

# Prevalence, incidence and molecular characterization of tape worms in Al Taif governorate, KSA and the effectiveness of *Spirulina platensis* as a biological control *in vitro*

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## Research

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# Abstract

## Background

Tapeworms are parasites that infect sheep and cattle and live in the small intestine, causing many problems, including diarrhea and weight loss, which leads to losses in livestock breeding. One of the most common tapeworms that infects sheep, goats and cattle *Moniezia expansa*, *Moniezia benedene*, *Avitellina centripunctata*, *Thysaniezia giardia* and *Stilesia hepatic*.

## Methods

A total (965) of small intestine were collected from postmortem sheep of slaughter house of Al Taif abattoir during the period from October 2018 to September 2019. The PCR product of *cox1* gene (364 bp) was sequenced and then data were aligned with the same fragment of *cox1* gene for other related helminths parasites. *In vitro* determination of the anthelmintic efficacy of *Spirulina platensis* on adult *Moniezia*.

## Results

The results reported that about 9.94% of selected sheep were infected with tape worms in native breed. Concerning the seasonal incidence of tape worms among sheep, the results revealed that the highest percentage was recorded during winter season (11.3%), while the lowest percentage was recorded during the spring (7.72%). The species of tape worms recorded were *Moniezia expansa*, *Moniezia benedene*, *Avitellina centripunctata*, *Thysaniezia giardia* and *Stilesia hepatic*. Molecular characterization of Al Taif *Moniezia* species *cox1* gene records four different species Me *Taif1*, Me *Taif2*, Me *Taif3* and Me *Taif4*, that have identity ranged from 90–99% to species in Senegal and Ethiopia (Me13, Me14 and Me22). *In vitro* treatment of *Moniezia* spp. with different concentrations (10, 20, 30 and 50 µg/ml) of *Spirulina platensis* extract (Blue-green Algae) affects the morphological architecture of tape worms by using scanning electron microscopy (SEM). The results revealed that *Spirulina platensis* extract treatment, especially for higher doses 30 and 50 µg/ml, caused the sucker opening to narrow as a result of scolex swelling more than usual, deformation of the proglottids occurred with circular areas of declare swelling along their margins and microtriches covering the whole tegument appears in several directions.

## Conclusions

As per of our knowledge it is the first time to record new species of *Moniezia* species in Al Taif governorate and the anthelmintic potential of *Spirulina platensis*.

## Background

Sheep plays an important role in the rural socio-economic conditions as well as in the national economy in the country. In rural hilly areas, comprehensive development cannot be achieved by neglecting to develop agricultural commodities such as goats, cattle and sheep. Parasites play an important role in reducing the production of sheep and goats around the world [1].

Helminths are considered one of the most dangerous internal parasites destroying vertebrate animals, including humans. Moreover, it reduces livestock productions as well as increases mortality rate. Sheep are considered as a valuable animal providing people with meat, milk and wool [2]. Sheep have received great attention as they are one of the most important livestock and the most preferred for human consumption in the Arab countries, especially the Gulf countries [3].

In countries famous for raising sheep, tape worms are considered one of the most aggressive parasites that cause outbreaks and constitute a major problem in their raising [4]. It causes intestinal obstruction, perforation, perineal abscess, hepatic abscess, cholecystitis, and appendicitis. Tape worms cause poor body condition, leading to body weight loss, difficulty in lambing and kidding and low birth weight. Animals become more susceptible to other health problems due to infection with these parasites that can lead to death [5].

The tape worms of the genus *Moniezia* Blanchard, 1891 (Cyclophyllidea, Anoplocephalidae) are predominantly parasites that infect cattle, goats, sheep, antelopes and deer. They belong to the ruminant artiodactylus although there are a few species also known from Swedes, equids, and rodents. There are currently 15 species of *Moniezia* [6]. Sheep and goats are the main host *Moniezia expansa*, but it can be found in cattle while cattle are the main host *Moniezia benedeni* and also it can be found in sheep and goats. Chronically, monieziosis is characterized by progressive weakness, anemic condition, reduce milk and meat production. This form is most common where mortality is low, but morbidity may reach high leads to weakness of animals and emaciation. However, heavy parasitic load may cause death [7]. Whereas *Avitellina centripunctata* is more common among sheep and goats than in cattle. *Thysaniezia giardia* is one of the tapeworms that live in the small intestine of ruminants, mainly in cattle and sheep, and oribatid mites is the intermediate host. *Stilesia hepatic* is called liver tapeworms because it lives in the bile ducts of sheep, goats, cattle and antelopes. It is also common in North and South America, Asia and South Africa, as well as in tropical regions.

