RESEARCH ARTICLE

Molecular Variations Based on RAPD among Oxyurids in Egypt

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ABSTRACT

Background: Pinworms are nuisance parasites of man and animals. In addition to the human pinworm *Enterobius vermicularis*, *Syphacia muris* has been reported to infect man. Morphological differences between pinworm species are difficult to determine. Polymerase chain reaction (PCR) based assays including random amplified polymorphic DNA (RAPD) have been used effectively as a complementary approach for fast and simple detection of genomic variability and similarity among parasites. Such information is essential for accurate identification and for finding appropriate experimental models for species under study.

Objective: The present study seeks to investigate the degree of similarity between the encountered pinworms aiming to find out the closely related species to *E. vermicularis* to be used as an experimental model. This is evaluated through RAPD-PCR using ten arbitrary primers to differentiate between the encountered

pinworm species. The results are statistically evaluated to reveal the significant variations and to specify the degree of similarity and divergence between the encountered species.

Materials and Methods: The pinworms included in this study are: *Syphacia obvelata* from the house mouse *Mus musculus*, *S. muris* from the black house rat *Rattus rattus*, *E. vermicularis* from infected children and *Aspiculuris tetraptera* from the white laboratory rats *Rattus norvegicus alba*. The degree of similarity between the encountered species was evaluated by PCR-RAPD using the following ten arbitrary primers: OPB-03 (5̀- CAT CCC CCT G-3̀), OPB-06 (5̀- CAT CCC CCT G-3̀), OPB-19 (5̀- ACC CCC GAA G-3̀), OPC-02 (5̀- GTG AGG CGT C-3̀), OPC-05 (5̀- GAT GAC CGC C-3̀), OPC-14 (5̀- TGC GTG CTT G-3̀), OPD-03 (5̀- GTC GCC GTC A-3̀), OPD-13 (5̀- GGG GTG ACG A-3̀), OPE-07 (5̀- AGA TGC AGC C-3̀), and OPE-12 (5̀- TTA TCG CCC C-3̀).

Results: PCR-RAPD technique produced common as well as species specific multiband fingerprints with the ten used arbitrary primers for *S. obvelata, S. muris, A. tetraptera* and *E. vermicularis*. These variations proved valid for differentiation between the oxyurid species in Egypt. OPC-02, OPC-14 and OPD-3 revealed higher similarity between *E. vermicularis* and *S. muris* than with the other pinworm species. Seven of the ten tested arbitrary primers (OPB-03, OPB-06, OPB-19, OPC-05, OPD-13, OPE-07, and OPE-12) revealed that the similarity

between *A. tetraptera* and *E. vermicularis* is higher than that between *E. vermicularis* and both *Syphacia* species.

Conclusion: The present study concluded that the primers used in the present study can be used to differentiate between the encountered species and that *A. tetraptera* and *S. muris* can be used as experimental models for studying enterobiasis.

Keywords: *Syphacia muris, Syphacia obvelata, Aspiculuris tetaptera, Enterobius vermicularis,* RAPD-PCR, *Egypt.*

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INTRODUCTION

Enterobiasis is most commonly asymptomatic; however, *E. vermicularis* has been associated with serious illness because of ectopic worm movements[(1)]. Enterobiasis is usually accompanied by localized perianal pruritis, insomnia, irritability, anorexia, and vulvovaginitis in girls[(2,3)]; more rarely, abdominal pain and even chronic peritonitis may occur[(4)]. In a case presenting with colon carcinoma, Lee *et al*.[(5)] reported chronic infiltration by *E. vermicularis* eggs. Significantly the rodent pinworm, *S. muris* has been reported as a zoonotic parasite[(6,7)].

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. Female *S. muris* and *S. obvelata* differ

only in the location of the vulva being slightly posterior in *S. muris* than that of *S. obvelata*[8,9]. The fact that these morphological differences are difficult to determine urge the need for easier and more reliable methods for the identification and differentiation between these pinworm species[9].

Molecular tools have been recently used to discriminate morphologically closely related species and have contributed effectively to taxonomical, phylogenic and epidemiological studies of many parasites[10-13]. Polymerase chain reaction (PCR) based assays including random amplified polymorphic DNA (RAPD) have been used effectively for the fast and simple detection of genomic variations among parasites. The use of PCR offers a complementary approach for parasite identification and by applying RAPD technique, the genetic variability of different organisms can be analyzed by generating a genomic fingerprint. Although the literature holds extensive information about using molecular techniques for the identification and phylogenic analysis of nematodes, yet few studies were made on pinworms. Several studies employed DNA sequence analysis to reveal evolutionary relationships among pinworms[14,15]. Another study used a high-fidelity PCR to amplify a large portion of the ribosomal gene complex of *S. obvelata*, *S. muris*, *A. tetraptera*, and *Passalurus ambiguous* collected from laboratory rodents and rabbits[16].

The present study was conducted aiming to reveal the degree of similarity and divergence between the encountered pinworm species through RAPD-PCR using

ten arbitrary primers aiming to contribute to the taxonomy of pinworms and to find the closely related species to *E. vermicularis* to be used as an experimental model.

SUBJECTS AND METHODS

Type of the study: Descriptive analytical study**.**

The study was conducted during the years 2008 and 2009. The molecular work was done in the laboratory of molecular biology, Faculty of Agriculture, Ain Shams University.

Parasites: Adult *S. obvelata* and *S. muris* were collected from the distal part of small intestine, caecum, colon and around anal opening of naturally infected house mouse *M. musculus* and black house rat *R. rattus*, respectively. *E. vermicularis* worms were recovered from medical laboratories during diagnosis from the perianal region of infected children; those of *A. tetraptera* were recovered from the caecum and colon of white laboratory rats *Rattus norvegicus alba*. Pinworm species were accordingly identified[17-22]. Differentiation between the two species of *Syphacia*[18,19] and between *S. obvelata* and *A. tetraptera*[23] was accordingly conducted. Frozen samples were kept at -20°C until further use.

