

**“Prevalence and Identification of Cestode Species Recovered from Infected Organs of Lizardfish (*Saurida undosquamis*)”**

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**Abstract**

192 specimens of the *Saurida undosquamis* were collected from the local market of Misurata city in Libya. the prevalence rate of cestode helminths in examined fish *Saurida undosquamis*, was high. Based on the morphological and morphometric characterization represented two species of cestodes were isolated. Of the total examined fish (169, 88%) were infected with (Tetraphyllidean sp. and Trypanorhyncha sp. larvae). The density of cestode helminths was around 6810, and they were collected from the stomach, anterior and posterior parts of the intestine in all seasons, the liver and muscles showed no cestode infection, and a non-significant difference ( $P > 0.05$ ) were found between cestode density infection of infected organs, this study found a high infection rate with Tetraphyllidean sp. and Trypanorhyncha sp. larvae in the *Saurida undosquamis*.

**Keywords:** Endoparasites, Lizardfishes, Tapeworms, Prevalence, Identification, Cestodes, *Saurida undosquamis*.

## 1. Introduction

Cestodes, or tapeworms, are also flatworms (Class Cestoidea) that are typically intestinal parasites of vertebrates when adults. Depending on the type of cestode, variations occur in the larval and juvenile stages and the type of hosts used. Those, which use zooplankton as intermediate hosts and infect fishes, belong to the orders Trypanorhyncha, Tetraphy Uidea, Pseudophyllidea and Proteocephalidea. Life cycles within these groups are similar. Eggs passed into the water are ingested, or hatch into free-swimming coracidia, which are ingested by invertebrates, where they develop into larvae or proceroids. In some cases, a second intermediate host ingests the intermediate host, where the cestode develops further, often into a worm-like plerocercoid that is infective to the definitive host. In others, the plerocercoid stage as well as the adult occur in the definitive host. The proceroid stage is typically associated with zooplankton. Fish may be intermediate hosts and carry plerocercoid, or definitive hosts and carry adult cestodes, most tapeworms generally do not induce severe damage to the fish digestive tract, provoking only destruction of the superficial layer of the intestinal wall at the point of scolex attachment. (Marcogliese, 1995).

Few previous studies have been done on *Saurida undosquamis*, to diagnose infection by Cestodes. From previous studies, many parasites affect the internal organs of *Saurida undosquamis* such as intestines, stomach, muscles, liver, and kidney, and often show symptoms of infection of these parasites, In the Mediterranean and north-East Atlantic regions, Tetraphyllidean sp. species is the most frequently reported in a wide range of fish different species can have similar larval morphology. Moreover, larvae can exhibit rather uniform morphology, which is completely different from their adult forms. Life-cycles of marine tapeworms of the orders Tetraphyllidea and Rhinebothriidea are poorly known primarily because their larvae typically lack

species level. Taxonomically distinguishing adult characteristics and using morphology they can be identified to genus, family or order only, the taxonomic characteristic of this order is Scolex, the hook or sucker that is used to attach the parasite to the body of the host. (Jensen, 2010), while Trypanorhyncha is taxonomically complex and considered the most confused group of tapeworms (Campbell and Beveridge, 1994). The adult of this species has a wide geographical distribution (Pereira and Boeger, 2005).

There were few studies about infection by parasitic helminths in *Saurida undosquamis* in Libya. This made it challenging us to identify the isolated species and compare the results of this study with similar studies. This study was aimed at the determination of the prevalence and identification of some cestode species from different infected organs in lizardfish in Misurata, Libya.

## 2. Materials and Methods

**Fish sampling:** The study included 192 lizardfish *Saurida undosquamis* ranging in weight (26-235 g) and length (14-32 cm). The current study was conducted from June 2018 to May 2019, and 15 to 20 fish were collected from the local market of Misurata, Libya. They were transferred in an icebox to the laboratory of the Zoology Department, Faculty of Science.