There are three major classes of commonly used anthelmintic drugs, the benzimidazoles, imidothiazoles and macrocyclic lactones, however there is a great resistance to all those three drug classes of sheep and goats throughout the world [8]. Therefore, there is a great demand to have a new alternative to or supplements for chemical control of parasitic cestode in grazing livestock.

Microalgae play a major role in future food supplies as they can be used directly as food or as a food ingredient [9] or indirectly as animal feed [10] or as sources of bio-fertilizers and bio-stimulants [11]. Microalgae have been used in other high-value applications including wastewater treatment and biodiesel production as well as in the cosmetic and pharmaceutical industries [12]. Two of the main types of cyanobacteria are called *Arthrospira platensis* and *Arthrospira maxima*. The International Association of

Applied Microbiology in 1967 recognized *Arthrospira* species as a future food source [13]. Microalgae are very high in protein, which amounts to 60% of the dry weight [14]. *Arthrospira* species are also used to develop biostimulants and bio-fertilizers due to their high protein content. Recent studies of the microalgae *Spirulina platensis* have shown the presence of a blue protein called phycocyanin, which belongs to the photosynthetic system, and has antioxidant and free radical scavenging properties in both in vitro and in vivo models [15].

The current study was aimed to report the spread, incidence and molecular characterization of cestodes among sheep at Al-Taif Governorate in Kingdom of Saudi Arabia (KSA) and their biological control *in vitro* by using *Spirulina platensis*.

## Methods

A total (965) of small intestine were collected from postmortem sheep of slaughter house of Al Taif abattoir during the period from October 2018 to September 2019. Routine examination of small intestine was carried out to check for the presence of cestodes. To remove any debris from collected adult worms, washed 2–3 times with a 0.9% saline solution [16, 17]. Adult worms of recovered *Moniezia* from the small intestine of slaughtered sheep at Taif abattoir were used for later techniques.

### Molecular characterization of *Moniezia* spp.

The whole DNA was extracted from a homogenized tape worm body according to Sambrook [18]. Then extracted DNA was subjected to PCR using forward primer (5-TTTTTTGGGCATCCTGAGGTTTAT-3) and reverse primer (5-TAAAGAAAGAACATAATGAAAATG-3) that targets cytochrome c oxidase subunit 1 (cox1) gene of mitochondrial DNA (mtDNA) with conditions: 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min, and then final strand elongation at 72 °C was done for an additional 7 min.

The PCR product of cox1 gene (364 bp) was sequenced and then data were aligned with the same fragment of cox1 gene for other related helminths parasites<sup>3</sup>.

### In vitro **determination of the anthelmintic efficacy of *Spirulina platensis* (Blue-green algae) on adult *Moniezia***

In a laminar flow cabinet, under sterile conditions, tape worms were washed in several changes of warm (37.8°C), sterile complete RPMI 1640 culture medium containing antibiotics (streptomycin, 50 µg/ml, penicillin, 50 µg/ml). To fresh culture medium containing 50% (volume/volume), 2% (volume/volume) red blood cells of rabbits and heat denatured rabbit serum, the tape worms were then transferred as according to Ibarra and Jenkins [19], The whole worms were incubated for 24 h at 37.8 °C in an atmosphere of 5% CO<sub>2</sub> with algae extracts from (*S. platensis*) in four different concentrations 10, 20, 30 and 50 µg/ml.

# Scanning electron microscope

After incubation, the whole tape worms were placed for 12 h in a 3:1 mixture of 4% (w/v) of glutaraldehyde in 0.12 M-Millonig's buffer solution pH 7.4 and aqueous osmium tetroxide to fixed the anterior end and segments were intact. Samples were then processed for SEM according to the method previously reported by Echeverria [20].

## Results

### The prevalence and incidence of tape worms among sheep in Al Taif governorate

From the total 965 small intestine of sheep that were collected from slaughtered house at Al Taif abattoir, only 96 (9.94%) infested intestine was found. It was reported that the highest percent of positively was recorded in October (20%), while the lowest percent was in September (5%). According to seasons, it was found that the incidence of tapeworms among sheep was high in winter (11.3), while it is least in spring season (7.72) as shown in **Table 1**.

