

A New Parasitic Nematode Species *Rhabdochona pakistanica*, New Species From *Cyprinion watsoni* (Osteichthyes: Cyprinidae) in Central Balochistan

Asmatullah-Kakar^{*1}, Fatima Mujib Bilqees² and Kafaitullah Khan¹

¹Department of Zoology, University of Balochistan, Quetta 87300, Pakistan

²Department of Zoology, Jinnah University for Women Karachi, Pakistan

Abstract.- A new species of nematode (Nematoda: Rhabdochonidae) is reported here from the stomach of a fish, *Cyprinion watsoni* (Day, 1872) caught at river Bolan, Sibi division, Balochistan. *Rhabdochona pakistanica* n. sp. is distinguished from all species of the genus *Rhabdochona* Railliet, 1916 in general and its closest allies (possessing 8 prostomal teeth) mainly in body size, spicules ratio, number and distribution of caudal papillae, shape of the egg and position of vulva and excretory pore.

Key words: Parasitic nematode, *Rhabdochona pakistanica* n. sp., *Cyprinion watsoni*, Sibi, Balochistan.

INTRODUCTION

The genus *Rhabdochona*, a perplexing group of parasites, contains a large number of species. Its representatives are distributed over all continents with exception of Australian zoogeographical region. Nematodes of this genus include more than 100 species that reside in the alimentary tract mostly of the freshwater fish species (Mejia-Madrid *et al.*, 2007). However, few species of *Rhabdochona* Railliet, 1916 are reported infesting marine fishes as well. According to Caspeta-Mandujano and Mejia-Mojica, 2004, 92 nominal species of *Rhabdochona* have so far been recognized from freshwater fishes. But Moravec *et al.* (2010) considered 96 species of the genus as possibly valid. However, an additional four species *R. gubernaculus* Asmatullah-Kakar *et al.*, 2010; *R. ictaluri* Aguilar *et al.*, 2010; *R. pseudomysti* and *R. thaiensis* Moravec and Yooyen, 2011 has been described from the fish hosts, *Cyprinion watsoni* (Day, 1872), *Ictalurus dugesii* (Bean, 1880) and *I. pricei* (Rutter, 1896) and *Pseudomystus siamensis* (Regan, 1913) respectively in Pakistan, Mexico and Thailand. Consequently, at present, the genus *Rhabdochona* Railliet, 1916 comprises more than 110 species (Cernoticova *et al.*, 2011) of which 20

are reported from Pakistani inland fishes (Asmatullah-Kakar *et al.*, 2010).

A Study has been carried out on endo-helminth fauna of cyprinids (Pisces: Cypriniformes) in central Balochistan. During this study a new nematode species belonging to the family Rhabdochonidae (Rhabdochoninae) Travassus, Artigas and Pereira, 1928, Skrjabin, 1946 has been found in the stomach of freshwater carp *Cyprinion watsoni* (Day, 1872) a favorable host and locality river Bolan. Previously two species of Rhabdochonid nematode were described from the same host and localities river Bolan and Khaisar valley, Nushki, Balochistan. An analysis of the material showed that this nematode parasite can not be assigned to any known species.

MATERIALS AND METHODS

Twenty eight fishes *Cyprinion watsoni* were collected in the month of March, 2007 from river waters at Gogurt, district Bolan. These were examined for nematodes. Of the Twenty eight, ten fishes were infected harbored twenty seven mature specimens (16 ♂ and 11 ♀) in the mucosa of stomach. Worms were preserved in 70% ethyl alcohol, fixed in a mixture having three part of the same fixative and one part of glycerin and cleared in lactophenol. These were mounted temporarily on glass slide in pure glycerin under cover slip. Diagrams were made with a camera Lucida. All measurements are given in millimeters. Mean and

* Corresponding author: asmardanzai@yahoo.com
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standard deviation of paratype are given in parentheses. All the specimens (holotype and paratype) are in the museum of Zoology Department, University of Balochistan at Quetta city and accessible to other scientists.

