



Rhabdochona spatulatum, New Species (Nematoda: Rhabdochonidae) from the Freshwater Cyprinid Fish *Cyprinion microphthalmum* (Day) in Quetta Division, Pakistan

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ABSTRACT

A new species of *Rhabdochona* is described from the intestine of cyprinid fish collected from Khessar stream (Hamun River basin) at Nushki, Quetta Division, Balochistan. It is characterized mainly by having a spatulate right spicule with ventral barb at posterior end, the left spicule has notched anterior and razor-sharp distal tips, finely striated caudal region, fifteen pairs (10 preanal, 5 postanal) papillae in males, bifurcate deirids and conical tail tips in both the sexes. The subgeneric definition of *R. (Rhabdochona)* Railliet, 1916, *R. (Filochona)* Saidov, 1954, and *R. (Globochona)* Moravec, 1972a which were based primarily on egg morphology are considered of little value in determining specific relationships within the genus. The new species may be included in the subgenus *Rhabdochona* (Morphological group A, Moravec, 1975) in the presence of thick-shelled (slightly floated) eggs, which is, therefore, described here as *Rhabdochona (Rhabdochona) spatulatum* n. sp. This nematode is the first of the genus recovered from *Cyprinion microphthalmum* (Day) from this locality in Balochistan.

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AK and FMB conceived the study. AK executed the experimental work and wrote the article.

Key words

Nematode parasite, *Rhabdochona spatulatum* n. sp., freshwater fish, Nushki, Pakistan

INTRODUCTION

The genus *Rhabdochona* Railliet, 1916 is a pathogenic parasite occurs in the digestive tract more often of freshwater fishes (Gonzalez-Solis *et al.*, 2014). It is found in all zoogeographical regions and the majority of its members exhibit high degree of specificity, and their hosts largely belong to the family Cyprinidae less to Bagridae and Siluridae (Moravec, 2010; Anjum, 2013; Asmatullah-Kakar *et al.*, 2014). The genus *Rhabdochona* was divided by Saidov (1954) into two subgenera namely: *R. (Rhabdochona)* Railliet, 1916, *R. (Filochona)* Saidov, 1954. Anderson *et al.* (1975) quoted *R. (Globochona)* Moravec, 1972 a third subgenus. Division of the three subgenera based primarily on the presence or absence of filaments or floats on the eggs. The taxonomic importance of these formations on the eggs of *Rhabdochona* species has generally been regarded as of generic, subgeneric and specific value (Janiszewaska, 1955; Yamaguti, 1961; Rasheed, 1965; Moravec, 1972). Later studies (Margolis *et al.*, 1975) considered these subgeneric taxa to be artificial primarily because species assigned to one subgenus were often most similar (except in egg morphology) to a member of another than to

species of their own subgenus. They indicated that confusion has resulted because of the past utilization of only these criteria for separation of subgenera, and proposed that more features be incorporated. Also, Moravec and Aria (1971) found that during manipulation of the eggs, polar filaments were easily lost. Kayton *et al.* (1979) observed both filamented and non-filamented mature eggs in the same specimen of *R. catostomi*, thus, indicate that character of eggs in this genus does not always reflect the actual relationship and that the division into subgenera is to a certain extent artificial.

Previously few *Rhabdochona* species belonging to the three subgenera have been described from fishes in other parts of Pakistan (Khan and Yaseen, 1969; Rehana and Bilqees, 1973a; Zaidi and Khan, 1975; Bilqees, 1979; Siddiqui and Khattak, 1984; Ghazi and Attau-Rahim, 1999; Akram and Khatoon, 2001; Ghazi *et al.*, 2003). The list of *Rhabdochona* species reported worldwide is very impressive, those added from Balochistan (Pakistan) are seventeen mostly from cyprinid fishes (See Table I). The present study was aimed to investigate the helminth fauna of fish. A new nematode belonging to subgenus *R. (Rhabdochona)* Railliet, 1916 has been recorded in *Cyprinion microphthalmum* (Cypriniformes) inhabiting Khessar stream from Nushki showing westward extension of its range of distribution in Balochistan. Khessar, is the only perennial stream in the district and has very rich fish fauna. To date, very little published data is available on the fish nematodes from this locality

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(Asmatullah-Kakar and Bilqeess, 2007a). Hence, many other congeneric species of *Rhabdochona* may be described in future in this little explored region of Balochistan.

MATERIALS AND METHODS

Specimens of *Cyprinion microphthalmum* Day (n =14) were caught by fishing net from Khessar stream (29° 33' 0" North, 66° 1' 0" East) in November, 2013 in Nushki, Balochistan. Nematodes recovered from the hosts' digestive tract were fixed in hot 70% ethanol and cleared in a mixture of alcohol and glycerin (3:1 ratio) in laboratory. For light microscopical (LM) examination, these were cleared with lacto phenol.

Specimens were mounted on slides in pure glycerin or lacto-phenol. *En face* view was prepared by decapitation and subsequent mounting in glycerin. The drawings were made with the aid of Zeiss drawing attachment. All measurements are in millimeters followed by means in parentheses.

The type specimens were deposited in helminth collection in the museum of Zoology Department, University of Balochistan at Quetta, Pakistan. The scientific name of fish follows Fish Base (Talwar and Jhingran, 1991).

RESULTS

Rhabdochona (Rhabdochona) spatulatum, new species (Figs. 1-3)

<i>Type host:</i>	<i>Cyprinion microphthalmum</i> (Cypriniformes: Cyprinidae)
<i>Site of infection:</i>	Intestine
<i>Type locality:</i>	Stream water, district Nushki, Balochistan.
<i>Prevalence and intensity:</i>	12 fish were infected/14 fish examined: 36 nematodes (maximum 7 in a single host).
<i>Holotype male:</i>	ZBU-N60
<i>Allotype female:</i>	ZBU-N61

Description

Description is based on 4 males and 3 females: Small-sized nematodes tapering at both ends, widened at the mid-body region. Tails of both sexes conical, striated in males, female's tail tip ends in a sharp spike. Caudal alae absent. Prostome funnel-shaped provided with 10 longitudinal ridges, pointed anteriorly terminating in anterior teeth. Lateral ridges (4) forming 2 teeth each (Fig.1b). Basal teeth absent. Mesostome wide, smooth. Cephalic papillae arranged in outer circle of 6 (2 median, 4 small sub-medians). Esophagus with anterior muscular