**Table (1): The relationship between total incidence of tape worms and seasonal changes among sheep in Al Taif governorate.**

Season	Number of total examined animals	Number of infested animals	% of infestation
Spring	220	17	7.72
Summer	300	30	10
Autumn	180	19	10.5
Winter	265	30	11.3
Total	965	96	9.94%

**Table (2)** shows that *Avitellina centripunctata* and *Moniezea* spp. were the most detected tape worms in infested intestine with percentage 39.5% and 36.4% from total, respectively. Moreover, it was found that the highest incidence of sheep with *Moniezea* spp. in Autumn with percentage 31.57%, while with *Avitellina centripunctata* highest incidence in summer (12%).

**Table (2): The relationship between total incidence of difference tape worm species and seasonal changes among sheep in Al Taif governorate.**

Season	<i>Avitellina centripunctata</i>	<i>Moniezia spp.</i>	<i>Thysaniezia giardia</i>	<i>Stilesia hepatic</i>
Spring	7.8%	5.8%	6.2%	3.2%
Summer	12%	10%	8%	0%
Autumn	9.04%	31.57%	0%	0.95%
Winter	11.78%	3.95%	7.3%	8.48%
Total	39.5%	36.4%	15.6%	8.3%

### Molecular characterization of *Moniezia spp.*

Four-different species of *Moniezia spp.* were initially determined by the BLAST program on NCBI (<http://www.ncbi.nlm.nih.gov/>) based on the (*cox1*) sequences of type strains. All isolates were identified with type strain of *Moniezia spp.* and it was labelled them as Me *Taif1*, Me *Taif2*, Me *Taif3* and Me *Taif4*, respectively. Those four different strains were all obtained from sheep in Al Taif governorate and their sequence were compared with different *Moniezia* species in Senegal and Ethiopia. In which Me13, Me14 were found in Ethiopia and Me22 was found in Senegal, that were already found in Genbank with accession numbers AB821384.1, AB821385.1 and AB821393.1, respectively [21, 22, 23]. **Table (3)** represents the identity and coverage percentage of the four-different isolated strains and the percentages of identity with other *Moniezia expansa* strains. The percentage of identity were ranged from 90–99%; in which Al Taif strains Me *Taif1*, Me *Taif3* and Me *Taif4* have identity percentage 99% in alignment to subjects Me13, Me13 and Me14, respectively. However, Me *Taif2* strain shows about 90% identity percentage for subject sequence Me22 as shown in **Figure (1)**.

Table (3): Identity and coverage percentage of different strains of *Moniezia expansa* from Al Taif governorate with most related parasite in Genbank according to the obtained mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) sequence.

Most related parasite in Genbank		Query covering %	Identities (%)	Site of change found in Al Taif strains
Suggested names	Name of gene and Accession No.			
Me <i>Taif1</i>	<i>Moniezia expansa</i> mitochondrial cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Me13. <b>AB821384.1</b> <sup>21</sup>	100	99	Me13 at bp 970 C  Me <i>Taif1</i> at the same site  T
Me <i>Taif2</i>	<i>Moniezia expansa</i> mitochondrial cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Me22. <b>AB821393.1</b> <sup>23</sup> .	100	90	Several sites as shown in <b>Fig. 1</b>
Me <i>Taif3</i>	<i>Moniezia expansa</i> mitochondrial cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Me13. <b>AB821384.1</b> <sup>21</sup>	100	99	Me13 at bp 936 T  Me <i>Taif3</i> at the same site  C
Me <i>Taif4</i>	<i>Moniezia expansa</i> mitochondrial cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Me14. <b>AB821385.1</b> <sup>22</sup>	100	99	Me14 at bp 1089 G  Me <i>Taif4</i> at the same site  A

In vitro **anthelmintic efficacy** of *Spirulina platensis* extract on *Moniezia* spp.