RESULTS

Rhabdochona (Rhabdochona) pakistanica, n. sp. (Figs. 1-2)

Description

Small, slender, transparent worms, attenuated at both ends. Cuticle thin, smooth and unstriated. Anterior end somewhat rounded, widest at the mid-body region in male and at the level of vagina in the female. Oral aperture roughly pentagonal, provided with two distinct cephalic papillae, one on each side of it. Prostom funnel-shaped, armed internally with 8 distinct small prostomal teeth (2 dorsal, 2 ventral and 4 lateral on either side). Esophagus very long, distinctly divided into slender, anterior muscular portion and thicker posterior glandular portion. Intestine simple devoid of any diverticula. Nerve ring encircling the muscular esophagus, located almost in the mid-portion of muscular esophagus in male and at the base in female specimens. Deirids medium-sized, spiniform, located close to base of muscular esophagus in male, and in female situated near middle of muscular esophagus or somewhat posterior to it. Excretory pore of both sexes post-equatorial. Male tail pointed at tip, curved ventrally, caudal papillae 12 pairs including 7 preanal and 5 postanal. Spicules unequal and dissimilar. Larger (left) spicule non-alate, tubular with markedly developed knob at proximal end, bluntly pointed at distal end. Smaller (right) spicule broad, rounded at proximal end, narrower in the middle, trophy-shaped, terminates in a triangular flat base. Female tail smooth, bluntly pointed. Vulva post-equatorial, vulval lips beak-like and somewhat elevated. Vagina strongly muscular, directed posteriorly from vulva. Basal region of vagina wider containing a mature, elongate egg devoid of filaments or floats.

Measurements

Holotype (male)

Length 3.1 mm, width 0.07; prostome 0.003; mesostome 0.005; anterior muscular esophagus

0.073; posterior glandular esophagus 0.142 in size; deirids 0.046, nerve ring 0.075, excretory pore 2.1 from the anterior end; left spicule 0.32; right spicule 0.04; spicules length ratio 1:8.0; tail 0.21.

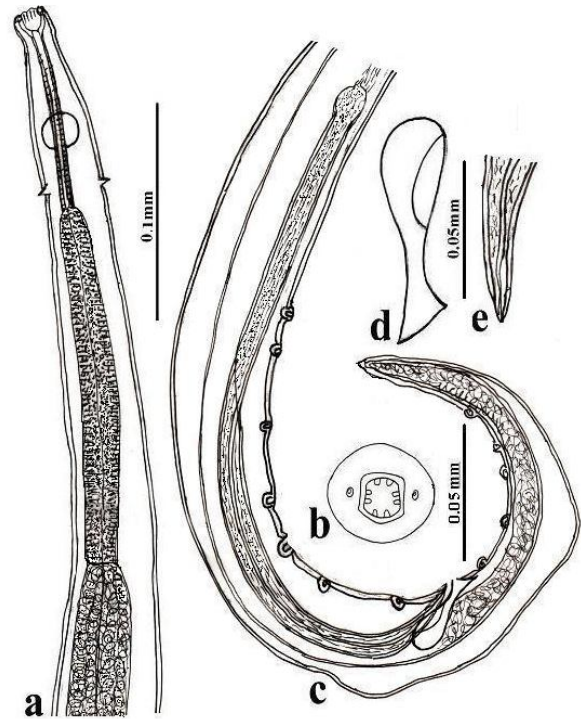


Fig. 1. *Rhabdochona (Rhabdochona) pakistanica* n.sp., holotype male, a, anterior region showing buccal capsule, muscular esophagus and anterior portion of intestine. Nerve ring and deirids are also seen; b, enface view showing prostomal teeth; c, posterior region showing large and small spicules, caudal papillae and tail; d, small spicule enlarged; e, tip of large spicule enlarged. Scale is same for a, c, d and e.

Male (n=15)

Length 1.05-3.24 (2.96 ± 0.34) mm; maximum width 0.031-0.09 (0.079 ± 0.023); prostome 0.001-0.005 (0.0047 ± 0.0013); mesostome 0.002-0.0086 (0.0049 ± 0.0017); muscular esophagus 0.026-0.092 (0.077 ± 0.028); glandular esophagus 0.03-0.175 (0.16 ± 0.07) in size; deirids 0.013-0.051 (0.049 ± 0.021), nerve ring 0.019-0.085 (0.073 ± 0.019), excretory pore 0.48-2.32 (1.99 ± 0.26) from anterior extremity; left spicule 0.072-0.37 (2.88 ± 0.41); right spicule 0.012-0.043 (0.039); spicules length ratio 1: 2.46-8.60 (6.72 ± 0.36). Tail 0.05-0.23 (0.2 ± 0.13).