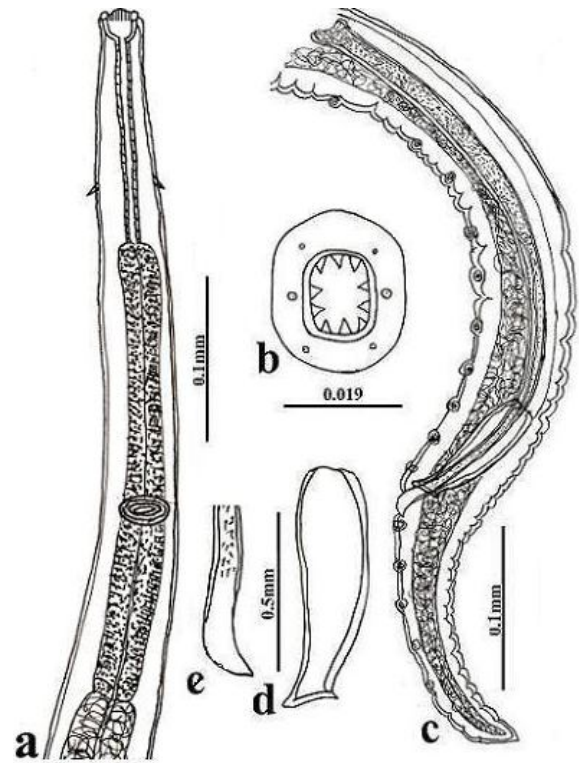


Fig. 1. *Rhabdochona (Rhabdochona) spatulatum* new species, holotype male. A, anterior body region showing buccal capsule, muscular and glandular esophagus and part of intestine. Nerve ring and deirids are also seen; b, enface view showing prostomal teeth; c, posterior region showing large and small spicules, caudal papillae and tail; d, small spicule enlarged; e, tip of large spicule enlarged.

and wider posterior glandular regions. Deirids small, bifurcate, lying slightly anterior to midvestibule. Nerve ring at level of anterior $\frac{1}{3}$ of muscular esophagus. Excretory pore of both sexes preequatorial. Spicules of male unequal and dissimilar. Left (large) spicule slender, curved ventrally, slightly notched at the anterior tip ending into a large spine directed backward. Right (small) spicule spatulate, wider than left spicule, flat anteriorly reflect with distal barb. Caudal papillae variable in number with 10 preanal and 5 postanal pairs, sub-ventral, second postanal pair (from cloaca) slightly more lateral. Female vulva preequatorial, consisting of two chitinous, spine-like structures curved posteriorly. Vagina muscular, strongly developed, broader anteriorly then becomes narrower proceeding into a broad posterior portion joining the uterus at the base. Mature eggs elongate, thick-walled (gelatinous flocks hardly visible).

Immature eggs smaller with irregular shells and two or three projections.

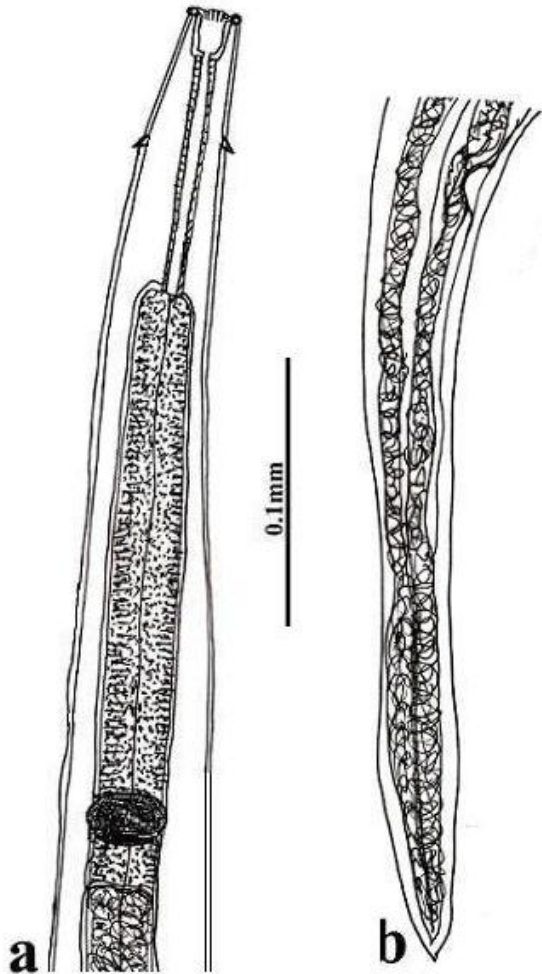


Fig. 2. *Rhabdochona (Rhabdochona) spatulatum* n.sp., allotype female. a, anterior region showing buccal capsule, muscular and glandular esophagus and part of intestine. Nerve ring and deirids are also seen; b, posterior region showing tail and associated structures.

Measurements

Male

(4 specimens; measurement of holotype in parenthesis): Body length 4.2-4.6 (4.37), greatest width 0.1-0.13 (0.12), prostome 0.002-0.004 (0.032) x 0.004-0.006 (0.051), mesostome 0.012-0.014 (0.013) x 0.008-0.01 (0.01), muscular esophagus 0.081-0.086 (0.084) x 0.004-0.005 (0.0043), glandular esophagus 0.198-0.227 (0.213) x 0.021-0.024 (0.022) in size. Length ratio

between muscular and glandular portions of esophagus 1: 2.45-3.38 Distance of nerve ring, deirids and excretory pore 0.0234-0.0236 (0.0241), 0.032-0.044 (0.035) and 3.7-3.92 (3.81) respectively from anterior end of body. Large spicule 0.345-0.383 (0.367) x 0.014-0.017 (0.015), small spicule 0.078-0.083 (0.081) x 0.018-0.025 (0.022). Spicule length ratio 1: 4.42-4.63 (1:4.57). Tail 0.09-0.15 (0.12) mm long.