Normal *Moniezia expansa* shows normal globular scolex with four oval suckers each sucker appears triangular shape, an elongated ribbon-like structure called strobila that consists of several proglottids and unidirectional microtriches covering the whole tegument as in **Figs. 2 (A,B), 3 (A,B) and 4 (A)**. However, treatment of *Moniezia* spp. adult tape worms with different concentrations of *Spirulina platensis* shows deformation especially for higher concentrations 30 and 50 µg/ml. It causes the scolex to become more swollen than normal with narrowing of sucker's opening, the proglottids appear deformed with circular areas of pronounced swelling occurred along their margins and microtriches covering the whole tegument appears in several directions **Figs. 2 (C,D), 3 (C,D,E) and 4 (B,D)**.

## Discussion

### Prevalence and incidence of tape worms in Al Taif governorate.

The present study was designed to assess an epidemiological study of cestodes among sheep at Al-Taif governorate throughout the whole year from October 2015 to September 2016 in KSA. In the current study the incidence of natural infection with tapeworms was (9.94%), which is in agreement with many other scientific reports [24, 25, 26, 27], while higher values were reported in this study than those reported by other authors [28, 29] and lower than that recorded by Kumar [30] to 28.5%. These differences may be due to host habits in different countries, parasites, vectors and many environmental factors.

It was found that the highest percent of positively was recorded in October (20%), while the lowest percent was in September (5%). According to seasons, it was found that the incidence of tapeworms among sheep was high in winter (11.3), while it is least in spring season (7.72) as shown in **Table (1)**. The results of seasonal incidence of tape worms among sheep recorded in this study were nearly the same all over the year, except for spring. This could be attributed to the climatic conditions including temperature, relative humidity and rainy weather which were favorable to the survival and development of larval stages which were strongly supported by Qamar [31], and the intermediate hosts (oribatid mites) on pasture in Al Taif governorate [32].

The worms that were identified in this study listed in **Table (2)**, *Avitellina centripunctata* (39.5%), *Moniezia expansa* and *Moniezia benedeni* (36.4%), *Thysaniezia giardia* (15.6%), and *Stilesia hepatica* (8.3%). With regard to parasite prevalence throughout the year, the highest values of infection with *Moniezia* spp. were recorded during the autumn season, and this was consistent with [33, 34].

### Molecular characterization of *Moniezia* spp.

The present results agreed with Diopa [35], they revealed that according to a phylogenetic analysis based on *cox1* gene sequences in tapeworms from goats/sheeps and that from cattle were genetically different from each other. The percentage of Al Taif *Moniezia* species *cox1* gene identity were ranged from 90–99%; in which Taif strains Me *Taif1*, Me *Taif3* and Me *Taif4* have identity percentage 99% in alignment to subjects Me13 (accession number: AB821384.1), Me13 and Me14 (accession number: AB821385.1),

respectively but Me *Taif2* shows 90% identity percentage for subject sequence Me22 (accession number: AB821393.1).

Diopa [35] reported that most *Moniezia* species *cox1* gene (375 bp) of Senegal and Ethiopia including Me13, Me14 and Me22 were already found in Japan tapeworms in the GenBank database with accession number: AB099693 in Genbank [36], but there is no host information in the database. They stated that no relevant papers were published and the data was only published in the database. In addition, they compared with the sequences obtained from *Moniezia* sp. (AB099693) and was equally genetically distant to all the sequences with the similarity of 86.7–87.7%.

The present study revealed the genetic diversity of Al Taif *Moniezia* sp. might be due to intraspecific variation. This suggestion agreed with Diopa [35], they suggest that the mitochondrial variability of *M. expansa* in Senegal is due to intraspecific variation rather than interspecific among cryptic species. For anoplocephalid cestodes no reports were found of intraspecific variation of the *cox1* gene sequences between them, but some other types of cyclophyllidean cestodes such as *Echinococcus multilocularis* and *Taenia solium* had genetic sub-group in the world with the listed as 1.9% and 1.3%, respectively [37, 38].

#### In vitro **anthelmintic efficacy of *Spirulina platensis* extract on *Monizia* spp.**

As per to our knowledge, it is the first time to assess the anthelmintic potential of *Spirulina platensis* extract on *Monizia* spp. that was reported by deformation of the tape worm morphology that was treated with *Spirulina platensis* extract at different concentrations by scanning electron microscopic examination.