DNA Extraction and RAPD-PCR: DNA was extracted and purified using chloroform-isoamyl alcohol DNA extraction method[24]. RAPD-PCR was conducted using 10 arbitrary 10-mer primers (Operon RAPD-PCR Inc.) (Table 1). The reaction conditions were optimized and mixtures were prepared (25 µl

total volumes) consisting of ready mix (Fermentas Dream Taq Green PCR Master Mix) 12.5 µl, Primer (10 mM) 2 µl, DNA template (10-20 ng/µl) 3 µl, double distilled H_2O 7.5 µl. Negative controls included all the components except DNA which was substituted with distilled water and the experiments were processed similarly. The reactions were performed in a sterile 0.2 ml volume PCR tube. Amplification was carried out in Teckne TC-312, which was programmed for step 1**:** initial denaturation (one cycle) at 95 ^{o}C for 5 minutes followed by 45 cycles (steps 2, 3, and 4); step 2: denaturation at 94^{o}C for 0.5 minute; step 3: primer annealing at 36^{o}C for 0.5 minute; step 4: primer extension at 72^{o}C for 2 minutes; step 5: final extension at 72^{o}C for 10 minutes; step 6: hold at 4^{o}C.

Table (1): List of the used RAPD primers and their nucleotide sequences according to Operon catalog (http://www.operon.com/)

Kit Name	Primer Name	Sequence	G+C content %
Kit B	OPB-03	5̀- CAT CCC CCT G -3̀	70%
	OPB-06	5̀- TGC TCT GCC C -3̀	70%
	OPB-19	5̀- ACC CCC GAA G -3̀	70%
Kit C	OPC-02	5̀- GTG AGG CGT C-3̀	70%
	OPC-05	5̀- GAT GAC CGC C -3̀	70%
	OPC-14	5̀- TGC GTG CTT G -3̀	60%
Kit D	OPD-03	5̀- GTC GCC GTC A -3̀	70%
	OPD-13	5̀- GGG GTG ACG A -3̀	70%
Kit E	OPE-07	5̀- AGA TGC AGC C -3̀	60%
	OPE-12	5̀- TTA TCG CCC C -3̀	60%

Gel electrophoresis[25]**:** Agarose (1.2%) ultra pure (Bioshope, Canada) was used for resolving the PCR products one Kb DNA ladder (0.1 µg/µl) (Fermentas). The ladder is composed of fourteen chromatography-purified individual DNA base pairs (bp): 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250, and three reference bands of (6000, 3000 and 1000 bp) for easy orientation.

Analysis of gel images: All DNA electrophoretic patterns were analyzed by Egy-gene gel-analyzer version 3 software to determine molecular size by bp and presence (1) or absence (0) of each fragment (http://www.geocities.com/egygene).

The amplification products were fragmented and the produced bands were classified into polymorphic (partially common between species); monomorphic (common in all species at equal molecular weights; MW); and positive (unique) marker (species specific). Polymorphism percentage is used to refer to the percentage of the total number of polymorphic and positive marker bands per the total number of bands. High percentage of polymorphism in a certain primer indicates high value in species discrimination. Cluster analysis of pair wise genetic similarity of different species was performed using un-weighted pair group method with the arithmetic mean (UPGMA). The calculations and dendrogram were performed by using SPSS version 14 software.

RESULTS

Random amplification polymerase chain reaction of the DNA of *E. vermicularis, S. obvelata, S. muris, and A. tetraptera* using ten arbitrary primers (OPB-03, OPB-06, OPB-19, OPC-02, OPC-05, OPC-14, OPD-03, OPD-13, OPE-07, and OPE-12) revealed the molecular weights of monomorphic fragments, polymorphic fragments, positive markers and polymorphism percentage (%).

Using primer OPB-03 resulted in one monomorphic and 3 polymorphic in addition to 3 positive markers (Table 2 and Figure 1a). The polymorphism percentage reached 85.7%. The proximity matrix showed a high degree of similarity (75%) between *E. vermicularis* and *A. tetraptera* and between *S. muris* and *A. tetraptera*. The un-weighted pair group method using arithmetic average (UPGMA) dendrogram based on genetic similarity index revealed two main clusters, the 1st nested a minor cluster incorporating *E. vermicularis* and *A. tetraptera* at similarity of 75% together with *S. muris*, while the 2nd included *S. obvelata* (Figure 1a). The similarity coefficient between *S. obvelata* and *S. muris* reached 57%.

OPB-06 pattern revealed two monomorphic fragments, two polymorphic fragments and five positive marker fragments (Table 2 and Figure 1b). The polymorphism percentage reached 77.8%. The highest proximity matrix was achieved between *E. vermicularis* and *A. tetraptera* (75%). UPGMA dendrogram comprised two main clusters, the first nested the two species of

Syphacia at similarity coefficient of 66.7%, and the second nested *E. vermicularis* and *A. tetraptera* (Figure 1b).

Primer OPB-19 pattern revealed one monomorphic, three polymorphic and five positive marker fragments (Table 2 and Figure 1c). The polymorphism percentage reached 88.9%. The proximity and the dendrogram (Figure 1c) resemble those obtained using primer OPB-03. The dendrogram nested two main clusters; the first included *E. vermicularis* and *A. tetraptera* in a minor cluster together with *S. muris*; and the other included *S. obvelata*. The similarity coefficient between *E. vermicularis* and *A. tetraptera* reached 80% while that between *S. obvelata* and *S. muris* reached 33.3%.

Primer OPC-02 pattern revealed one monomorphic, three polymorphic and two positive marker fragments (Table 2 and Figure 1d). The polymorphism percentage reached 83.3%. The proximity matrix showed that *E. vermicularis* is closely similar to *S. muris* (85.7%) than to the other pinworm species. UPGMA dendrogram comprised two main clusters, one of which nested *S. muris* and *E. vermicularis* in a minor clustered together with *A. tetraptera* and the other included *S. obvelata* (Figure 1d). The similarity coefficient between *S. obvelata* and *S. muris* reached 33.3%.

Primer OPC-05 pattern revealed seven polymorphic and two positive marker fragments (Table 2 and Figure 1e). The polymorphism percentage reached 100%. The proximity matrix showed that *E. vermicularis* is closely related to *A.*

tetraptera (72.7%) than to the other pinworm species. Using UPGMA dendrogram showed a pattern similar to that obtained by OPB-03 and OPB-19 (Figure 1e).

