Rhabdochona kharani sp. n. (Nematoda: Rhabdochonidae) From The Fish Labeo gedrosicus Zugmayer, 1912 from Garruk, District Kharan, Balochistan, Pakistan

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SUMMARY: A new nematode species, *Rhabdochona kharani* sp. nov., has been isolated from the intestine, stomach and swim bladder of the fish *Labeo gedrosicus* Zugmayer, 1912 (Fam. Cyprinidae: Cypriniformes) from spring water at Garruk, District Kharan, Balochistan. The new species is characterized largely by the presence of eight anterior teeth in the prostom, inconspicuous deirids, by the shape and length of unequal spicules (0.08 - 0.09 and 2.9 - 3.1 mm), and by 17-18 pairs of caudal papillae including 6-7 postanal, one adanal, and 10 -11 preanal. Other characteristics include a pointed tail tip, non filamented eggs, a bulbous, voluminous, vaginal complex that is directed backward, and a vulva that is post-equatorial with a triangular large anterior lip and a smaller posterior lip.

Key Words: Rhabdochona kharani sp. n., Labeo gedrosicus, intestine, stomach, swim bladder, Garruk, Kharan, Balochistan.

Balochistan'ın Kharan Bölgesi Garruk'tan Labeo gedrosicus Balığında Saptanan Rhabdochona kharani

ÖZET: *Rhabdochona kharani* sp. nov. adı verilen yeni bir tür, kaynak suyunda yaşayan *Labeo gedrosicus* Zugmayer, 1912 (Fam. Cyprinidae: Cypriniformes) adlı balığın barsağı, midesi ve yüzme kesesinden izole edilmiştir. Yeni türün en belirgin özellikleri şunlardır: Prostomdaki sekiz ön diş; belirgin olmayan sefalik papiller; eşit olmayan spiküllerin şekil ve uzunluğu (0.08 - 0.09 ve 2.9 - 3.1 mm); 6-7 postanal, bir adanal ve 10-11 preanal'dan oluşan 17-18 kaudal papil çifti. Diğer özellikleri arasında, sivri bir kuyruk ucu; filamanlı olmayan yumurtalar; şişkin, geniş ve arkaya doğru uzanan vajina kompleksi; büyük üçgen bir ön dudağı ve daha küçük bir arka dudağı olan, post-ekvatoryal bir vulva yer alır.

Anahtar Sözcükler: Rhabdochona kharani sp. n., Labeo gedrosicus, Bağırsak, mide, yüzme kesesi, Garruk, Kharan, Balochistan, Pakistan

INTRODUCTION

The genus *Rhabdochona* Railliet 1916, is worldwide in distribution and is found mostly in fresh water fishes (3, 5, 8, 10-12, 15, 19, 20, 36, 38-41, 43, 47, 51, 52, 59, 62, 65). A large number of species, more than 32 are known from India (36). Recently, *Rhabdochona* sp. are reported from Thailand (9) and Arizona (19). A few species have been described from fishes of Pakistan (2, 22-24, 33, 53, 60, 67), including one species from a marine fish (6). Species of *Rahabdochona* have also been reported from Islamabad (31) and Jummu and Kashmir (48). Here a new species, *Rhabdochon kharani* is described. Present description is based on both male and

female specimens from a fish *Labeo gedrosicus* from Garruk, District Kharan, Balochistan. This is the first record of genus *Rhabdochona* from Balochistan.

MATERIALS AND METHODS

On January 24, 2004, ten freshwater carps, *Labeo gedrosicus*, Zugmayer, 1912, were collected from spring water at Garruk, District Kharan, Balochistan. Of these, seven were males and three females. Fishes were caught in scoop and drag nets (4). The samples were preserved in 10% formalin and were taken to the laboratory of Zoology, University of Balochistan, Quetta. Dissection and examination for parasites were performed under dissecting microscope, using standard methods (27, 28). The dissection procedure include, opening of the body cavity on the ventral midline of the fish from its anus to head and removing the entire digestive tract, stomach and swim-bladder. All the organs were placed in Petri-dishes

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separately, containing physiological saline. The internal organs were opened lengthwise and each parasite was isolated carefully.

Eleven nematodes (8 females and 3 males) were recovered from five fishes, maximum number of parasites were three in a fish. Parasites were fixed in 70% alcohol and glycerol (1:1) (7). To expose key anatomical features and outer integument of nematodes, lactophenol was used (66). Parasites were mounted between slide glass and cover glass using 2 to 3 drops of glycerol. Nail polish was pasted around the sides of cover slips to hold them firmly. Diagrams were made with the help of camera lucida and measurement are given length by width in millimeters. Holotype and paratype specimens were deposited in the Fish Parasitology section in the department of zoology, university of Balochistan, Quetta.