The outer surface or tegument is the area of contact between the environment in the host and *Monizia*, so the tegument has many functions and features, which have made the study of great importance, because the outer surface of the tape worm is the main target site for different natural anthelmintic products and synthetic drugs as proved by ultrastructural and histomorphological studies [39, 40]. These changes depend on the concentration of the extract and the incubation period, leading to deformation and destruction in the scolex and proglottis as well as in the cuticle tegumental architecture of *M. expansa*. One of the main characteristics of parasite cuticle is metabolically active as well as specialized in the selection and absorption of nutrients and the osmoregulation process. Therefore, the deformities and destructive changes on the parasite surface are responsible for the passive diffusion of anthelmintic through the cuticle [41, 42].

Mossa [43] reported that using different plant extracts of Kingdom of Saudi Arabia have high efficacy in the treatment of Ascariasis and fascioliasis. It had also a bactericidal effect and molluscicidal activity of *Schistosoma mansoni* and also with less side effects and less expensive than other chemical drugs.

The effect of ethanolic extract of *Calligonum comosum* and TCBZ on adult worms of *Fasciola gigantica* was studied by Degheidy [44] through biochemical and hematological examination. Their results showed

a decrease in the number of eggs in the feces and also the blood parameter returned to its normal levels after treatment, which led to the improvement of the health condition of the sheep. In addition, in Al Taif Governorate, Kingdom of Saudi Arabia, Degheidy and Al-Qtaibi [45] studied biological control of adult tapeworms (*Monizia* spp.) by brown algae (*Fucus vesiculosus*) and their results confirmed the effectiveness of all concentrations, especially 30 µg/ml after one hour.

In the light of our present findings, we conclude that the *Spirulina platensis* could be a good anthelmintic for moniziasis in sheep. To validate our findings and improve our knowledge on its potential as an anthelmintic drug used for tape worms or in combination of other chemical drug to improve its therapeutic effect more studies are needed.

## Declarations

### Ethics approval and consent to participate

The parts used were taken from animals that were already slaughtered in the slaughterhouse in Al Taif Governorate, Saudi Arabia, under the supervision of a veterinarian

### Consent for publication

Not applicable

### Competing interests

The authors confirm that there are no conflicts of interest.