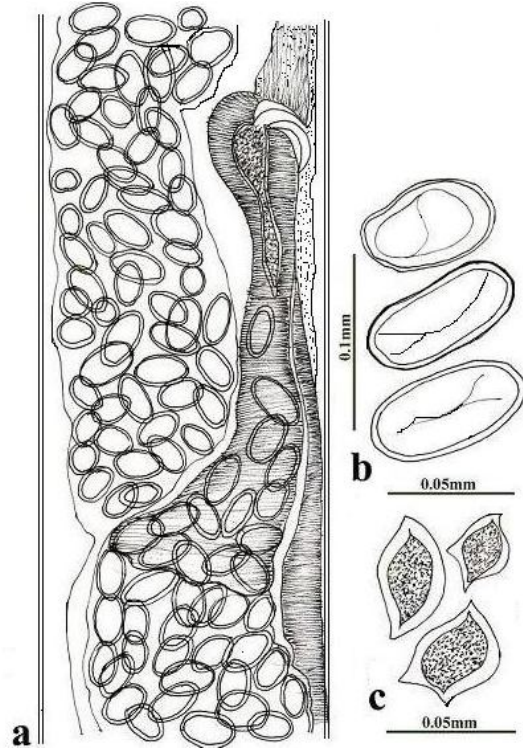


Fig. 3. *Rhabdochona (Rhabdochona) spatulatum* n.sp., allotype female. a, region of genital opening showing submarginal vulva, vagina and smooth eggs; b, mature eggs elongate and thick-walled; c, immature eggs are smaller with irregular shells with two or three projections.

Female

(3 specimens; measurement of allotype in parenthesis): Body length 5.3-6.1 (5.81), greatest width 0.11-0.18 (0.16), prostome 0.002-0.003 (0.0029) x 0.007-0.009 (0.008), mesostome 0.012-0.015 (0.0124) x 0.007-0.01 (0.009), muscular esophagus 0.079-0.085 (0.083) x 0.005-0.009 (0.006), glandular esophagus 0.194-0.212 (0.2) x 0.02-0.03 (0.03) in size. Length ratio between muscular and glandular portions of esophagus 1: 2.46-2.49. Distance of nerve ring, deirids and excretory pore 0.211-0.237 (0.225), 0.041-0.045 (0.043) and 4.1-4.6

(4.43) respectively from anterior end of body. Vulva 0.041-0.045 x 0.02-0.03. Distance of vulva from the posterior end of body 3.11-3.29 (3.18). Vagina 0.02-0.03 (0.03) x 0.088-0.098 (0.092). Mature eggs 0.016-0.037 (0.033) x 0.011-0.021 (0.017) in size. Tail 0.23-0.25 (0.25) mm long.

Etymology

The species name *R. (R.) spatulatum* refers to spatulate small spicule.

DISCUSSION

On the basis of morphological features pertaining to the egg structure, the present specimens can be placed in the subgenus *Rhabdochona (Rhabdochona)* Railliet, 1916 (Morphological group A) in which the females produce either smooth-shelled eggs or thick-shelled eggs bearing an irregular flock-like coating, and possess somewhat conical tail tips. The worms belonging to this group have 10 anterior prostomal teeth, bifurcate deirids, basal teeth present or absent, and lack the lateral alae (Moravec, 1975). The new specimens can easily be compared with its congeneric reported from Balochistan (Tables I, II) viz., *R. kharani* Asmatullah-Kakar *et al.*, 2006; *R. nushkiai* and *R. watsoniai* Asmatullah-Kakar and Bilqees, 2007a (based on males only); *R. uvaginus* Asmatullah-Kakar and Bilqees, 2007b; *R. hingoli* and *R. bifidum* Asmatullah-Kakar and Bilqees, 2007c (based on males only); *R. magnavesicula* Asmatullah-Kakar and Bilqees, 2008; *R. cephalodiverticola* and *R. bolani* Asmatullah-Kakar *et al.*, 2008a (based on males only); *R. milesi* Asmatullah-Kakar *et al.*, 2008b; *R. mujibi* Asmatullah-Kakar and Bilqees, 2009; *R. (F.) gubernaculus* Asmatullah-Kakar *et al.*, 2010; *R. (G.) spinicauda* Asmatullah-Kakar *et al.*, 2012a; *R. bifurcatum* Asmatullah-Kakar and Bilqees, 2012; *R. annai* Asmatullah-Kakar *et al.*, 2012b; *R. pakistanica* Asmatullah-Kakar *et al.*, 2012c and *R. haspani* Asmatullah-Kakar *et al.*, 2014.

Perusal of Tables I and II evidently distinguish *R. spatulatum* n. sp. from aforesaid forms in morphometric data. In addition, the males of new species are unique in having tail finely striated dorso-ventrally, and spicules, right one spatulate provided with distal barb, left one slightly notched anteriorly ends in a large spine. In contrast, such structures of spicules and tails lacking in males of above mentioned forms. It may be differed in other features like deirids shape and eggs morphology. The present species is characterized by having bifurcate deirids differ from *R. magnavesicula*, *R. milesi*, *R. mujibi*, *R. annai*, *R. pakistanica* and *R. haspani*. *R. cephalodiverticola*, *R. (G.) spinicauda* and *R. bifurcatum*

because they have simple (non-bifurcate) deirids. It also differs from all of them in having thick-walled eggs; and with respect to vulva position from *R. kharani*, *R. uvaginus*, *R. magnavesicula*, *R. milesi*, *R. mujibi*, *R. annai*, *R. pakistanica* and *R. haspani*. In these entire eight species vulva is postequatorial (Table II). Females of *R. hingoli*, *R. bifidum*, *R. nushkiai*, *R. watsoniai*, *R. cephalodiverticola* and *R. bolani* are unknown. Beside above mentioned differences, it has close similarities with *R. (F.) gubernaculus* and *R. (G.) spinicauda* in the shape of deirids. The Tables also highlight similarities of *R. spatulatum* n. sp. with *R. milesi* males and *R. spinicauda* females tail length (0.09-0.15 mm) and (0.23-0.25 mm) respectively, in the number of anterior teeth (*R. spinicauda*, *R. magnavesicula*, *R. milesi*, *R. gubernaculus*, and *R. nushkiai*), in the number (15) and arrangements (10+5) of caudal papillae pairs (*R. annai*, *R. cephalodiverticola*), in the distance of nerve ring (0.0234-0.0236) and excretory pore (3.7-3.92) of male worms measured from anterior end of body (*R. milesi*).