DESCRIPTION

Rhabdochona (Rhabdochona) kharani sp. n. (Figs. 1-7)

| Host | : Labeo gedrosicus |
|-----------------------------|--------------------------------------------------------|
| Location | : Intestine, stomach and swim bladder |
| Locality | : Stream water, Kharan, Balochistan |
| Number of host examined | : 10 |
| Number of host infected | : 5 |
| Number of specimens recover | : 3 male & 8 female |
| Date of collection | : 24.01.2004 |
| Cat. No | : ZMUB N-I, Holotype male ZMUB N-2, Paratype female |

Description is based on mature male and female worms . A total of 3 males and 8 females were recovered, including 6 from intestine, 2 from stomach and 3 were in swim bladder. In the swim bladder all the 3 (2 females and 1 male) were immature. In the intestine (2 males and 4 females) and in the stomach (1 male and 1 female) was present. Microscopic study shows the body smooth, long, delicate, narrow anteriorly and posteriorly. Anterior extremity rounded , posterior pointed, widest at the post-eusophageal region. Prostome with 8 teeth, mesostome almost triangular in shape. Two cephalic papillae are present, one at each side of the prostome. Muscular esophagus is two and half to three times smaller than glandular eusophagus. Excretory pore is pre-equatorial. Males are smaller than females. Spicules are very unequal, wider at the distal end and pointed at the proximal end. Long spicule is more than three times longer than the short spicule (1: 3.6 / 3.8), with thick cuticular margins throughout the length. Caudal papillae 17-18 pairs including 6-7 pairs postanal, 1 adanal and 10-11 preanal. In female there is a small intestinal cecum, vulva is post-equatorial with a large prominent, almost triangular anterior lip, vagina muscular, long, enclosed in a large glandular structure forming a voluminous, bulbous, vaginal complex. This structure is

prominent even in the immature female specimens (Fig. 7). Uterus is long, extending anteriorly to the base of glandular esophagus and posteriorly terminates far anterior to the anal opening. Tail is relatively short, pointed. Eggs are numerous, large, oval, smooth and non filamented.

Male: Body length 3.5 - 4.3, width 0.2 - 0.4, prostome 0.020 - 0.021 wide, mesostom 0.020 - 0.024 in length, 0.011 - 0.012 wide, anteriorly, muscular esophagus 0.016 - 0.064 x 0.011 - 0.014, glandular esophagus 0.170 - 0.175 x 0.016 - 0.018, excretory pore at a distance of 2.4 to 2.6 from the anterior extremity, spicules 0.08 - 0.09 and 2.9 - 3.1 mm. Tail 0.25 - 0.28 in length.

Female: body length 8.5 - 9.2, width 0.025 - 0.027, prostome 0.020 - 0.022 wide, mesostome 0.022 - 0.025 in length, 0.021 - 0.022 wide anteriorly, muscular esophagus 0.090 - 0.093 in length, 0.012 - 0.013 in width, glandular esophagus 0.24 - 0.26 in length, 0.025 - 0.027 in width, intestinal cecum approximately 0.026 in length, vulva at a distance of 5.8 - 6.3 from the anterior end. Anterior lip of vulva $0.06 - 0.063 \times 0.037 - 0.039$ in mature female. Vaginal complex $0.18 - 0.19 \times 0.060 - 0.066$, vagina $0.16 - 0.17 \times 0.030 - 0.031$. Eggs $0.040 - 0.059 \times 0.019 - 0.021$. Tail 0.17 - 0.19 in length.

Etymology: The species name refers to the locality of the fish host.

DISCUSSION

The classification of the genus *Rhabdochona* Railliet 1916, and its subgenera based on morphology of eggs, have been discussed by several workers (11, 18, 26, 29, 30, 38-40, 52, 54). Character of eggs has been emphasizes for diagnosis of subgenera.

Saidow (1953) subdivided the genus into two genera *Rhabdochona* without egg filaments and *Filochona* with egg filaments. Janiszewska (30) created a new subfamily Rhabdochoniae for the species having filamented eggs and the character was taken generic diagnosis. Yamaguti (65) elevated *Filochona* to a generic rank based on the presence of egg filaments. But generic rank of *Filochona* was not accepted by several workers (20, 38-40, 52, 54, 56, 61).