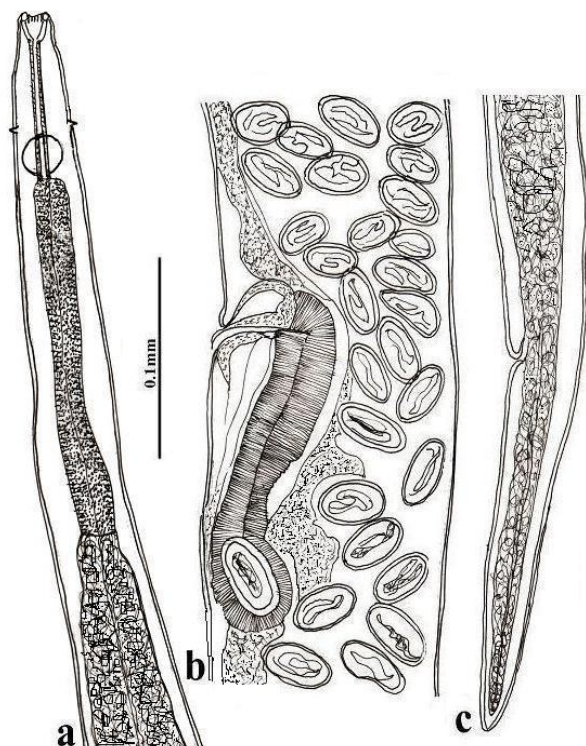


Fig. 2. *Rhabdochona (Rhabdochona) pakistanica* n.sp., allotype female, a, anterior region showing buccal capsule, muscular and glandular esophagus and part of intestine. Nerve ring and deirids are also seen; b, region of genital opening showing marginal vulva, vagina and smooth eggs; c, posterior region showing tail, and associated structure. Scale is same for a, v and c.

Female (n=11)

Length 2.16-4.23 (3.57 ± 0.46) mm; maximum width 0.05-0.14 (0.11 ± 0.09); prostome 0.001-0.0036 (0.003 ± 0.0012); mesostome 0.003-0.01 (0.007 ± 0.002); muscular esophagus 0.034-0.08 (0.06 ± 0.024); glandular esophagus 0.12-0.192 (0.17 ± 0.05) in size; deirids 0.026-0.057 (0.05 ± 0.017), nerve ring 0.028-0.054 (0.047 ± 0.03), excretory pore 1.51-2.68 (2.41 ± 0.31) from anterior extremity. Vulva length 0.027-0.045 (0.039 ± 0.015); anterior vulval lip 0.11-0.19 (0.17 ± 0.04), posterior vulval lip 0.021-0.047 (0.043 ± 0.027); vulva from posterior end 1.23-1.85 (1.74 ± 0.24); vagina length 0.13-0.171 (0.15 ± 0.03); mature eggs 0.014-0.034 (0.03 ± 0.012) in size. Tail 0.143-0.183 (0.174 ± 0.051).

Etymology

The species name *Rhabdochona (Rhabdochona) pakistanica* refers to the country name from where the fish hosts were collected.

Taxonomic summary

Type host:	<i>Cyprinion watsoni</i> (Cyprinidae)
Site of infection:	Stomach
Type locality:	River Bolan, Balochistan
Number of specimens:	A total 27 worms, 16 male and 11 female from 10 out of 28 hosts examined. Maximum 5 male and 4 female from a single fish.
Holotype male:	ZBU-N58
Allotype female:	ZBU-N59

DISCUSSION

Based on the number of anterior prostomal teeth, the Pakistani species of *Rhabdochona* can be separated into 6 groups. Those with 3 teeth (*R. magna* Khan and Yaseen, 1969 (based on female only) and *R. cavasius* Rehana and Bilqees, 1973a); 4 (*R. chanawanensis* Zaidi and Khan, 1975 (based on male only); 6 (*R. bifidum*, *R. hingoli* Asmatullah-Kakar and Bilqees, 2007c (based on male only) and *R. cephalodiverticola* Asmatullah-Kakar *et al.*, 2008a (based on male only); 8 (*R. sarana* Karve and Naik, 1951, Akram and Khatoon, 2001 (based on females only), *R. kharani* Asmatullah-Kakar *et al.*, 2006, *R. watsoniai* Asmatullah-Kakar and Bilqees, 2007a (based on male only); *R. uvaginus* Asmatullah-Kakar and Bilqees, 2007b and *R. mujibi* Asmatullah-Kakar and Bilqees, 2009). Those possessing 10 teeth (*R. megasacculata* Ghazi and Atta-u-Rahim, 1999 (based on female only), *R. hellichi* Sramek, 1901, Akaram and Khatoon, 2001; *R. nushkiai* Asmatullah-Kakar and Bilqees, 2007a (based on male only); *R. milesi* Asmatullah-Kakar *et al.*, 2008b, *R. magnavesicula* Asmatullah-Kakar and Bilqees, 2008 and *R. gubernaculus* Asmatullah-Kakar *et al.*, 2010); 10-12 (*R. charsaddiensis* Siddiqi and Khattak, 1984 and *R. rahimi* Gahzi *et al.*, 2003) and 16 (*R. parastromatei* Bilqees, 1979) from a marine fish. In contrast to all of them (except *R. sarana*, *R. kharani*, *R. watsoniai*, *R. uvaginus* and *R. mujibi*), the present new species *Rhabdochona pakistanica* n. sp. has 8 prostomal teeth.