Of the previously reported ten Pakistani species six belong to subgenus *Rhabdochona*: *R. (Rhabdochona) magna* Khan and Yaseen, 1969 (based on female only); *R. (R.) cavasius* Rehana and Bilqees, 1973; *R. (R.) chanawanensis* Zaidi and Khan, 1975 (based on male only); *R. (R.) parastromatei* Bilqees, 1979 (from a marine fish); *R. (R.) megsacculata* Ghazi and Atau-Rahim, 1999 (based on single female); *R. (R.) sarana* (Karve and Naik, 1951) Akram and Khatoon, 2001 (based on female only). The subgenus *Filochona* include three: *R. (Filochona) charsaddiensis* and *R. (F.) schizothoracis* Siddiqui and Khattak, 1984; *R. (F.) helichi* (Sramek, 1901), Akram and Khatoon, 2001, and subgenus *Globochona* include one reported species, *R. (Globochona.) rahimi* Ghazi *et al.*, 2003. The differences which the first six species have with *R. spatulatum* n. sp. are the long esophagus of females, longer body size of both the sexes (except *R. megsacculata*), shape of egg, and shorter length ratio between male spicules (except *R. parastromatei*). *R. magna* has 1:5.42-10.13 mm ratio between muscular and glandular part of esophagus, it is 1:16.20-16.33 in *R. cavasius*, body of the former (based on female only) is 20.79-23-82 mm long, of the later 8.74 mm for the single male and 16.33-18.28 for the females, spicular ratio being 1: 2.86 compare to the esophagus ratio (1:2.46-2.49), body size (males = 4.2-4.6, females = 5.3-6.1) and spicular ratio (1:4.42-4.63) of new species. The species *R. chanawanensis*, *R. parastromatei* and *R. sarana* also larger than new species in body dimension (males=8.72-9.55, females not described), (males=10.53-15.61, females=10.53.61-15), and (males= unknown, females= 8.94-12.22 mm long) respectively, larger esophagus ratio being 1:11.01-11.85, 1:8.76-26.5, 1:9.74-10.31 mm for

A NEW NEMATODE SPECIES

Table I.- Morphometric comparison of male *Rhabdochona* species in various fish hosts from Balochistan (Pakistan).

<i>Rhabdochona</i> species	Host/ locality	Body size	No. of teeth	Distance from anterior end of body		Length ratio			No. of caudal papillae			Tail length	
				Nerve ring	Deirids	Excretory pore	Esophagus	Spicules	Pre- anal	Ad- anal	Post anal		Total
<i>R. (Rhabdochona.) kharani</i>	<i>Labeo gedrosicus</i> Kharan	3.4-5.3	8	Not observed	Not observed	2.4-2.6	1: 2.62-2.73	1: 3.44-3.62	10-11	1	6-7	17-18	0.25-0.28
<i>R. (R.) wvuginus</i>	<i>Tor putitora</i> Bolan	3.04	8	Not observed	Not observed	2.23	1: 1.13	1: 2.31	8	0	5	13	0.02
<i>R. (R.) amrai</i>	<i>Tor putitora</i> Bolan	3.91	8	0.058-0.065	0.09-0.113	2.34-3.42	1: 3.33-3.73	1: 4.68	10	0	5	15	0.09-0.2
<i>R. (R.) biferacutum</i>	<i>Cyprinion watsoni</i> Bolan	3.11-3.25	8	0.13-0.14	0.081-0.092	1.21-1.22	1: 3.14-3.41	1: 3.97-4.06	4	0	5	9	0.14-0.16
<i>R. (R.) pakistanica</i>	<i>C. watsoni</i> Bolan	1.05-3.24	8	0.046-0.051	0.075-0.085	2.1-2.32	1: 1.15-1.90	1: 2.46-8.60	5	0	7	12	0.21-0.23
<i>R. (R.) mujibi</i>	<i>Tor putitora</i> Bolan	4.44-4.6	8	0.234-0.236	0.03-0.04	3.7-3.9	1: 2.44-2.64	1: 1.40-4.48	9	0	6	15	0.2-0.3
<i>R. (R.) haspami</i>	<i>C. watsoni</i> Sibi	2.89-3.45	8	0.05-0.06	0.03-0.37	1.0-1.2	1: 1.08-1.5	1: 4.08-4.47	8	0	5	13	0.1-0.16
<i>R. (R.) bolani</i>	<i>Tor putitora</i> Bolan	2.89-3.06	8	0.079-0.092	0.11-0.12	1.68-1.82	1: 2.64	1: 5.49-5.91	12	0	5	17	0.13-0.25
<i>R. (R.) watsoniati</i>	<i>C. watsoni</i> Nushki	2.33	8	Not observed	0.038	Not observed	1: 2.88	1: 4.14	12	0	5	17	0.22
<i>R. (R.) cephalodiverticula</i>	<i>Tor putitora</i> Bolan	3.55	6	0.062	0.11	1.73	1: 2.74	1: 4.56	10	0	5	15	0.11-0.15
<i>R. (R.) bifidum</i>	<i>Tor putitora</i> Bolan	2.16-3.35	6	0.01-0.02	0.071-0.082	1.3-1.9	1: 1.65	1: 3.4-3.6	8	0	5	13	0.098-2.1
<i>R. (R.) hingoli</i>	<i>C. milesi</i> Awaram	3.96-4.37	6	0.048-0.056	0.03-0.04	1.98-2.1	1: 2.84-3.12	1: 4.02-4.12	6	0	6	12	0.21-0.23
<i>R. (F.) gubernaculus</i>	<i>Cyprinion watsoni</i> Bolan	2.7-3.5	10	0.131-0.0175	0.55-0.62	1.14-2.22	1: 2.25-4.8	1: 3.5-3.90	9	0	5	14	0.142
<i>R. (G.) spincanda</i>	<i>T. putitora</i> Bolan	3.41-3.45	10	0.02-0.03	0.06-0.07	1.99-2.0	1: 3.40-3.52	1: 2.46-2.55	9	0	6	15	0.1-0.21
<i>R. (R.) mushkai</i>	<i>Cyprinion milesi</i> Nushki	2.5	10	Not observed	0.32	Not observed	1: 1.93	1: 4.06	7	0	8	15	0.12-0.15
<i>R. (R.) milesi</i>	<i>C. milesi</i> Nushki	4.9-5.06	10	0.097-0.11	0.028-0.035	2.6-2.9	1: 2.10-2.82	1: 3.48-3.55	12	0	7	19	0.09-0.15
<i>R. (R.) magrawesicula</i>	<i>Schizocypirus brucei</i> Musakhel	7.86-9.15	10	0.053-0.057	0.17-0.21	4.072-5.054	1: 4.12-4.17	1: -2.652-8.4	4	0	5	9	0.18-0.2

Table II.- Morphometric comparison of female *Rhabdochona* species in various fish hosts from Balochistan (Pakistan).