Primer OPC-14 pattern revealed five polymorphic fragments and two positive marker fragments (Table 2 and Figure 1f). The polymorphism percentage reached 100%. The proximity matrix showed high degree of similarity (85.7%) between *S. obvelata* and *S. muris* than the other pinworm species. UPGMA dendrogram comprised two main clusters, one of which nested three species including *S. obvelata*, *S. muris*, and *E. vermicularis*, while the other cluster included *A. tetraptera* (Figure 1f).

Primer OPD-03 pattern revealed one monomorphic, four polymorphic and three positive marker fragments (Table 2 and Figure 1g). The polymorphism percentage reached 87.5%. The proximity matrix showed high degree of similarity (88.9%) between *E. vermicularis* and *S. muris*. Similar to the results obtained for OPC-02, the dendrogram revealed two main clusters, the first encompassing *S. muris*, *E. vermicularis* in a minor cluster together with *A. tetraptera* and the other nested *S. obvelata* (Figure 1g).

Primer OPD-13 pattern revealed two polymorphic fragments and nine positive marker fragments (Table 2 and Figure 1h). The polymorphism percentage reached 100%. The proximity matrix showed 40% similarity between *E. vermicularis* and *A. tetraptera* with no similarity to the two species of *Syphacia*.

Using UPGMA dendrogram revealed similarity between this dendrogram and that obtained by using OPB-06, where the dendrogram showed the presence of two main clusters, the first nested *E. vermicularis* and *A. tetraptera*, and the other clustered *S. obvelata* and *S.muris* (Figure 1h).

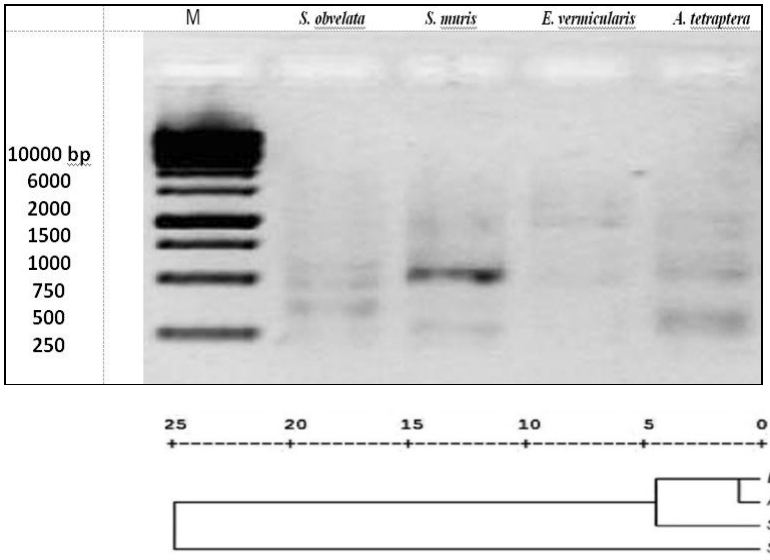
Primer OPE-07 pattern revealed two monomorphic, five polymorphic and four positive marker fragments (Table 2 and Figure 1i). The polymorphism percentage reached 81.8%. The proximity matrix revealed that *E. vermicularis* closely resembles *A. tetraptera* where the similarity coefficient reached 60%. UPGMA dendrogram is similar to that obtained by OPB-06 and OPD-13 revealing two main clusters, the first encompassing *E. vermicularis* and *A. tetraptera* and the other encompassing *S. obvelata* and *S. muris* (Figure 1i).

Primer OPE-12 pattern revealed one monomorphic, two polymorphic and three positive marker fragments (Table 2 and Figure 1j). The polymorphism percentage reached 83.3%. The proximity matrix revealed that *A. tetraptera* is the closest to *E. vermiculuris* where the similarity coefficient reached 66.7%, while that between *E. vermicularis* and either *Syphacia* species reached 50%. UPGMA dendrogram revealed the presence of two main clusters, the first nested *E. vermicularis*, *A. tetraptera* in a minor cluster together with *S. muris* and the other enclosed *S. obvelata* (Figure 1j).

Table (2): Random Amplified Polymorphic DNA (RAPD) marker fragments of *E. vermicularis, S. obvelata, S. muris, and A. tetraptera*

Primers	Mono	Positive markers				Poly	%
		S. obvelata	*S. muris*	*E. vermicularis*	*A. tetrapetra*		
OPB-03	522.9	468.4	–	–	785.7	1039.5	85.7
		317.1				668.8	
						235.6	
OPB-06	1245.1	358.2	990.2	240.7	787.6	853.9	77.8
	540.1		284.9			777.1	
OPB-19	792.9	525.2	437.6	–	594.3	1974.2	88.9
			302.1		293.3	1183.1	
						442.8	
OPC-02	725.1	2427.8	–	–	427.7	1998.7	83.3
						1246.4	
						270.4	
OPC-05	–	319.5	548.2	–	–	2203.5	100
						1621.7	
						1272.4	
						1069.3	
						953.7	
						711.6	
						399.8	
OPC-14	–	–	–	413.6	292.5	1943.9	100
						1434.5	
						1177.1	
						912.6	
						636.4	
OPD-03	835.5	577.8	–	–	1178.2	1310.3	87.5
					626.8	1046.3	
						438.9	
						327.2	
OPD-13	–	530.5	681.2	422.2	1029.6	1052.2	100
		433.1	474.1	274.3	507.9	748.5	
		286.5					
OPE-07	1065.1	–	1221.3	1240.1	1351.6	1768.2	81.8
			478.2			878.5	
	661.4					292.5	
						526.5	
						785.8	
OPE-12	630.1	1497.1	–	–	–	986.6	83.3
		449.2				513.5	
		230.2					

(a)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S. obvelata
S. muris
E. vermicularis
A. tetraptera
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6000
2000
1500
1000
750
500
250