Moravec (39) divided the genus into three subgenera based on the type of eggs which was accepted by some workers (16, 21, 38). But, Moravec (40) considered that only egg character is not sufficient to divide the genus into three subgenera and other characters must also be included, such as number and arrangements of teeth in the prostome, presence of cervical alae, shape of the female tail tip and shape of deirids. Based on these characters, he raised the number of subgenera into four including *Rhabdochona*, *Globochona, Globochonaides* and *Sinonema*. This was accepted by Bilqees (6); Soota and Sarkar (63) and Soota (1983). *Rhabdochona* with 10-14 or 16 anterior teeth, tail tip of female conical, ending in sharp



Figs. 1-7. Rhabdochona (R) kharani new species

Fig. 1. Anterior region of male holotype, showing prostome, mesostome, cervical papillae, esophagus, excretory pore and part of intestine;
Fig. 2. Posterior region of some holotype, showing spicules, caudal papillae, cloaca and tail. Scale bar 0.5mm; Fig. 3. Posterior region of male at higher magnification, showing details of papillae, small spicule and part of long spicule. Scale bar on figs. 1-3, 0.01mm; Fig. 4. Anterior region of female paratype, showing prostome, mesostome, esophagus and part of intestine. Note the two cephalic papillae and a small intestine cecum at the junction of esophagus and intestine; Fig. 5. Posterior region of female, showing anal opening and tail. Scale bar on figs. 4 and 5, 0.2 mm.; Fig. 6. Region of vulva of female, showing vulvar lips, voluminous , vaginal complex, uterus with numerous non-filamented eggs. Note the large anterior triangular lip. Scale bar 0.1 mm; Fig. 7. Region of vulva of an immature female, showing developing vaginal complex, large anterior lip of vulva and immature eggs. Scale bar 0.1 mm.

cuticular spike or rounded; egg with smooth surface, or with very fine, irregular gelatinous flocks, filaments or special polar swelling. But Chabaud (16) mentioned eggs smooth or with thin illdefined gelatinous covering only. *Globochona* with 8 or 12 anterior teeth; tail tip of the female widely rounded, often with numerous spins, mueronate points or tooth like processes, egg smooth or with lateral globules or swellings. But Chabaud (16) only mentioned eggs with special hemispherical floats. *Globochonoides* Moravec 1975; with 8 anterior teeth, tail tip of female with crown of minute tooth like processes, tail tip of male rounded and egg smooth. Chabaud (16) did not consider this as valid subgenus.

Sinonema Moravec, 1975; with 20-22 anterior teeth, tail tip of female rounded; egg surface not described. According to Chabaud (16) there are only three subgenera of the genus including *Rhabdochona (Rhabdochona)* Railliet 1916, *Rhabdochona (Filochona)* Saidov, 1953; and *Rhabdochona (Globochona)* Moravec, 1972; mainly based on morphology of eggs. Now, Chabauds' classification is widely accepted and followed here.

As mentioned above, species of the genus *Rhabdochona* reported from Pakistan are *R. (R) magna* Khan and Yaseen, 1969, *R. (R) cavasius* Rehana and Bilqees, 1973; *R. (R) Chanawensis* Ziadi and Khan, 1975; *R. (R) parastomate*i Bilqees, 1979; *R. (F) charsaddiensis* Siddiqi and Khattak, 1984; *R. (F) shizothoracis* Siddiqi and Khattak, 1984; *R. (R)*

megasacculata Ghazi and Rahim, 1999; and *R. (R)* rahimi Ghazi et al., 2003. All these species are from fresh water fishes except *R. (R) parastromaei* described from *Parastromateus niger* of Karachi coast (Bilqees, 1979). Description of *R. (R) megasacculata* is based on a single female specimen only.

We have collected several male and female specimens from the fish Labeo gedrosicus collected from Garruk, District Kharan, Balochistan. The nematods were found in the stomach, intestine and swim bladder of the fish. This is the first report of the genus from Balochistan. One female specimen reported from Barilius vagra of Islamabad by Ghazi and Atta-ur-Rahim (23), also has peculiar morphology of female terminal genitalia consisting of simple vulva, leading to a voluminous, bulbous, saccular structure containing muscular vagina, a structure close to the present specimens. But the present specimens are different in having prominent vulva with a large triangular anterior lip. The cephalic papillae are present and there is a small intestinal cecum which is absent in R. megasacculata is based on a single female specimen. The male of present new species are different from all other species of the genus reported so far in combination of characters, such as presence of cephalic papillae, 8 teeth in the prostom, glandular portion of esophagus relatively much longer than anterior muscular part, spicules very unequal and dissimilar, 17-18 pairs of caudal papillae including 10-11 preanal, 1 adanal and 6-7 postanal, tail relatively long, conical.

An intestinal cecum is present in female which has not been described previously in other species of the *Rhabdochona*. Gupta and Srivastava (25) described *R. chitala R. bagarie, R. chhaparai* and *R. tori*, they followed Rasheed (52). Agrawal (1) described *R. garuai*, Moravec (16) transferred it under *Rhabdochona (Globochona)*, Sahay (55) described *R. basei* and Rai (1969) *R. baylisi*. But Sahay and Narayan (57) treated *R. baylisi* conspecipic of *R. basei*. But Moravec (16) suppressed both *R. baylisi* and *R. basei* under *R. (G) garuai*. Gupta and Masoodi (1985) agreed with Moravec . They described *R. (G) garuai*. Agrawal, 1965 from the fish *Wallago attu* from Kanpur with morphological variations.