<i>Rhabdochona</i> species	Fish host / Locality	No. of teeth	Body size	Distance from anterior end of body			Position of excretory pore		Vulva from posterior end	Size of egg	Tail length
				Nerve ring	Deirids	Excretory pore	Esophagus length ratio	Excretory pore			
<i>Rhabdochona</i> (R.) <i>kharani</i>	<i>Labeo gedrosicus</i> Kharan	8	5.5-9.2	Not observed	Not observed	Not observed	Preqtl*	Poseqtl**	2.12-2.55	0.04-0.059	0.17-0.19
<i>R. (R.) waguinus</i>	<i>Tor putitora</i> Bolan	8	6.05	Not observed	Not observed	2.52	Poseqtl**	Poseqtl**	2.3-2.72	0.003-0.013	0.03
<i>R. (R.) annai</i>	<i>Tor putitora</i> Bolan	8	4.43-6.56 Holotype	0.099-0.175	0.097-0.114	3.32-4.12	Poseqtl**	Poseqtl**	2.27-2.87	0.009-0.21	0.1-0.25
<i>R. (R.) bifercatum</i>	<i>Cyprinion watsoni</i> Bolan	8	3.89-4.13	0.087-0.095	0.011-0.012	2.8-2.9	Poseqtl**	Poseqtl**	1.82-1.93	0.24-0.25	0.24-0.25
<i>R. (R.) pakistanica</i>	<i>C. watsoni</i> Bolan	8	2.16-4.23	0.051-0.057	0.049-0.054	2.5-2.68	Poseqtl**	Poseqtl**	1.69-1.85	0.014-0.034	0.175-0.183
<i>R. (R.) mujibi</i>	<i>Tor putitora</i> Bolan	8	3.3-3.6	0.201-0.203	0.038-0.042	3.2-3.4	Poseqtl**	Poseqtl**	1.14-1.32	0.09-0.1	0.09-0.1
<i>R. (R.) haspani</i>	<i>C. watsoni</i> Sibi	8	3.68-4.43	0.06-0.07	0.03-0.04	1.0-1.2	Poseqtl**	Poseqtl**	2.1-2.13	0.013-0.035	0.23-0.27
<i>R. (F.) gubernaculus</i>	<i>Cyprinion watsoni</i> Bolan	10	3.4-4.22	0.098-0.14	0.047-0.053	1.35-2.82	Poseqtl**	Preqtl*	1.21-2.13	0.012-0.037	0.1-0.2
<i>R. (G.) spinicauda</i>	<i>T. putitora</i> Bolan	10	3.87-4.22	0.049-0.053	0.138-0.141	2.68-275	Poseqtl**	Preqtl*	2.15-2.17	0.012-0.018	0.23-0.25
<i>R. (R.) milesi</i>	<i>C. milesi</i> Sibi	10	4.65-5.0	0.1-0.12	0.03-0.04	1.72-1.75	Poseqtl**	Poseqtl**	2.39-262	0.01-0.013	0.08-0.1
<i>R. (R.) magnavesicula</i>	<i>Schizocyprius brucei</i> Musakhel	10	12.74-15.72	0.049-0.052	0.014-0.17	7.14-8.72	Poseqtl**	Poseqtl**	4.74-6.22	0.041-0.052	0.5-0.6

Note: *Preqtl indicates Preequatorial and **Poseqtl indicates Postequatorial position of excretory pore and vulva. Female species of *R. (R.) bolani*; *R. (R.) cephalodiverticula*; *R. (R.) bifidum*; *R. (R.) hingoli*; *R. (R.) nushkai* are unknown.

females respectively, but have shorter spicular ratio in case of *R. chanawanensis* (1:1.98-2.22 mm). These may be distinguished in other characters and fish hosts, but have some close affinities with *R. spatulatum* n. sp. in that in all these tails are conical. Other affinities include postanal papillae pair's number, and tail length in both the sexes of *R. cavasius*, and position of vulva and excretory pore in *R. sarana*.

The first three species of subgenus *Filoochona* can be set apart from *R. spatulatum* n. sp. and from *R. (G.) rahimi* in the following characteristic features: Eggs provide with long filaments, right spicules widened with reflected barb at distal tips, tail tips possess a short blunt spine-like structures in both the sexes of *R. (F.) charsaddiensis* and *R. (F.) schizothoracis*, those in *R. (F.) helichi* bluntly pointed. In contrast, *R. rahimi* is characterized by having eggs with lateral cuticular floats, boat-shaped right spicule, bluntly rounded tail tips, in both the sexes and known to have 10-12 prostomal teeth as in *R. charsaddiensis*. Number of teeth is not determined. In *R. schizothoracis*, this nematode is unique among all the known Pakistani species (except *R. cavasius*) in possessing the smallest (0.20-0.30 mm) left spicules (Siddiqui and Khattak, 1984). In addition to above cited or other differences if any, the present species is, however, close to *R. charsaddiensis* in the structure of left spicule; nearly to *R. schizothoracis* males body size (4.33-4.76 mm vs 4.2-4.6 of new species), right spicule length (0.073-0.076 vs 0.078-0.083), and in possessing 15 caudal papillae pairs; to *R. helichi* in having 10 anterior teeth, 0.021-0.037 mm vs 0.16-0.037 mm eggs size, 5 pairs postanal papillae, and vulva located a little before the equatorial region.

R. spatulatum n. sp. markedly differs from all described species from North America except *R. kisutchi* Margolis *et al.*, 1975; *R. catostomi* Kayton *et al.*, 1979; *R. paxamni*, *R. salmonis* Maggneti *et al.*, 1992; *R. lichtenfelsi* Sanchez Alvarez *et al.*, 1998; *R. mexicana* Caspeta-Mandujano *et al.*, 2000; *R. aheuheullensis* Mejia-Madrid and Pérez-Ponce de León, 2003 by having 10 anterior teeth and bifurcate deirids. *Rhabdochona kisutchi* differs from *R. spatulatum* n. sp. chiefly in left spicules shape, character of eggs. Left spicule of the former slender, has shaft representing its distal half, lanceolate distal tip with wide cuticular membrane, and the eggs bear polar caps with variable formations, in some eggs a long filament (0.45 mm) arising from corner of one polar caps also present. No eggs have so far been observed among hundreds of thousands from several reported *Rhabdochona* female species to have such shape of eggs as sometimes observed among eggs of *R. kisutchi*. It also is longer in body size (males, 6.61-8.41mm; females,