R. pakistanica n. sp. is easily distinguished from all so far known and closely related species of

Rhabdochona by having trophy-shaped smaller (right) spicule. This feature is unique within Rhabdochonidae. Among (above) known species (*R. cavasius*, *R. rahimi*), and a closely related form (*R. mujibi*), all the three possess boat-shaped smaller spicule without dorsal barb at distal end. Some of the known species e.g., *R. chanawanensis*, *R. bifidum* and *R. cephalodiverticola* have simple, smaller spicule terminates in bifid tips. Some of them (*R. hingoli*, *R. watsoniai*, *R. nushkiai* and *R. milesi*) possess smaller spicule with bifid anterior tips and triangular flat base at distal tips. While in *R. magnavesicula* this spicule is spindle-shaped with thread-like proximal portion, and in *R. gubernaculus* it is slightly concave on ventral side with notched anterior tip. In other related forms (*R. uvaginus* and *R. kharani*), the smaller spicule is spatulate in the former, it is flat anteriorly with blunt distal tip in the latter. The smaller spicule of *R. hellichi* has distinct dorsal barb, whereas those of *R. charsaddiensis* with reflected barb at distal end. Similarly, in *R. parastromatei* (from marine fish), it is bow-shaped with arrow like structure at anterior tip. A remarkable character of *R. pakitanica* n. sp. is its unusual larger (left) spicule provided with markedly developed knob at anterior tip which is not present in the above mentioned species (however, the male of *R. magna*, *R. megasacculata* and *R. sarana* is unknown).

The species under study is very different from these nematodes in that its deirids (of males) are very close to the posterior extremity of muscular esophagus; the female tail tip is blunt, those of male pointed; spicular ratio in the male is rather high (1: 8.0-8.60); there are 12 pairs (7+5) of caudal papillae: *R. pakistanica* n. sp. is very similar to *R. kharani* in female tail length (0.175-0.18 mm), *R. sarana* (based on females only) in size of eggs (0.032-0.034), *R. rahimi* in female nerve ring distance (0.51-0.57) from anterior extremity, *R. hingoli* in number of preanal papillae (7 pairs), and to *R. nushkiai* (based on single male) in tail length (0.21) and distance of excretory pore (2.5) from anterior extremity; but differ in that the former has larger (left) spicule (0.32-0.37) than the latter's (except *R. kharani*), (0.29-0.31), (0.314-0.327) and (0.329), and bears 7 instead of 10-11 and 11 pairs of preanal papillae. In *R. kharani* length of larger

spicule is 2.9-3.1. Other species such as, *R. cavasius*, *R. bifidum*, *R. cephalodiverticola* and *R. gubernaculus* have 9 pairs of preanal papillae respectively, whereas *R. chanawanensis* has (11), *R. watsoniai* (12), *R. magnavesicula* (4), *R. hellichi* (8), *R. charsaddiensis* (10-11), and *R. parastromatei* (3-4). The relative lengths of their larger (left) spicule are also distinctly smaller than in *R. pakistanica* n. sp. The evident beak-like, elevated vulval lips in the new species are another unique feature within *Rhabdochona*.

