys natalensis and *Gerbilliscus vicinus* in Tanzania. J. Parasitol. 99 (5), 868–875. https://doi.org/10.1645/12-151.1.

Ribas, A., Wells, K., Morand, S., Chaisiri, K., Agatsuma, T., Lakim, M.B., Yuh Tuh, F.Y., Saijuntha, W., 2020. Whipworms of south-east Asian rodents are distinct from *Trichuris muris*. Parasitol. Int. 77, (April). https://doi.org/10.1016/j.parint.2020.102128 102128.

Robles, M.D.R., 2011. New species of trichuris (Nematoda: Trichuridae) from akodon montensis thomas, 1913, of the paranaense forest in Argentina. J. Parasitol. 97 (2), 319–327. https://doi.org/10.1645/GE-2434.1.

Robles, M.D.R., Cutillas, C., Panei, C.J., Callejón, R., 2014. Morphological and molecular characterization of a new trichuris species (nematoda-Trichuridae), and phylogenetic relationships of Trichuris species of cricetid rodents from Argentina. PLoS One 9 (11). https://doi.org/10.1371/journal.pone.0112069.

Schmid, R., Wilson, D.E., Reeder, D.M., 1993. Mammal species of the world: a taxonomic and geographic reference. Taxon 42 (2), 512. https://doi.org/10.2307/1223169.

Stepek, G., Lowe, A.E., Buttle, D.J., Duce, I.R., Behnke, J.M., 2006. In vitro and in vivo anthelmintic efficacy of plant cysteine proteinases against the rodent gastrointestinal nematode, *Trichuris muris*. Parasitology 132 (5), 681–689. https://doi.org/10.1017/S003118200500973X.

Stepek, G., Lowe, A.E., Buttle, D.J., Duce, I.R., Behnke, J.M., 2007. The anthelmintic efficacy of plant-derived cysteine proteinases against the rodent gastrointestinal nematode, Heligmosomoides polygyrus, in vivo. Parasitology 134 (10), 1409–1419. https://doi.org/10.1017/S0031182007002867.

Swar, S.O., Shnawa, B.H., 2021. Recent advances in molecular characterization of Sarcocystis species in some meat producing animals: an updated review. Asian J. Agric. Biol. 2021 (1). https://doi.org/10.35495/ajab.2020.09.502.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30 (12), 2725–2729. https://doi.org/10.1093/molbev/mst197.

Tattersall, F.H., Nowell, F., Smith, R.H., 1994. A review of the endoparasites of wild House Mice *Mus domesticus*. Mammal Rev. 24 (2), 61–71. https://doi.org/10.1111/j.1365-2907.1994.tb00135.x.

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

Tritten, L., Silbereisen, A., Keiser, J., 2012. Nitazoxanide: In vitro and in vivo drug effects against *Trichuris muris* and *Ancylostoma ceylanicum*, alone or in combination. Int. J. Parasitol. Drugs Drug Resist. 2, 98–105. https://doi.org/10.1016/j.ijpddr.2012.02.004.

Van Megen, H., Van Den Elsen, S., Holterman, M., Karssen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2009. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11 (6), 927–950. https://doi.org/10.1163/156854109X456862.

van Wyk, J.A., Malan, F.S., Randles, J.L., 1997. How long before resistance makes it impossible to control some field strains of Haemonchus contortus in South Africa with any of the modern anthelmintics? Vet. Parasitol. 70 (1–3), 111–122. https://doi.org/10.1016/s0304-4017(96)01147-8.

Varady, M., Praslicka, J., Corba, J., Vesely, L., 1993. Multiple anthelmintic resistance of nematodes in imported goats. Vet. Rec. 132 (15), 387–388. https://doi.org/10.1136/vr.132.15.387.

Will, L., 2005. Genetic variability between populations of the critically endangered frog *Microbatrachella capensis*, Boulenger 1910 (Anura: Ranidae Cacosterninae) by. Methods 1910 (October).

Xu, Z.-M., Li, Z.-S., Wen, M.-X., Peng, R.-Y., Sun, L., Wu, X.-Y., Zhou, L.-X., Tao, Y.-P., Yang, L., 2008. In vitro effect of medicinal scorpion on the larvae of *Ancylostoma caninum*. Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi = Chin. J. Parasitol. Parasitic Dis. 26 (5). 387–388,391.

Yacoub, T., Rima, M., Karam, M., Sabatier, J.M., Fajloun, Z., 2020. Antimicrobials from venomous animals: an overview. Molecules 25 (10). https://doi.org/10.3390/molecules25102402.

Yavuzcan Yildiz, H., Phan Van, Q., Parisi, G., Dam Sao, M., 2019. Anti-parasitic activity of garlic (*Allium sativum*) and onion (*Allium cepa*) juice against crustacean parasite, Lernantropus kroyeri, found on European sea bass (*Dicentrarchus labrax*). Ital. J. Anim. Sci. 18 (1), 833–837. https://doi.org/10.1080/1828051X.2019.1593058.