812.04-1738 mm), a longer tail: (male, 0.300-0.390, female, 0.351-450) and numerous caudal papillae pairs (16). *Rhabdochona catostomi* can be distinguished from new species by their male and female body length (8.9-14.3 mm, 15.3-16.7 mm) respectively, esophagus ratio (males=1:10.72-12.90, females= 1:13.25-14.82), shorter spicular ratio 1:3.61-3.64, eggs morphology (filamentous) and by vulva position. It, however, resembles *R. spatulatum* n. sp. by having 5 pairs postanal papillae, conical caudal end with terminal spike, lacking basal teeth, and by the location of deirids and excretory pore measured from anterior end. *Rhabdochona paxmani* differ from *R. spatulatum* n. sp. females by longer length (9.8-13.8 mm), less anterior deirids (0.7-0.11 vs 0.041-0.045) and more anterior excretory pore (0.25-0.36 vs 4.1-4.6); the males by smaller prostome (0.018-0.025 vs 0.002-0.004), longer muscular esophagus (0.290-0.41 vs 0.081-0.086), longer left spicule (0.482-0.579 vs 0.345-0.383), and by a well-developed distal barb of right spicules. Instead of these differences, both species resembling in nerve ring location (lying at level of anterior third of muscular esophagus), vulva position (preequatorial), and to some extent in spicular ratio (1:3.8-4.6 vs 1: 4.42-4.63). *Rhabdochona salmonis* can be differentiated from *R. spatulatum* n. sp. mainly by feebly developed tip of right spicule has a sharply indented dorsal gorges, and usually indistinct in new species. The tail also differ, conical with rounded tip in the former versus tail with sharp spike at distal tip in the later form. In contrast with new species described above, it relates closely in vulva position, character of eggs, placement of nerve ring and excretory pores in both the sexes, and in spicular ratio (1:4.42-4.63 vs. 1:4.2-4.6).

Rhabdochona lichtenfelsi, *R. mexicana* and *R. aheuheullensis* differs from *R. spatulatum* n. sp. in having lateral amphids and basal teeth. Moreover, *R. spatulatum* n. sp. differs distinctly from them in the form of left spicules' distal end, tail shape, and character of eggs in addition to some other features. Left spicule of *R. mexicana* is bifurcate distally, with narrow cuticular membrane, almost bifurcate in *R. lichtenfelsi*, being shorter (0.12-0.24) and tricuspid in *R. aheuheullensis* compare to razor-sharp distal end of new species. Tails are conical, with a terminal cuticular point in *R. lichtenfelsi*, bluntly rounded in *R. mexicana* and *R. aheuheullensis*. Eggs of *R. spatulatum* n. sp. thick-walled, in contrast, eggs of the former and later species bear polar filaments, and of *R. mexicana* has irregular flock-like coating. It differs also in the number of preanal papillae (10 vs. 7-8 4-6, 8-11, on each side, respectively), and body lengths (males= 4.2-4.6, females= 5.3-6.1 vs. 6.475-8.525, 9.137-14.875, 4.55-9.23, 8.90-18.13; 3.16-6.36, 2.75-8.92 mm long, respectively). Among above

mentioned species, *R. aheuheullensis* resembled *R. spatulatum* n. sp. in the presence of 5 pairs of postanal papillae, preequatorial vulva and excretory pore. *R. lichtenfelsi* close nearly in ratio of right to left spicule length (1:3.78-4.46). Another North American species described from rainbow trout in California: *Rhabdochona californiensis* Maggneti, et al., 1992 also is differ from *R. spatulatum* n. sp. in the number of teeth, which in the former is reportedly 14, by the absence of egg ornamentation, and the tail tip possess spinose mucron. *R. californiensis* also is longer in body size (males= 5.4-8.1 mm; females= 8.72-10.7mm), has a more posterior excretory pore (males= 0.265-0.336, females= 310-380), a longer tail: (male= 0.310-0.430; female= 0.320-0.410) and lower spicular ratio (1:3.3- 4.4). *R. californiensis* is, however, come closest to *R. spatulatum* n. sp. nearly in eggs size (0.03-0.037), in having excretory pore anterior to mid-body region, and nerve ring at level 1/3 of muscular esophagus.

Species of *Rhabdochona* form world other regions strongly differ from *R. spatulatum* n. sp. by having caudal projections on the tail tip arranged in a crown-like appendage either in females or both the sexes: those harbour these structures on tail tip of both sexes include *R. ovifilamenta* Weller, 1938 from the perch, *Perca fluviatilis* from Michigan, USA; *R. equispiculata* Moravec and Scholz, 1991 from the fish, *Hampala macrolepidota* and *H. dispar* in Vietnam; *R. Kurdistanensis* Moravec et al., 2012a from *Luciobarbus kersin* in Iraq; and *R. thaiensis* Moravec and Yooyen, 2011a from *Mystacoleucus marginatus* in Thailand. whereas in *R. barusi* Majumdar and De, 1971 from *Barilius* sp. in India; *R. onychostomi* and *R. brevichona* Guitang et al., 1994 from the hosts *Onychostoma simum* and *Rectoris luxiensis* respectively in China; *R. chodukini* Osmanov, 1957 from *Luciobarbus brachycephalus* and *L. capito* in Tadjhikistan; *R. tricuspudata* Moravec and Jirku, 2014 from *Raiamas christyi* in the Central African Republic caudal projections are found on the tail only of females. Other differences these species have with *R. spatulatum* n. sp. are the right spicules of different shape, large, deeply bifurcate deirids, almost with three prongs in *R. tricuspudata* (vs. slightly bifurcate deirids of new species), variable number of preanal papillae (9, 15, 12, 11, 8, 8, 8 respectively vs. 10), and less prostomal teeth in exception to *R. barusi* (possess probably 12 teeth). Right spicule of *R. ovifilamenta* is scoop-shaped, barbed with blunt distal tip, elongate, narrow, with pointed distal tip in *R. equispiculata*, bent ventrally and pointed at base in *R. chodukini*, short and tapering in *R. barusi*, thick and short in *R. onychostomi*, curved dorsally bear blunt distal tip. In *R. Brevichona*, and in *R. thaiensis* and *R. tricuspudata* it is boat-shaped without dorsal barb at distal

tip. *R. equispicula* distinctly differs from all above mentioned species in possessing similar, almost equally long spicules. In contrast to *R. spatulatum* n. sp. described above all aforesaid forms appear to differ in the relative length of spicules, tails, and esophagus, by the size of egg, and in the more posterior location of vulva and more anterior position of nerve ring. However, *R. ovifilamenta*, *R. equispiculata*, and *R. brevichona* are very similar to *R. spatulatum* n. sp. in the presence of 5 pair's postanal papillae. Right spicule of *R. onychostomi* is 0.070-0.080 mm long similar nearly to those of new species.