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20
15
10
5
0
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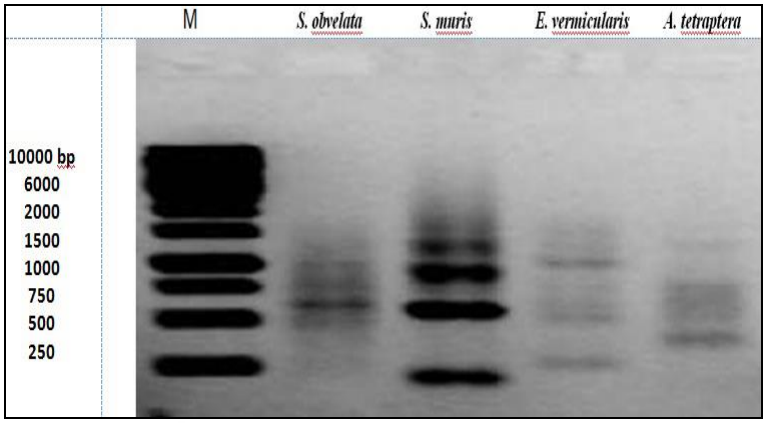
Aspiculuris tetraptera
Syphacia muris
Syphacia obvelata

(b)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E. vermicularis
A. tetraptera
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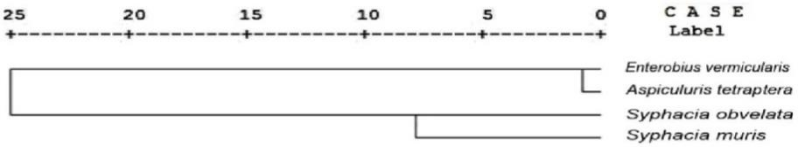
25
20
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CASE
Label
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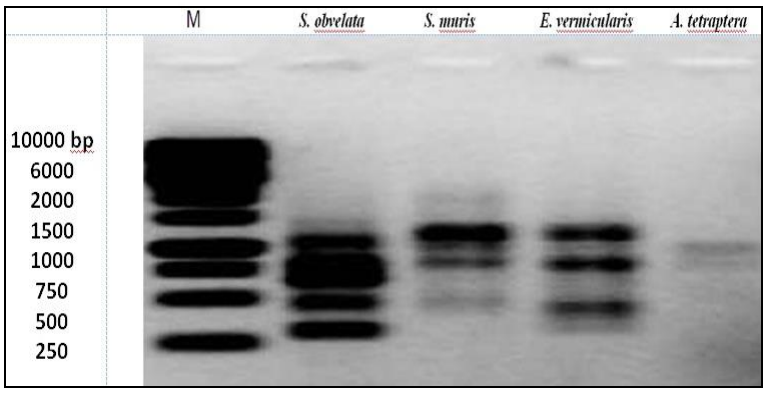
Aspiculuris tetraptera
Syphacia obvelata
Syphacia muris

(c)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S. muris
E. vermicularis
A. tetraptera
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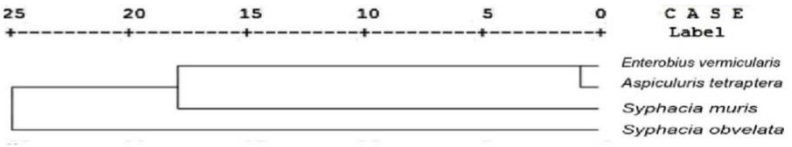
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CASE
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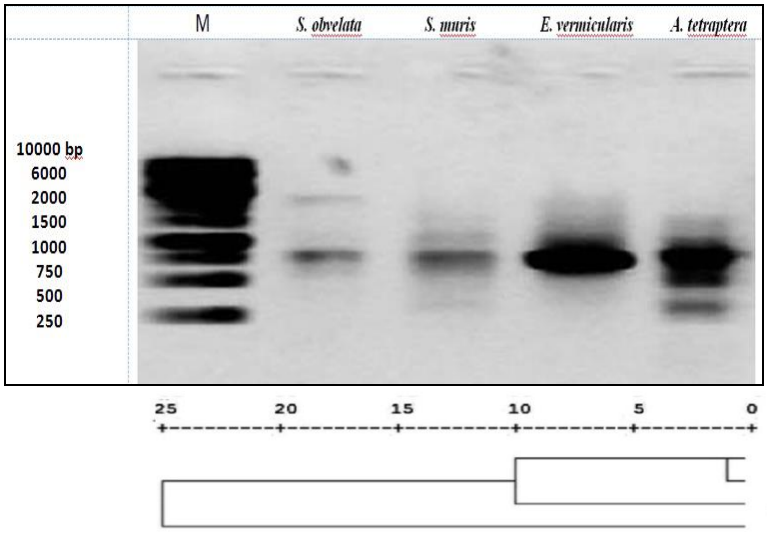
Aspiculuris tetraptera
Syphacia muris
Syphacia obvelata

(d)


M
S. obvelata
S. muris
E. vermicularis
A. tetraptera
10000 bp
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25
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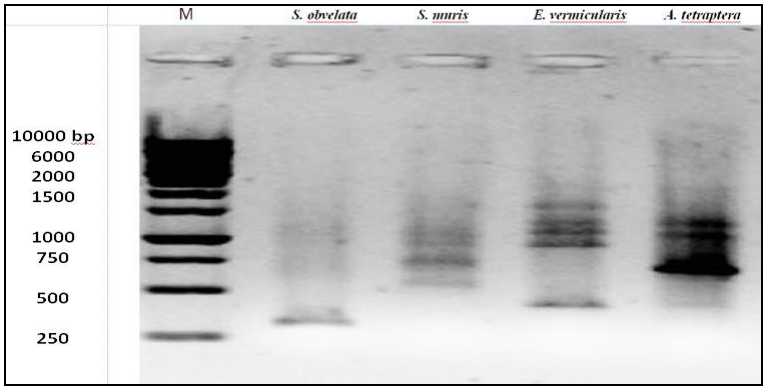
Enterobius vermicularis
Aspiculuris tetraptera
Syphacia obvelata