### Funding

Not applicable

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## Figures

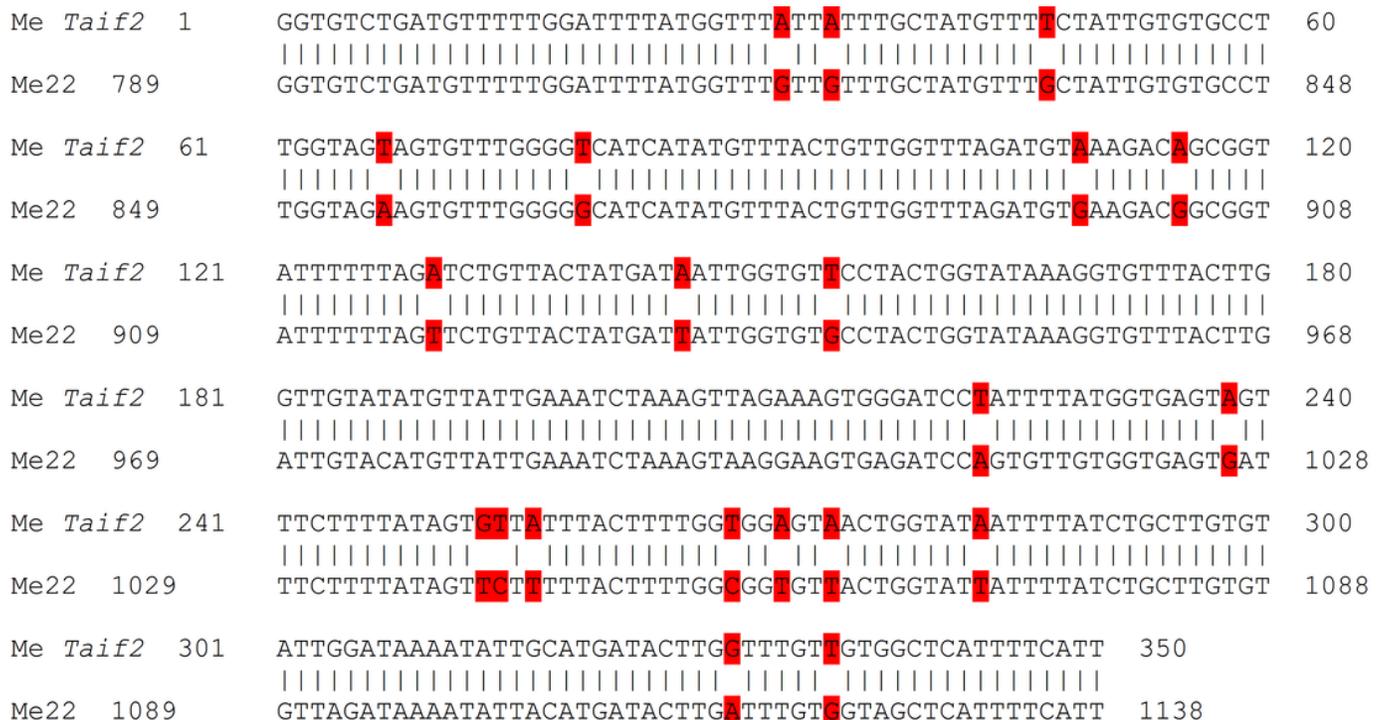
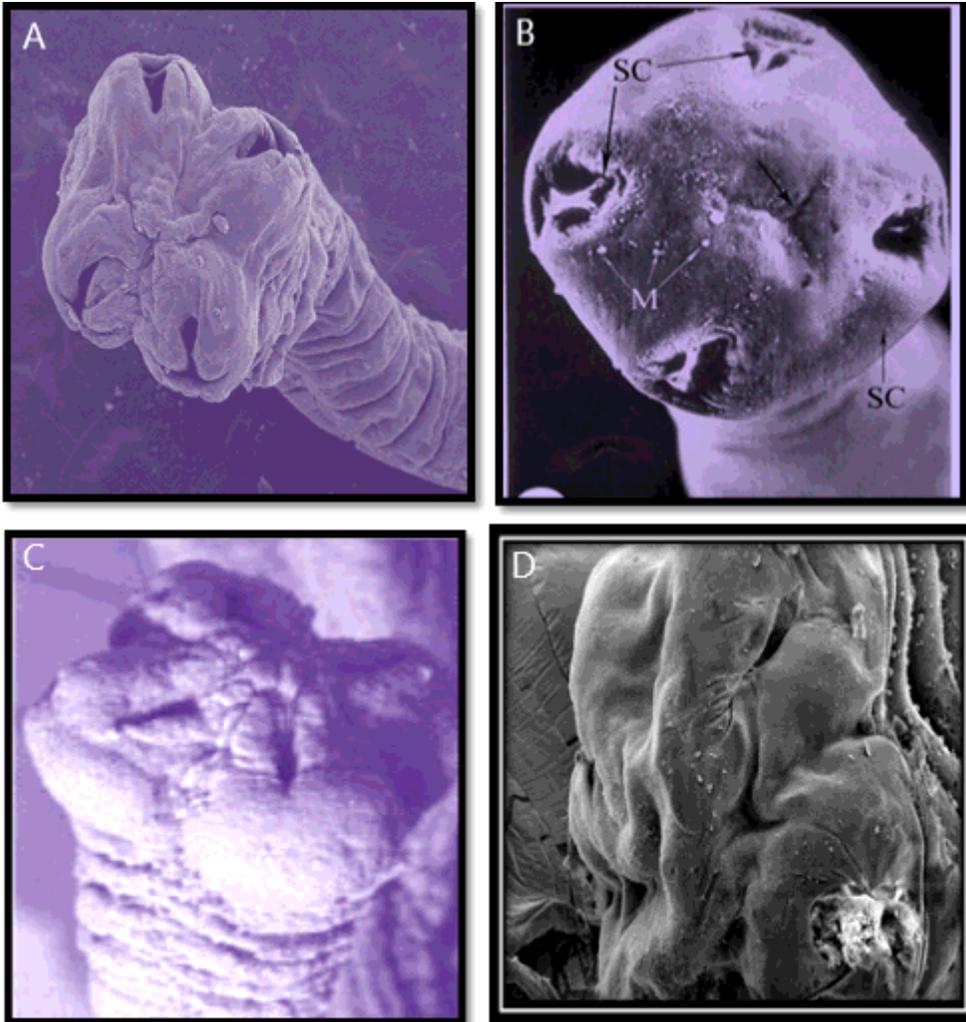