Lizardfishes were examined only for internal parasites. Fish were dissected, and the whole body cavity, muscles, liver, and digestive system were carefully examined at first by the naked eye for the presence of helminths. On the other hand, the organs (gills, liver, stomach, anterior intestines, and posterior intestines) were separated by dissecting scissors and forceps and placed in Petri dishes containing the normal saline. Then, a scalpel and forceps were used to scrape fish contents gently. All Petri dishes were carefully examined for the presence of Cestodes by dissecting and light

microscopes. Finally, the cestodes were isolated in a sterile container containing normal saline, (Hoffman, 1967).

After collection, the Cestodes were washed several times with normal saline to remove any attached mucus. The different species of Cestodes were kept separately in sterile glass bottles containing 70% ethanol and were labelled with all details regarding each sample. The adult worms of Cestodes were fixed in warm ethanol (70%). Then, the worms were flattened gently, followed by dehydration and then stained in acetic acid alum carmine dye for 2-10 minutes. In addition, they were cleared in xylol, to identify the internal structures of the worms, and finally added to the slides using DPX. (Rasheed, 1989)

After mounting worms, they were examined by microscope (Buffalo, N.Y. 1420 U.S.A.) at 10X and 40X and drawn by sketch photos to facilitate identification and classification. The Morphometric measurements of cestodes included the total lengths of cestodes, All the morphological and morphometrical features were identified using the general keys as illustrated by (Yamaguti, 1959, Carus, 1863, Linton, 1890).

The data analysis compared helminth abundance between the different parasite species using one-way ANOVA at a 5% significance level. The relationship between lengths and weights of Lizardfish with parasite density was determined using linear correlation at a 5% level of significance. The comparisons of parasite abundance with seasonal variations were carried out using one-way ANOVA. This test was performed using the SPSS computer software.

### 3. Results

From examined fish were infected with Tetraphyllidean sp. and Trypanorhyncha sp. larvae 169 (88%). the anterior part of the intestine demonstrated the highest infection (42.6%), followed by the posterior part (35.8%), whereas the stomach showed the lowest infection rate (21.4%). In contrast, the liver and muscles showed no cestode infection. On the other hand, the stomach had the highest infection rate in spring (30.5%), followed by the anterior and posterior regions (46.5, 41.3% respectively) in summer as illustrated in Table (1). In addition, the stomach had the lowest cestode infection rate in summer (12%). Based on the statistical analysis, there was a non-significant difference ( $P > 0.05$ ) between all different infected organs with cestode. In addition, a moderate relationship was found between the stomach and the anterior and posterior parts of the intestines according to the correlation coefficient( $r$ ), ( $r = 0.682^*$ ,  $0.647^*$ , respectively). In contrast, a significant difference was found between the anterior and posterior parts of the intestine ( $P \leq 0.01$ ) ( $r = 0.878^{**}$ ).

Table (1): Total prevalence rate of Tetraphyllidean sp. and Trypanorhyncha sp. larvae with the seasonal variations of different organs.

Organs Seasons	Stomach	Anterior intestine	Posterior intestine	Total
Winter	21 (21.8%)	41 (42.7%)	34 (35.4%)	96
Spring	33 (30.5%)	42 (38.8%)	33 (30.5%)	108
Summer	7 (12%)	27 (46.5%)	24 (41.3%)	58
Autumn	15 (16.3%)	41 (44.5%)	36 (39.1%)	92
Total	76 (21.4%)	151 (42.6%)	127 (35.8%)	354

Regarding the density infection of Tetraphyllidean sp. and Trypanorhyncha sp. larvae in different infected organs, the anterior part of the intestine showed the highest density infection (68.1%), followed by the posterior region (22.6%). In addition, the lowest density infection was in the stomach (9.1%). The infection was not observed in the gills, liver, and muscle organs, as shown in Table (2). Moreover, based on the one-way ANOVA, a non-significant difference ( $P > 0.05$ ) was found between Tetraphyllidean sp. and Trypanorhyncha sp. larvae density infection of infected organs. There was a strong relationship between the abundance of Cestode in the anterior and posterior parts of the intestine ( $r = 0.744^{**}$ ).

Table (2): Density infection rates of Tetraphyllidean sp. and Trypanorhyncha sp. larvae in *Saurida undosquamis* according to infected organs.