The above mentioned differences in the diagnostic features are sufficient to set a part the present materials as a new species for which the name *R. (R.) spatulatum* is proposed referring to spatulate small (right) spicule.

REFERENCES

- Akram, M. and Khatoon, N., 2001. *Schizothorax plagiostomus*, a new host of nematode infection of *Rhabdochona* species from Gilgit, Pakistan. *Pakistan J. Zool.*, **33**: 77-79.
- Anderson, R.C., Chabaud, A.G. and Willmott, S., 1975. *CIH Keys to the nematode parasites of vertebrates No. 4. Keys to the order Spirurida*. Commonwealth Agriculture Bureaux, England., pp. 1-27.
- Anjum, A., 2013. A new species and first host record of *Rhabdochona* Railliet, 1916 (Nematoda: Thelazoidea) in fresh water fish *Tor putitora* from Poonch river of Jammu & Kashmir state, India. *Int. J. Adv. Biol. Res.*, **3**: 110-115
- Asmatullah-Kakar, Bilquees, F.M. and Kakar, J.K., 2006. *Rhabdochona kharani* sp.n. (Nematoda: Rhabdochonidae) from the fish *Labeo gedrosicus* Zugmayer, 1912 from Garruk, District Kharan, Balochistan. *Acta Parasitol. Turc.*, **30**: 63-68.
- Asmatullah-Kakar and Bilquees, F.M., 2007a. Two new species of the genus *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochonidae) from the fishes *Cyprinion milesi* Day, 1880 and *C. watsoni* Day, 1872 of Nushki, Balochistan. *Pakistan J. Nematol.*, **25**: 147-155.
- Asmatullah-Kakar and Bilquees, F.M., 2007b. *Rhabdochona uvaginus* new species (Nematoda: Rhabdochonidae) from the fish *Tor putitora* of River Bolan, Balochistan. *Pakistan J. Zool.*, **39**: 51-55.
- Asmatullah-Kakar and Bilquees, F.M., 2007c. Two new species of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochonidae) from the fishes of Hingol River and Bolan River of Balochistan. *Proc. Parasitol.*, **44**: 29-39.
- Asmatullah-Kakar and Bilquees, F.M., 2008. *Rhabdochona magnavesicula* new species (Nematoda: Rhabdochonidae) from the fish *Schizocyprus brucei* Regan, 1914 of River Loni, Musakhel, Balochistan, Pakistan. *Proc. Parasitol.*, **46**: 49-65.
- Asmatullah-Kakar, Bilquees, F.M. and Saeed, A., 2008. Two new species of the genus *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochonidae) from the fish *Tor putitora* (Cyprinidae) of Bolan, Balochistan, Pakistan. *Pak. J. Nematol.*, **26**: 21-28.
- Asmatullah-Kakar, Bilquees, F.M. and Nawaz, M., 2008. A new species of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochonidae) from a freshwater fish in Khaisar valley, Balochistan, Pakistan. *Proc. Parasitol.*, **45**: 53-66.
- Asmatullah-Kakar and Bilquees, F.M., 2009. A new nematode species *Rhabdochona (Rhabdochona) mujibi* (Nematoda: Rhabdochonidae) in *Tor putitora* from fresh waters in Balochistan, Pakistan. *Proc. Parasitol.*, **47**: 93-103.
- Asmatullah-Kakar, Bilquees, F.M., Kamran, K. and Mukhtar, G.E.S., 2010. *Rhabdochona (Filochona) gubernaculus* new species (Nematoda: Rhabdochonidae) from reverine fish *Cyprinion watsoni* (Day, 1872) in Balochistan, Pakistan. *Pakistan J. Zool.*, **42**: 75-80.
- Asmatullah-Kakar and Bilquees, F.M., 2012. A new Rhabdochonid species (Nematoda: Rhabdochonidae) parasitizing *Cyprinion watsoni* (Cyprinidae) in Pakistan. *Pakistan J. Zool.*, **44**: 545-550.
- Asmatullah-Kakar, Bilquees, F.M., Mukhtar, G.S. and Ahmad, S., 2012a. *Rhabdochona (Globochona) spinicauda* new species (Nematoda: Rhabdochonidae) from putitor Mahseer, *Tor putitora* in Pakistan. *Pakistan J. Zool.*, **44**: 95-99.
- Asmatullah-Kakar, Bilquees, F.M. and Khan, A., 2012a. On a new species of spirurid nematode *Rhabdochona (Rhabdochona) annai* from freshwater Mahseer, *Tor putitora* (Ham). *Pakistan J. Zool.*, **44**: 845-849.
- Asmatullah-Kakar, Bilquees, F.M. and Khan, A., 2012b. A new parasitic nematode species *Rhabdochona pakistanica* (Nematoda: Rhabdochonidae) new species from *Cyprinion watsoni* (Osteichthyes: Cyprinidae) in central Balochistan. *Pakistan J. Zool.*, **44**: 851-857.
- Asmatullah-Kakar, Bilquees, F.M., Khan, A. and Khan, K., 2014. *Rhabdochona haspani*, New Species (Thelazoidea: Rhabdochonidae) from Stream Fish (Actinopterygii Klein, 1185) of Sibi Division, Balochistan. *Pakistan J. Zool.*, **46**: 891-895.
- Bilquees, F.M., 1979. *Rhabdochona parastromatei* sp. n. (Nematoda: Rhabdochonidae) from The fish *Parastromateus niger* (Bleeker) of the Karachi coast. *Zool. Scrip.*, **8**: 107-110.
- Capseta-Mandujano, J.M., Moravec, F. and Salgado-Maldonado, G., 2000. *Rhabdochona mexicana* sp. n. (Nematoda: Rhabdochonidae) from the intestine of characid fishes in Mexico. *Folia Parasitol.*, **47**: 211-215.
- Ghazi, R.R. and Atau-rahim, M., 1999. Proposal for a new species *Rhabdochona megasacculata* (Nematoda: Rhabdochonidae) from a freshwater fish *Barilius vagra* (Ham. 1889) caught from the Chattar stream, Islamabad. *Proc. Parasitol.*, **28**: 61-65.
- Ghazi, R.R., Noor-U-Nissa and Bilquees, F.M., 2003. First report of the genus *Rhabdochona (Globochona) rahimi* sp. n. from a freshwater fish, *Brilius vagra* in Pakistan. *Acta Parasitol. Turc.*, **27**: 217-221.