In contrast to *R. pakistanica* n. sp., the eggs of *R. hellichi*, *R. charsaddiensis* and *R. gubernaculus* bear filaments, whereas those of *R. rahimi* are provided with cuticular floats. All the remaining species possess simple smooth egg similar to present new species. Moravec (1975) considered these features as of generic importance. Position of vulva of all known and related forms (except *R. magna*) is post-equatorial resembling thus *R. pakistanica* n. sp., whereas in *R. magna* it is equatorial (however, female of *R. chanawanensis*, *R. bifidum*, *R. hingoli*, *R. cephalodiverticola*, *R. watsoniai* and *R. nushkiai* is unknown). Except for *R. gubernaculus* (has bifurcate deirids); all other forms (excluding *R. magna*, *R. megasacculata*, *R. sarana*, *R. kharani* and *R. uvaginus*) have simple, spine-like deirids as in the species under reference. In *R. magna*, *R. megasacculata*, *R. sarana*, *R. kharani* and *R. uvaginus* deirids are inconspicuous. The shape and size of deirids are considered to be one of the taxonomically most important morphological features in *Rhabdochona* spp. (Mejia-Madrid *et al.*, 2007; Moravec, 1975; Moravec *et al.*, 2008).

Based on 8 prostomal teeth, *R. pakistanica* n. sp., most closely resemble to its congeners reported from other regions include *R. thaiensis* Moravec and Yooyen, 2011; *R. brevichona* Guitang *et al.*, 1994; *R. versterae* Boomker and Petter, 1993; *R. coronacauda* Belouss, 1965; *R. chabaudi* Mawson 1956; *R. paski* Baylis, 1928; *R. beatriceinsleyae* Holloway and Klewer, 1969; *R. ovifilamenta* Weller, 1938; *R. chodukini* Osmanov, 1957; *R. congolensis* Campana-Rouget, 1961; *R. sarana* Karve and Naik, 1951; *R. fotedari* Katoch and Kalia, 1993; *R. moraveci* Duggal and Kaur, 1987; *R. garuai* Agrawal, 1965b and *R. barusi*

Majumdar and De, 1971. The new species is characterized by the absence of filaments or floats on eggs resembling thus *R. thaiensis*, *R. versterae*, *R. fotedari*, *R. moraveci*, *R. garuai* and *R. sarana*, but differing *R. congolensis*, *R. brevichona*, *R. chabaudi*, *R. paski*, *R. chodukini*, *R. ovifilamenta*, *R. coronacauda* and *R. beatriceinsleyae* in which the eggs are equipped with filaments. While eggs of *R. barusi* has floats on its surface. *R. paski* can be easily differentiated from above named species and *R. pakistanica* n. sp. in having larger body size: the male measures 15-20 mm, the female 28-35 mm in length. This nematode appears to be somewhat larger than any of the species of *Rhabdochona* hitherto described (Aguilar *et al.*, 2010). In contrast to the new species, *R. ovifilamenta* is characterized by the presence of a gubernaculum (a rare feature in the genus *Rhabdochona*), a pre-equatorial vulva, 9 pairs of preanal papillae, and by spicular ratio (1:9.0 mm); whereas *R. thaiensis*, *R. versterae*, *R. chabaudi*, *R. paski*, *R. ovifilamenta*, *R. chodukini*, *R. congolensis*, *R. fotedari*, *R. garuai* and *R. barusi* differ distinctly in shorter spicular ratio and numerous pairs of preanal papillae: 1:2.35 (9 pairs), 1:2.11-2.74 (12-15), 1:4.08-4.09 (8), 1:2.14-2.61 (10-13), 1:3.42-3.50 (8), 1:2.37 (15-16), 1:3.1-3.27 (8), 1:2.25-3.39 (13), and 1:5.5 (8) vs 1:8.0-8.60 (7). *R. beatriceinsleyae* and *R. moraveci* also differ from the new species in that both have less numerous pairs (6) of preanal papillae and shorter spicular ratio (1:4.0-5.4 and 1:2.5-3.8) respectively. *R. brevichona* and *R. coronacauda* are very close to *R. pakistanica* n. sp. in having (7 pairs) preanal papillae, differ remarkably in length ratio of spicules 1:4.67-6.53 and 1:4.82-5.0 respectively.

In addition to above mentioned differences, the two named species (*R. thaiensis* and *R. garuai*) distinctly different from *R. pakistanica* n. sp. in the shape of deirids: whereas the deirids of these two species are bifurcate. All other species (except *R. beatriceinsleyae*, *R. ovifilamenta*, *R. chabaudi* and *R. sarana*, in which deirids are unknown) have spine-like deirids present in the new nematodes. *R. pakistanica* n. sp. can further be distinguished from them in the relevant length of body, morphology of vagina, sizes of egg and esophagus etc. Consequently, the nematodes described in the present communication are considered to belong to

new species and they are named *R. (R.) pakistanica*.

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