(e)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S. muris
E. vermicularis
A. tetraptera
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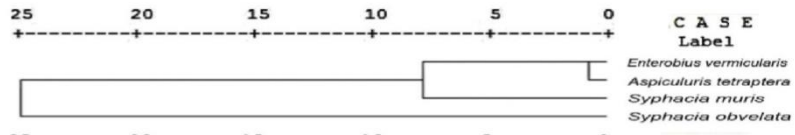
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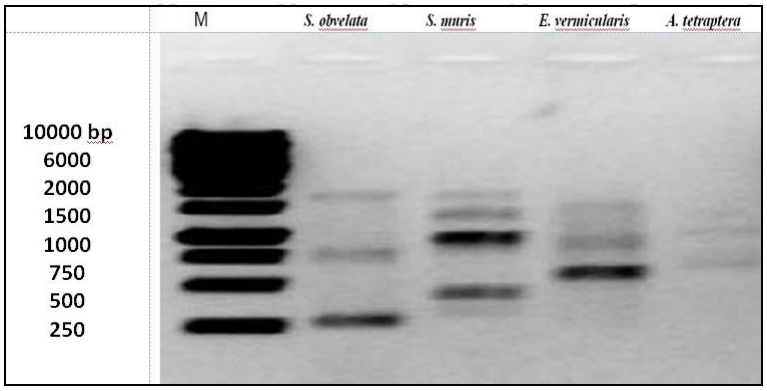
Syphacia muris
Syphacia obvelata

(f)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S. muris
E. vermicularis
A. tetraptera
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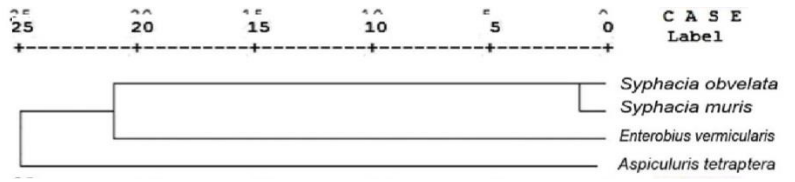
25
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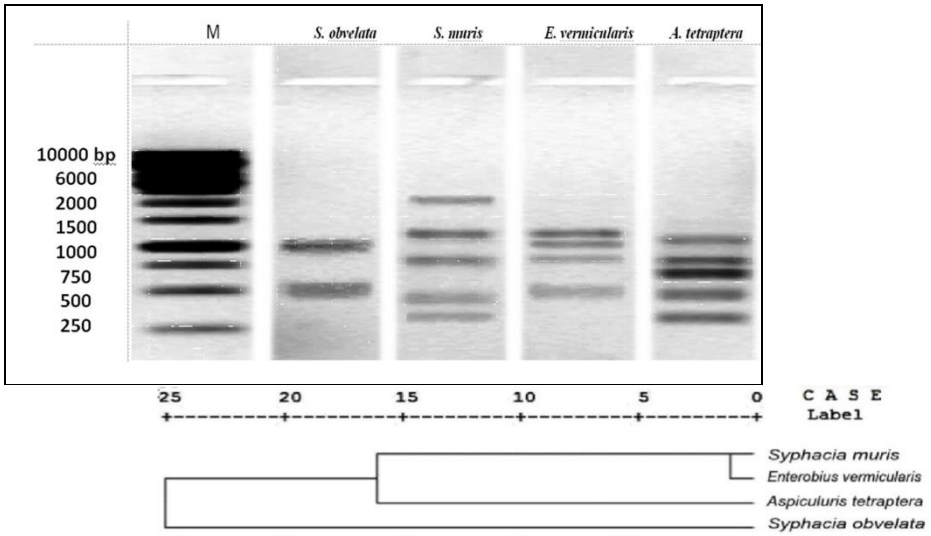
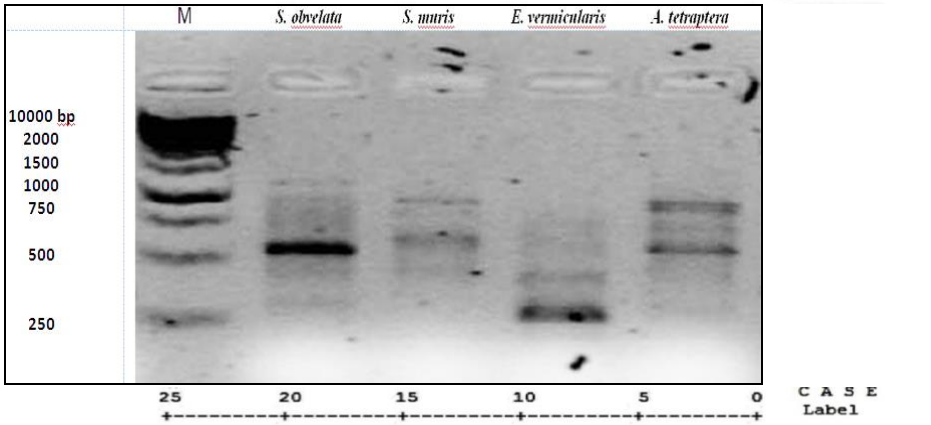
Aspiculuris tetraptera

(g)


M
S. obvelata
S. muris
E. vermicularis
A. tetraptera
10000 bp
6000
2000
1500
1000
750
500
250
25 20 15 10 5 0
CASE
Label
Syphacia muris
Enterobius vermicularis
Aspiculuris tetraptera
Syphacia obvelata