Organs \ Seasons	Stomach	Anterior intestine	Posterior intestine	Total Density rate
Winter	155 (10%)	1061 (68.7%)	327 (21.1%)	1543 (23%)
Spring	380 (20%)	1210 (63.6%)	310 (16.3%)	1900 (28%)
Summer	45 (4.9%)	678 (74%)	193 (21%)	916 (13.4%)
Autumn	43 (1.7%)	1695 (69.1%)	713 (29%)	2451 (36%)
Total	623 (9.1%)	4644 (68.1%)	1543 (22.6%)	6810 (36.3%)

According to the morphological and morphometric features of Tetraphyllidean sp. and Trypanorhyncha sp. larvae, collected from different regions of *Saurida undosquamis*, The Trypanorhyncha sp. was found in the anterior part of the intestine during winter and spring. Trypanorhyncha sp. (Linton, 1890): These larval stages

had a small body with a smooth tegument. The length of the anterior part was approximately 68.74  $\mu\text{m}$ , and the width was 20.61  $\mu\text{m}$  Figure 1 (A, B). Recent publications present diverse opinions concerning the classification and taxonomy of *Trypanorhyncha* sp. and the following keys were consulted for the identification: Phylum: Platyhelminthes, Sub Phylum: Rhabditophora, Superclass: Neodermata, Class: Cestoda, Subclass: Eucestoda and Order: Trypanorhyncha.

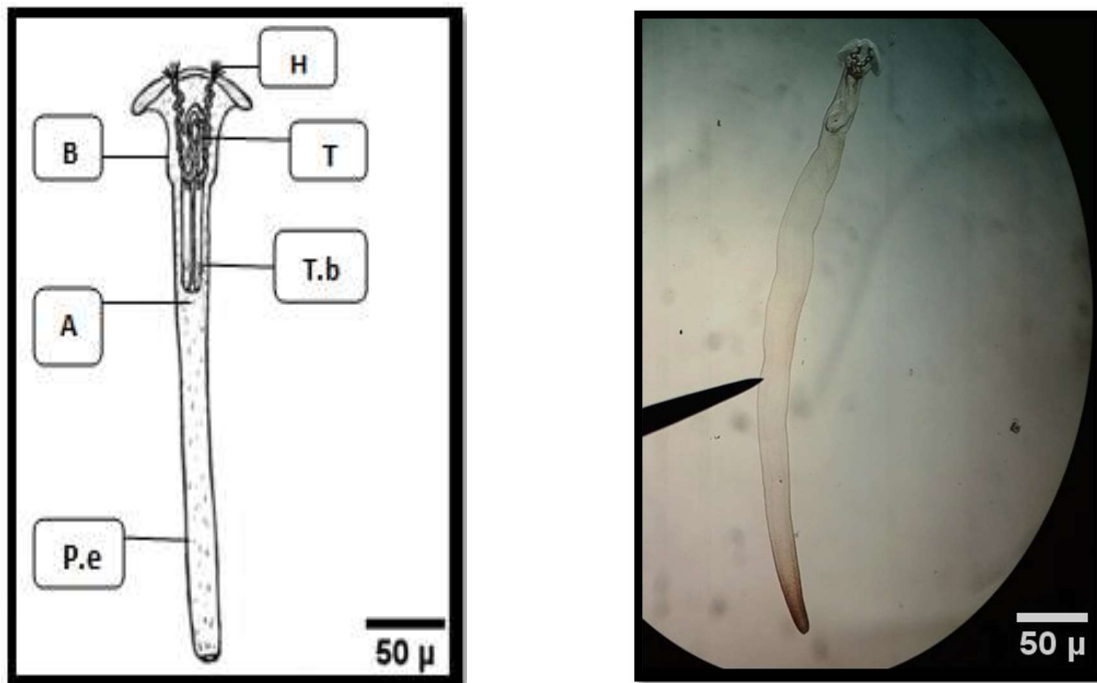


Figure 1 (A, B): A general view of the larval stage of *Trypanorhyncha*. Sp. from *Saurida undosquamis*. H, Hooks; B, Bulbs organ; T, Tentacles with retracted hooks; T.b, Tentacle bulb; A, Appendix; P.e, Posterior end.