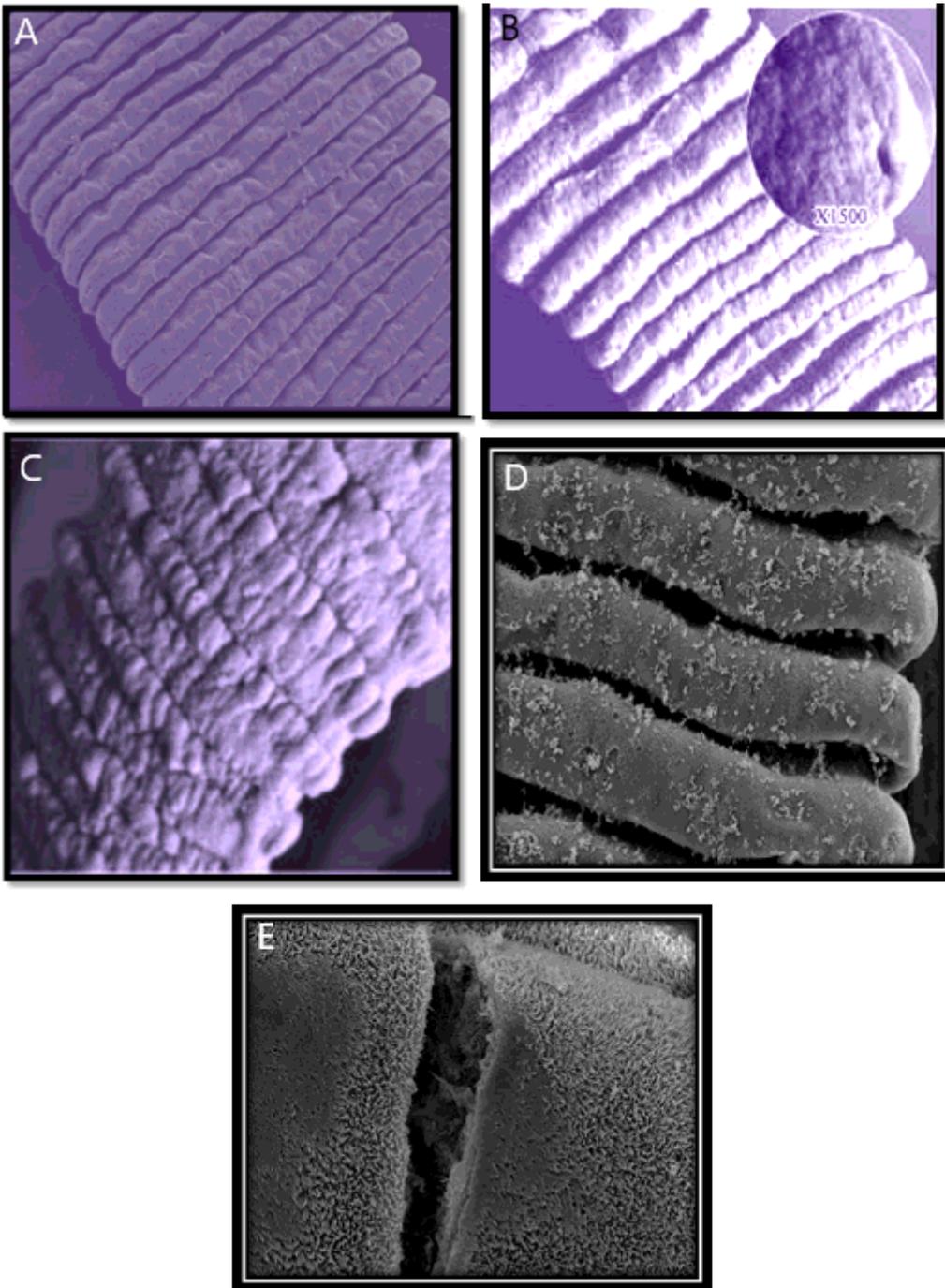
Figure 1

Alignment of the partial sequences of cytochrome c oxidase subunit 1 (cox1) gene between Me Taif2 and Me22 showing 90% identity using BLAST.