(h)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S. muris
E. vermicularis
A. tetraptera
10000 bp
2000
1500
1000
750
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CASE
Label
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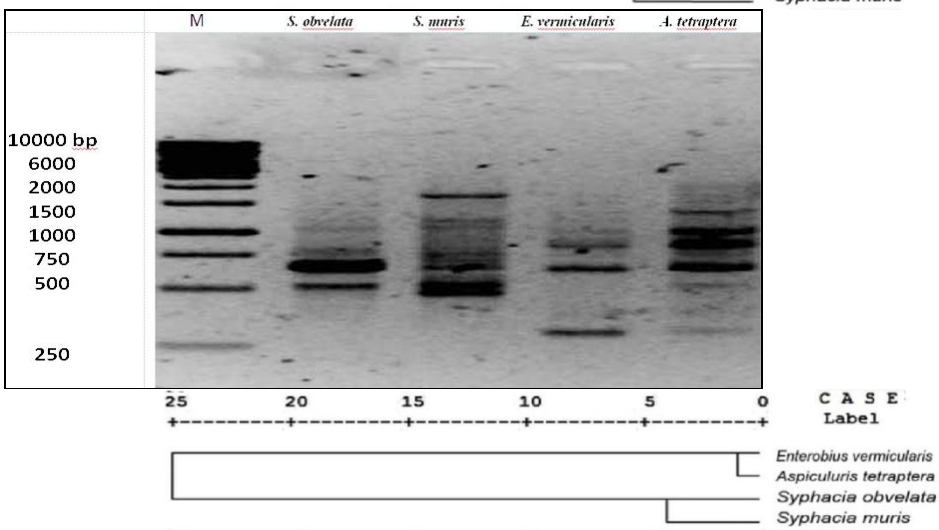
Aspiculuris tetraptera
Syphacia obvelata
Syphacia muris

(i)


M
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S. muris
E. vermicularis
A. tetraptera
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2000
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CASE
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Aspiculuris tetraptera
Syphacia obvelata
Syphacia muris

(j)

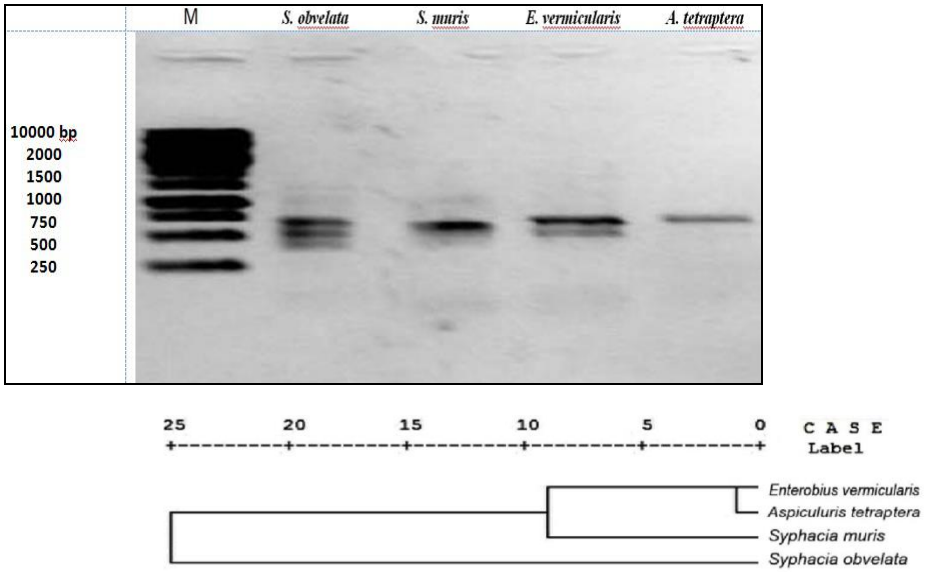

Figure (1): RAPD-PCR banding pattern and Un-weighted pair group method using arithmetic average (UPGMA) dendrogram based on genetic similarity index values calculated from data of (a) OPB-03, (b) OPB-06, (c) OPB-19, (d) OPC-02, (e) OPC-05, (f) OPC-14, (g) OPD-03, (h) OPD-13, (i) OPE-07, (j) OPE-12 arbitrary primers for the four pinworms species; *S. obvelata*, *S. muris*, *E. vermicularis* and *A. tetraptera*. M= molecular weight marker.

RAPD-PCR using ten arbitrary primers: The RAPD-PCR profile, using ten arbitrary primers, revealed its validity for differentiation between *S. obvelata*, *S. muris*, *E. vermiculuris* and *A. tetraptera* species. Using primers OPB-03, OPB-06, OPB-19, OPC-05, OPD-13, OPE-07 and OPE-12 revealed a similarity coefficient between *E. vermiculuris* and *A. tetraptera*, while the similarity coefficient between *E. vermiculuris* and *S. muris* was revealed by using primers OPC-02 and OPD-03 (Table 4). The incorporation of the results of the ten primers using UPGMA dendrogram revealed two main clusters. The first nests *E. vermiculuris* and *A. tetraptera* in a minor cluster at similarity coefficient of

61.7% together with *S. muris*, while the second includes *S. obvelata* (Figure 2). Results also showed that *E. vermicularis* is closer to *S. muris* (54.3%) than to *S. obvelata* (33.3%).

Table (3): Proximity matrix using SPSS version 14 calculated from data of 10 arbitrary primers to show the degree of similarity within the four pinworm species under study. *S. obvelata*, *S. muris*, *E. vermicularis* and *A. tetraptera*.

Case	**Matrix File Input**		
	S. obvelata	***S. muris***	***E. vermicularis***
S. muris	**0.519**		
E. vermicularis	**0.333**	**0.543**	
A. tetraptera	**0.338**	**0.512**	**0.617**

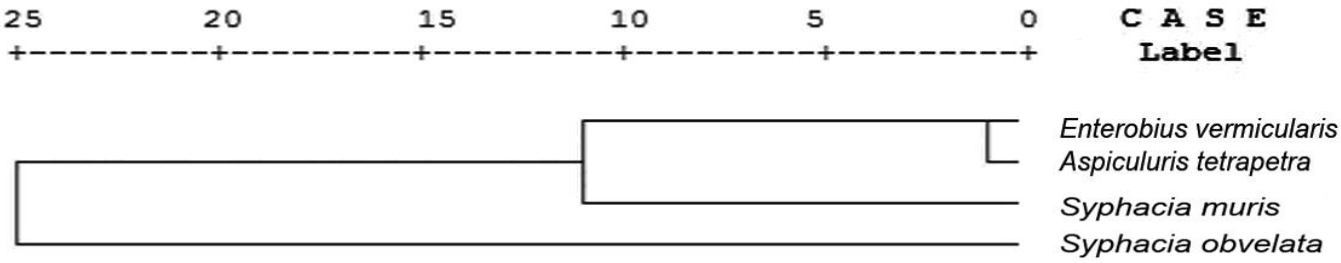

Figure (2): Un-weighted pair group method using arithmetic average (UPGMA) dendrogram based on genetic similarity index values calculated from data of ten arbitrary primers for the four pinworms species; *S. obvelata*, *S. muris*, *E. vermicularis* and *A. tetraptera*.