These results showed that this Tetrphyllidean sp. can be found widely at its larval stage in *Saurida undosquamis* fishes. The identities of these larvae remain indeterminate at all levels. The transformation of the larvae into an adult; thereby establishing its identity. The larval stages of Tetrphyllidean sp. were found in 169 (88%) infected fish, with a density of 6810, collected from the stomach, anterior and posterior intestines in all seasons. the high-density rate in autumn (36%), followed by spring (28%).

The morphological characteristics were described as a very small body with smooth tegument, and the total body length was 107.70  $\mu\text{m}$  Figure 2 (A1, A2). The other larval stage had a very small body with a smooth tegument. The total body length was 75.16  $\mu\text{m}$ , and the width was 33.23  $\mu\text{m}$ . The sucker had a length of 11.40  $\mu\text{m}$  and a width of 11.59  $\mu\text{m}$  Figure 3 (B1, B2). Another larval stage demonstrated a total body length of 120.78  $\mu\text{m}$  and a width of 37.09  $\mu\text{m}$ . The sucker had a length of 7.41  $\mu\text{m}$  and a width of 6.90  $\mu\text{m}$  Figure 4 (C1, C2). Moreover, another larval stage showed a very small body, with a smooth tegument and a total body length of 280.86  $\mu\text{m}$  Figure 5 (D1, D2). Recent publications present diverse opinions concerning the classification and taxonomy of Tetrphyllidean sp. and the following keys were consulted for the identification: Phylum: Platyhelminthes, Sub Phylum: Rhabditophora, Superclass: Neodermata, Class: Cestoda, Subclass: Eucestoda, Order: Tetrphyllidea, Carus, 1863 and Family: Tetrphyllidea.



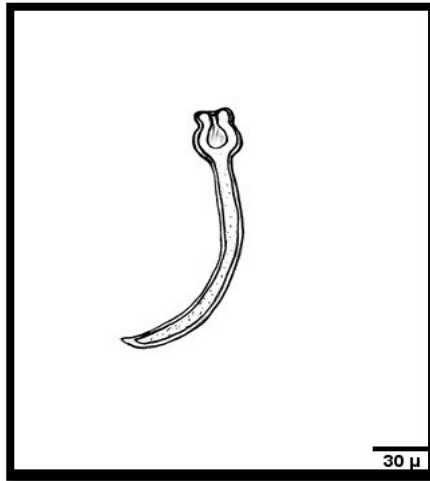


Figure 2 (A1, A2): A general view of the larval stage of Tetracystid sp. from *Saurida undosquamis*.

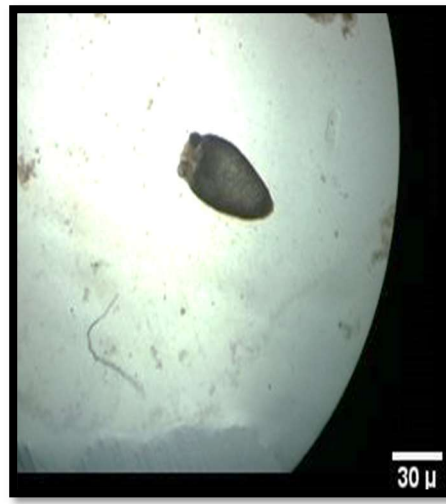
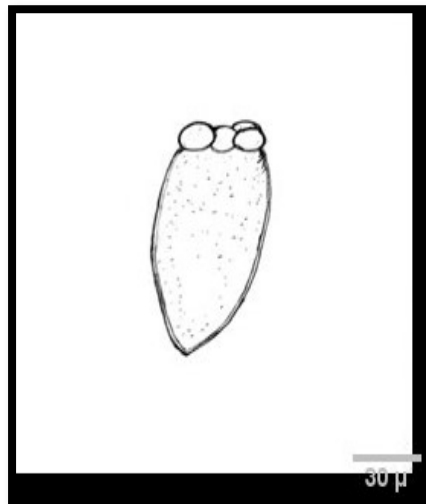