Lakshmi and Sudha (35) described *R. marina* from a marine fish *Pemperis vanicolensis* and later Lakshmi (36) described *R, indiana* from the same fish host. Kalyanker (32) reported *R. labeonis* and Rauleta and Malhotra *R. nemacheli* also from India.

The species *R.* (*G*) chodukini Oshmanov, 1957 and *R.* (*Globochonoides*) coronacauda Belouss, 1965 have small finger-like projections at the tip of tail which was referred as crown like structure by Rahimo and Al- Din (51). They have also reported a *Rhabdochona* sp. larva with these characteristic finger-like structures, similar to a crown at the tail tip.

These finger-like structures similar to a crown, are not found in the present species and the tail is simple with pointed end. Other species of adult Rhabdochona reported from Iraq include R. tigrae from Varoicorhinus trutta (49); R. similis from Glyptothorax sp. and R. denudata from Cyprinoin macrostomus (44); R. helichi from Barbus xanthopterus, Hetropneustus fossilis and Mystus halepensis (3); R. mesopotamica, R. grandipapillata from Cyprinion macrostomus (50). These species have no characteristic crown-like structure at the posterior end of female tail. The number of caudal papillae in the present specimens are 17-18 pairs. These are more numerous than in other species of the subgenus reported previously except R. (R) chanawensis from Pakistan. There are 14 pairs in R. (R) cavasius Rehana and Bilgees (53). In R. (R) chanawensis Zaidi and Khan, 1975, caudal papillae are 20, R. (R) magna Khan and Yaseen, 1969 is known only by females. The length of the unequal spicules in R. (R) cavasius is 0.12 and 0.42 mm, in R. (R) chanawensis 0.230 - 0.381 and 0.510 - 0.525. In R. (R) parastromatei which is from a marine fish, the spicules measure 0.10 - 0.12and 0.48 - 0.50 and the number of caudal papillae are 10 - 11pairs.

The Indian species of the genus *Rhabdochona*, *R.* (*R*) papillata Arya and Johnson, 1977 has 4 pairs of caudal papillae; *R.* (*R*) glyptoyhorcis Karve and Naik, 1951 has 17 pairs of caudal papillae, 10 preanal and 7 postanal and no adanal papillae and eggs are with numerous filament on both sides while eggs are smooth and one pair of adanal papillae are found in the present new species. In *R.* (*R*) helichi Sramek, 1901, Chitwood, 1933, there are 14 - 18 pairs of caudal papillae with 7 - 12 preanal and 5-9 postanal and the eggs in female have long filament. In *R. (R) hospeti* Thapar, 1950 (64) there are 12 - 18 pairs of caudal papillae in male with 7 - 12 pairs preanal and 5 - 6 postanal and the long spicule is much smaller (0.55 - 0.71) as compared to the present specimens (2.9 - 3.1). *R. (R) smythi* Agrawal, 1965 has 17 pairs of caudal papillae in male including 10 preanal and 7 postanal. The long spicule is also much shorter (0.62 - 0.68) than in the present species and in the female, eggs have filament at one end while eggs are smooth in the present species.

The recently described species of *Rhabdochona* from other parts of the world, *R. ahuehuellensis* Mejia-Madrid and Perez-Ponce de Leon, 2003 is also different in the shape and size of spicule and in having eggs with sub-polar filaments. *R. guerreroensis* (13) has different number of teeth in prostom (12), different spicule morphology and filamented eggs. *R. mexicana* (12) has 10 teeth in the prostom and eggs are with irregular flock-like coating, the spicule in male have a different peculiar shape. *R. lechtenfelsi* (58) has 10 large teeth, tip of tail short, spicule is bifurcated, while in the present specimen, there are 8 teeth and spicule is not bifurcated. *R. xiphophori* (14) is peculiar in having unique structure of the prostom, 6 anterior teeth, dorsal and ventral broad with two lateral horns.

Non of the above mentioned species have a voluminous vaginal complex with triangular large anterior lip of vulva, which is the main characteristic of the female in the present species. This character is not found in any of the species of *Rhabdochona* reported so far with relative spicule length of the large spicules in male. These characters are peculiar in the present new species and justify the proposal of a new species for which the name *R*. *(R) kharani* is suggested. *Rhabdochona* species has not been reported previously from *Labeo gedrosicus* and the nematode genus represent a new geographic distribution, as there is no previous record from Balochistan

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