DISCUSSION

Molecular biology tools have contributed effectively in association with traditional morphological techniques to differentiate between closely related species. RAPD has been used successfully for the fast and simple detection of genomic variations among parasites. It has the advantage of being used with limited genetic information of the encountered species, and the produced results are analyzed efficiently to generate easily interpreted data.

The traditional keys for nematode classification[26] include the species under study in the order Oxyurida. Members of the genera *Syphacia* Seurat[27] and *Enterobius* Leach[28] are included in family Oxyuridae[29], while *Aspiculuris* Schultz[30] is included in family Heteroxynematidae[31].

RAPD-PCR technique using 10 arbitrary primers, undertaken in the present study, proved to be effective in discriminating *S. obvelata, S. muris, E. vermicularis* and *A. tetrapetra*. The technique revealed the degree of relationship between species as presented by the similarity coefficient and the UPGMA dendrograms. The produced clusters represent the sharing of one or more specific genotypic characteristic which may or may not be related to morphological similarities. The study also revealed the validity of using the primers under study as expressed by the polymorphism percentage.

The literature holds little information on molecular discrimination between pinworms. Parel *et al.*[9] differentiated between *S. obvelata, S. muris* and *A. tetraptera* based on their ribosomal DNA (rDNA) sequences. Using restriction endonucleases allowed them to investigate unique regions in the ITS-2 of the three species.

Using OPC-14 confirmed the traditional taxonomy of this group where two clusters were produced, one of which nested *S. obvelata, S. muris* in a minor group together with *E. vermicularis* in a major cluster, while *A. tetrapetra* was included in another cluster. The use of this primer showed that the degree of

similarity between *E. vermicularis* and *S. muris* reaches 50%. The high polymorphism percentage (100%) for this primer denotes its validity for species discrimination.

Using OPB-03, OPB-19, OPC-05, and OPE-12 in RAPD-PCR revealed analogous results that disagree with the classical taxonomy based on morphological characteristics, where the produced dendrograms nested two main clusters, the first included *E. vermicularis* and *A. tetrapetra* in a minor group together with *S. muris* to form the first main cluster and the second cluster encompasses *S. obvelata*. The similarity coefficient was higher between *E. vermicularis* and *A. tetrapetra* (75%, 80%, 72.7%, and 66.7% for the four primers, respectively) than between *E. vermicularis* and *S. muris*. The polymorphism percentage reached 85.7%, 88.9%, 100% and 83.3% respectively, indicating that OPC-05 is more reliable for species differentiation than OPB-19, OPB-03 and OPE-12.

Primers OPB-06 and OPD-13 were revealed as positive markers indicating their value as species specific markers. The dendrogram of these primers nested *E. vermicularis* and *A. tetrapetra* at similarity coefficient of 75% and 40%, respectively, in one cluster and *S. obvelata* and *S. muris* in the other cluster. Finally, the last 2 primers (OPC-02 and OPD-03) revealed high similarity coefficient between *E. vermicularis* and *S. muris*.

Using OPB-03, OPB-06, OPB-19, OPC-05, OPD-13, OPE-07, and OPE-12 primers in RAPD-PCR technique revealed a high degree of similarity (75%, 75%, 80%, 72.7%, 40%, 60%, and 66.7%, respectively) between *A. tetrapetra* and *E. vermicularis* than to *Syphacia* species which nominates *A. tetrapetera* as a possible experimental model for human *Enterobius*. The previous finding coincides with the total cumulative UPGMA analysis of products from the ten arbitrary primers used in the present study, where the dendrogram revealed two main clusters, the first nested *E. vermicularis* and *A. tetrapetra* in a minor cluster (where the similarity coefficient reached 61.7%) together with *S. muris* forming a main cluster. Results of using OPC-02 and OPD-03 primers suggest that *S. muris* can be nominated as another model for *E. vermicularis.*

In conclusion, future studies are recommended to include advanced molecular and antigenic characteristics of the nominated species.

Author contribution: AI Khalil suggested and planned the experiments, analyzed results and shared in writing the manuscript. **GH Morsy** and **GH Lasheen** supervised collection of material and revised the manuscript. **DI Abd El-Mottaleb** collected, identified and examined the study specimens; helped in molecular biology analysis and in writing the manuscript. **AA Sharaf** performed the molecular biology analysis.

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التباين الجزيئى بين أنواع الديدان الدبوسية للقوارض فى مصر

باستخدام تفاعل البلمرة الجزيئية العشوائى المتسلسل

آمال اسكندر خليل[1] و جزاء حسن مرسى[2] و جيهان حسين لاشين[2] و دينا إبراهيم عبدالمطلب[2] و عبدالله شرف[3]

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مقدمة: تعتبر الديدان الدبوسية من الطفيليات التى تنتشر وتسبب ازعاج للإنسان والحيوان .وقد سجلت الدراسات أن سيفشا ميورس التى تصيب الفأر يمكن أن تصيب الإنسان أيضا بالإضافة إلى انتروبيوس فيرميكيولارس. ونظرا لوجود بعض المشكلات فى التفرقة بين الديدان الدبوسية، فقد اهتمت الدراسات بالبحث عن طرق للتفريق بين هذه الديدان كما اهتمت الدراسات بالبحث عن نموذج معملى يمكن استخدامة لدراسة هذه الطفيليات. وقد بينت دراسات سابقة أهمية استخدام التقنيات الحديثة مثل تحليل الحامض النووى (DNA) من خلال تفاعل سلسلة البلمرة العشوائى كنهج تكميلى سريع ودقيق لدراسة مظاهرالتشابه والإختلاف الجزيئى بين الديدان الطفيلية.