Figure 3 (B1, B2): A general view of the larval stage of Tetracystid sp. from *Saurida undosquamis*

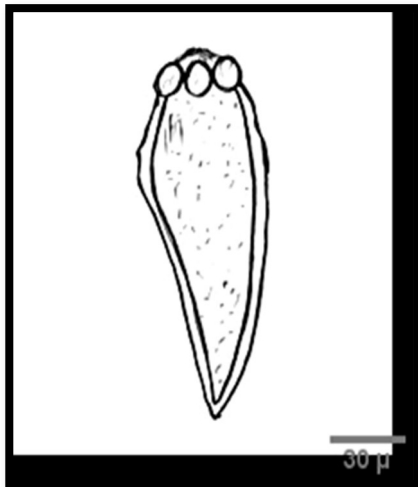


Figure 4 (C1, C2): A general view of the larval stage of Tetrphyllidean sp. from *Saurida undosquamis*.

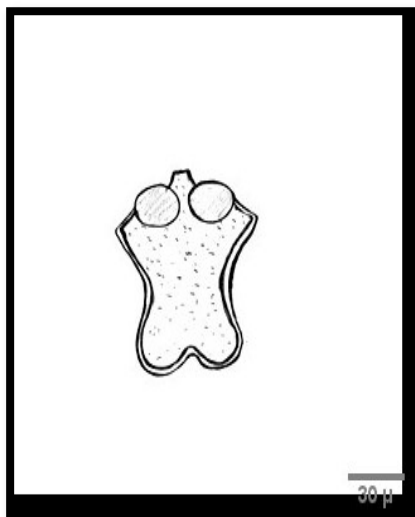


Figure 5 (D1, D2): A general view of the larval stage of Tetrphyllidean sp. from *Saurida undosquamis*.

## 6. Discussion

From this study, we encountered great difficulty in distinguishing between types of cestode larvae based on morphological and standard characteristics only, especially with the presence of high infection, as well as the lack of studies in Libya that can be referred to and relied upon in the classification of types of worms isolated from *Saurida undosquamis* from Misurata, Libya.

The present study provides the endo helminthic parasitic infestation in 192 *Saurida undosquamis* fish from June 2018 to May 2019, which was reported in Tetrphyllidean sp. and Trypanorhyncha sp. larvae. They demonstrated no pathognomonic clinical signs and were in good health. However, all the fish examined were infected with Tetrphyllidean sp. and Trypanorhyncha sp. in all seasons (100%). Few previous studies illustrated the prevalence of infection rates of Tetrphyllidean sp. and Trypanorhyncha sp. from different organs of *Saurida undosquamis*. In this study, about 88% of examined fishes were infected with Tetrphyllidean sp., which were collected from different organs such as stomach, anterior and posterior intestine, suggesting that the distribution of this species within the fish tissue was related to the availability of nutrients. In the current study, 1.6% of *Saurida undosquamis* infected with three larval stages of Trypanorhyncha sp. were collected from the anterior intestine of infected fish. This finding was similar to Bannai's (2008) study, which found a 0.6% infection rate. Furthermore, 88% of *Saurida undosquamis* were infected with larval Tetrphyllidean metacestodes collected from the stomach, anterior and posterior intestines; El-Ekiaby (2019) reported a high infection with their metacestodes (59.6%). This might be attributed to the difference in location, feeding ways, the number of examined fish, and environmental conditions. Tetrphyllidean sp. species is the most frequently reported in a wide range of fish different species

could have similar larval morphology. Moreover, larvae can exhibit rather uniform morphology, which is completely different from their adult forms; Life cycles of marine tapeworms of the orders Tetrphyllidea and Rhinebothriidea are poorly known primarily because their larvae typically lack species level. taxonomically distinguishing adult characteristics and using morphology they can be identified to genus, family or order only, the taxonomic characteristic of this order is Scolex, the hook or sucker that is used to attach the parasite to the body of the host. (Jensen, 2010). The Conclusion of this study, these findings revealed the highest infection with Tetrphyllidean sp. and Trypanorhyncha sp. larvae in *Saurida undosquamis*.

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