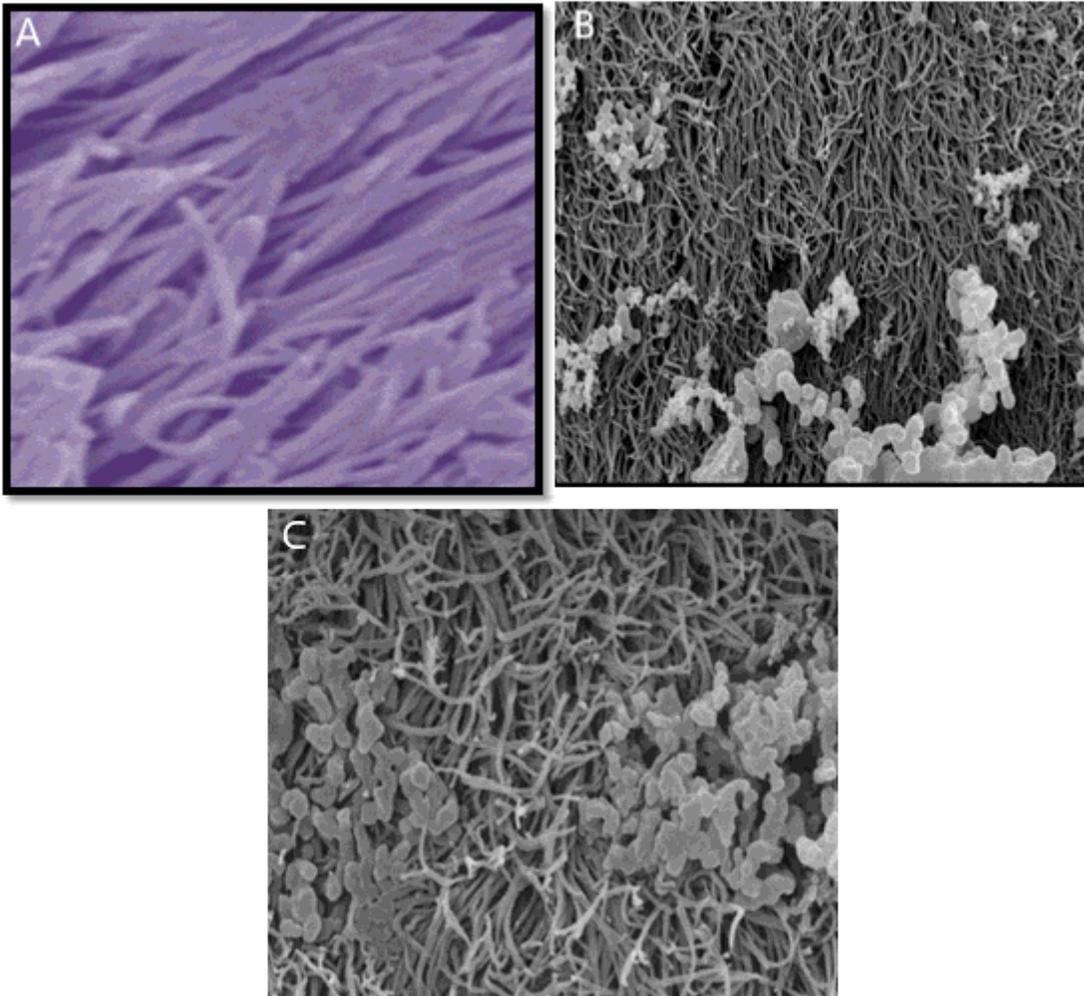
**Figure 2**

Morphological characterization of *M. expansa* scolex by scanning electron microscope (SEM). A and B show normal negative control *M. expansa*, C shows scolex of *M. expansa* treated with 30 µg/m *Spirulina platensis* and D refers *M. expansa* treated with 50 µg/m *Spirulina platensis*.



**Figure 3**

Morphological characterization of *M. expansa* strobilla that consists of several proglottids by scanning electron microscope (SEM). A and B shows normal negative control *M. expansa*, C shows proglottids of *M. expansa* treated with 30 µg/m *Spirulina platensis* and D and E refer to *M. expansa* treated with 50 µg/m *Spirulina platensis*.



**Figure 4**

Morphological characterization of *M. expansa* showing microtriches covering the whole tegument by scanning electron microscope (SEM) for negative control (A) and *M. expansa* treated with 50 µg/m *Spirulina platensis* (B and C).

## Supplementary Files

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