هدف البحث: تهدف هذه الدراسة لتحديد درجة التشابه والتباين بين الديدان الدبوسية محل الدراسة بهدف ايجاد النوع الأقرب إلى انتروبيس فيرميكيولارس لكى يستخدم كنموذج تجريبى. وذلك من خلال استخدام تفاعل سلسلة البلمرة العشوائى باستخدام عشرة بادئات عشوائية للتفرقة بين أنواع الديدان الدبوسية محل الدراسة وتقييم النتائج احصائيا للكشف عن القيمة المعنوية لهذة الاختلافات ولتحديد درجة التشابه والإختلاف بين هذه الأنواع.

المواد والطرق: تشتمل الديدان الدبوسية محل الدراسة على سيفشا اوبفيلاتا من الفؤير المنزلى وسيفشا ميورس من الفأر المنزلى و انتروبيوس فيرميكيولارس من أطفال مصابين و اسبيكيولارس تيترابيترا من الجرذ المعملى الأبيض. وقد تم تقييم درجة التشابه بين الأنواع محل الدراسة باستخدام تفاعل سلسلة البلمرة العشوائى باستخدام عشرة بادئات عشوائية وهى:

OPB-03 (5̀- CAT CCC CCT G -3̀), OPB-06 (5̀- CAT CCC CCT G -3̀), OPB-19 (5̀- ACC CCC GAA G -3̀), OPC-02 (5̀- GTG AGG CGT C-3̀), OPC-05 (5̀- GAT GAC CGC C -3̀), OPC-14 (5̀- TGC GTG CTT G -3̀), OPD-03 (5̀- GTC GCC GTC A -3̀), OPD-13 (5̀- GGG GTG ACG A -3̀), OPE-07 (5̀- AGA TGC AGC C -3̀), and OPE-12 (5̀- TTA TCG CCC C -3̀.

النتائج: أظهرت النتائج باستخدام عشرة بادئات عشوائية من خلال تقنية تفاعل سلسلة البلمرة العشوائى وجود بصمات مشتركة وكذلك محددة بين الأربعة أنواع محل الدراسة وهى سيفشا اوبقيلاتا وسيفشا ميورس وانتروبيوس فيرميكيولارس واسبيكيولارس تيترابيترا، كما أظهرت الدراسة أن هذه الإختلافات صالحة للتفرقة بين أنواع الديدان الدبوسية المختلفة. فباستخدام كل من البادئة (OPC-14) و(OPC-02 وOPD-03) تبين أن سيفشا ميورس هى الأقرب إلى انتروبيوس فيرميكيولارس عن أنواع الديدان الدبوسية الأخرى. ولقد أثبتت الدراسة أيضا باستخدام سبعة بادئات عشوائية وهى OPB-03 و OPB-06 و OPB-19 و OPC-05 و OPD-13 و OPE-07 و OPE-12 أن أسبيكيولارس تيترابيترا هى الأقرب إلى إنتروبيس فيرميكيولارس عن كلا نوعى السيفشا.

الاستنتاجات: خلصت الدراسة إلى امكانية استخدام هذه البادئات للتفرقة بين الأنواع محل الدراسة وإلى امكانية استخدام أى من اسبيكيولارس تيترابيترا وسيفشا ميورس كنموذج معملى لدراسة أمراض الديدان الدبوسية.

Table (4): RAPD-PCR using ten arbitrary primers to differentiate between *Syphacia obvelata*, *Syphacia muris*, *Enterobius vermicularis* and *Aspiculuris tetraptera*.

Primer name	Polymorphism percentage	Dendrogram		Similarity Coefficient of *E. vermicularis* with either			Positive Marker							
							S. obvelata		*S. muris*		*E. vermicularis*		*A. tetraptera*	
		First cluster	Second cluster	*S. obvelata*	*S. muris*	*A. tetraptera*	Band No.	Molecular weights	Band No.	Molecular weights	Band No.	Molecular weights	Band No.	Molecular weights
OPB-03	85.7%	*(E. vermicularis, A. tetraptera)* and *S. muris*	*S. obvelata*	28.6%	66.7%	75%	5 6	468.4 317.1	—	—	—	—	2	785.7
OPB-06	77.8%	*(E. vermicularis* and *A. tetraptera)*	*S. obvelata* and *S. muris*	50%	44.4%	75%	6	358.2	2 8	990.2 284.9	9	240.7	4	787.6
OPB-19	88.9%	*(E. vermicularis, A. tetraptera)* and *S. muris*	*S. obvelata*	33.3%	50%	80%	5	525.2	7 8	437.6 302.1			4 9	594.3 293.3
OPC-02	83.3%	*(S. muris, E. vermicularis)*, and *A. tetraptera*	*S. obvelata*	40%	85.7%	57%	1	2427.8	—	—	—	—	5	427.7
OPC-05	100%	*(E. vermicularis, A. tetrapetr)a* and *S. muris*	*S. obvelata*	22.2%	66.7%	72.7%	9	319.5	7	548.2	—	—	—	—
OPC-14	100%	*(S. obvelata, S. muris)* and *E. vermicularis*	*A. tetraptera*	28.6%	50%	28.6%	—	—	—	—	6	413.6	7	292.5
OPD-03	87.5%	*(S. muris, E. vermiculuris)*, and *A. tetraptera*	*S. obvelata*	33.3	88.9%	44.4%	8	577.8	—	—	—	—	3 7	1178.2 626.8
OPD-13	100%	*E. vermicularis* and *A. tetraptera*	*S. obvelata* and *S. muris*	0%	0%	40%	5 8 10	530.5 433.1 286.5	4 7	681.2 474.1	9 11	422.2 274.3	2 6	1029.6 507.9
OPE-07	81.8%	*E. vermicularis* and *A. tetraptera*	*S. obvelata* and *S. muris*	33.3%	22.2%	60%	—	—	4 9	1221.3 478.2	3	1240.1	2	1351.6
OPE-12	83.3%	*(E. vermicularis, A. tetraptera)* and *S. muris*	*S. obvelata*	50%	50%	66.7%	1 5 6	1497.1 449.2 230.2	—	—	—	—	—	