- Gonzalez-Solis, D., Chavan, S.P., Kannewad, P. and Gyananath, G., 2014. A new species of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochoniidae) from cyprinid fishes in the Western Ghats Region, India. *Syst. Parasitol.*, **7**: 273–281.
- Guitang, W., Yi, Y. and Huisheng, W., 1994. Studies on two new species of *Rhabdochona* (parasitic nematodes) from Wuling mountain region. *Acta Hydrobiol. Sin.*, **14**: 24–28.
- Janiszewska, J., 1955. *Rhabdochonoid des barbi* g. n., sp. n., subfamily Rhabdochonoidinae subfam.n. (Fam. Rhabdochoniidae Skrjabin) an intestinal parasite in cyprinid fish. *Acta Parasitol. Pol.*, **3**: 233–244.
- Karve, J. and Naik, G.G., 1951. Some parasitic nematodes of fishes. II. *J. Univ. Bombay*, **19**: 1–37.
- Khan, D. and Yaseen, T., 1969. Helminth parasites of fishes from East Pakistan. Nematoda I. *Bull. Dept. Zool. Univ. Punjab (N.S.)* **4**: 1–33.
- Kayton, R.J., Kritsky, D.C. and Tobias, R.C., 1979. *Rhabdochona catostomi* sp. n. (Nematoda: Rhabdochoniidae) from the intestine of *Catostomus* spp. (Catostomidae). *Proc. helminthol. Soc. Washington.*, **46**: 224–227.
- Maggenti, A.R., Abdel-rehman, F.R. and Cid Del Prado-vera, I., 1992. New species of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochoniidae) from rainbow trout in California streams. *J. Nematol.*, **24**: 379–390.
- Manjumdar, G. and De, N.C., 1971. *Rhabdochona barusi* sp. new. from the fish *Barilius* sp. with the key to the Indian species of this genus. *Folia Parasitol.*, **18**: 381–184.
- Margolis, L., Moravec, F. and McDonald, T.E., 1975. *Rhabdochona kisutchi* sp. nov. (Nematoda: Spiruroidea) from coho salmon, *Onchorynchus kisutch* (Walbaum) of western. *Can. J. Zool.*, **53**: 960–966.
- Mejia-madrid, H.H. and Pérez-Ponce De León, G., 2003. *Rhabdochona ahuehuellensis* n. Sp. (Nematoda: Rhabdochoniidae) from the balsas goodeid, *Ilyodon whitei* (Osteichthyes: Goodeidae) in Mexico. *J. Parasitol.*, **89**: 356–361.
- Moravec, F., 1972a. General characterization of the nematode genus *Rhabdochona* with a revision of the South-American species. *Vestn. Cesk. Spol. Zool.*, **36**: 29–48.
- Moravec, F., 1975. Reconstruction of the nematode genus *Rhabdochona* Railliet, 1916, with a review of the species parasitic in fishes of Europe and Asia. In: *Fishes of Europe and Asia. Studie. CsAV.* No. 8. Academia, Prague. p.104.
- Moravec, F., 2010. Some aspects of the taxonomy, biology, possible evolution and biogeography of nematodes of the spirurine genus *Rhabdochona* Railliet, 1916 (Rhabdochoniidae, Thelaziioidea). *Acta Parasitol.*, **55**: 144–160.
- Moravec, F. and Aria, H.P., 1971a. *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochoniidae) of fishes, including *Rhabdochona canadensis* sp. nov. *J. Fish Res. Bd.Can.*, **28**: 1645–1662.
- Moravec, F. and Jirku, M., 2014. *Rhabdochona* spp. (Nematoda: Rhabdochoniidae) from fishes in the Central African Republic, including three new species. *Folia Parasitol (Praha)*, **2**: 157–172
- Moravec, F. and Scholz, T., 1991. Observations on some nematodes parasitic in freshwater fishes in Loas. *Folia Parasitol.*, **38**: 163–178.
- Moravec, F. and Yooyen, T., 2011a. Two new species of *Rhabdochona* (Nematoda: Rhabdochoniidae) from freshwater fishes in Thailand. *Folia Parasitol.*, **58**: 224–232.
- Moravec, F., Bilal, S.J. and Abdullah, S. M. A., 2012a. Two species of *Rhabdochona* (Nematoda:Rhabdochoniidae) from the cyprinid fish *Luciobarbus kersin* (Heckel) in northern Iraq, including *R. (Globochona) kurdistanensis* sp.n. *Folia Parasitol.*, **59**: 139–147.
- Osmanov, S.O., 1957. A new nematode from fishes of Amu-Darya. *Dokl. AN. Uzbk. SSR.* No. **1**. pp. 63–65 (in Russian).
- Railliet, A., 1916. La Famille des Thelaziidae. *J. Parasitol.*, **2**: 99–105.
- RashEed, S., 1965. A preliminary review of the genus *Rhabdochona* Railliet 1916, with description of a new and related genus. *Acta Parasitol. Pol.*, **13**: 407–424.
- Rehana, R. and Bilqees, F. M., 1973a. *Rhabdochona* sp. n. (Nematoda: Rhabdochoniidae) from a freshwater fish *Mystus cavasius* (Ham.) of Kalri lake Sind, Pakistan. *J. scient. indust. Res.*, **16**: 110–111.
- Saidov, Y.U.S., 1954. Revision of the family Rhabdochoniidae Skrjabin, 1946 and the subfamily Cyclozoninae Sobolev, 1949, Sb. “*Rabory po gelmintologii*” k 75-letiyu Akad. K. I. Skrajabina, Moscow. pp. 622–635 (in Russian).
- Sanchez-Alvarez, A., Garcia-Prieto, L. and Perez-Ponce De Leon, G., 1998. A new species of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochoniidae) from endemic goodeids (Cyprinodontiformes) from two Mexican lakes. *J. Parasitol.*, **84**: 840–845.
- Siddiqi, M.N. and Khattak, A. R., 1984. Three new species of the family Rhabdochoniidae Skrjabin 1946, from fishes of N.W.F. P. *Pakistan. J. Zool.*, **16**: 181–188
- Sramek, A., 1901. Helminthen der an der zoologischen Station in Podiebrad (Bohmen) untersuchen Fische. *Arch. Naturw. Landesdurchfor. Bohmen.*, **11**: 94–118. (Czech transl. in 1903).
- Talwar, P.K. and Jhingran, A.G., 1991. Inland fishes of India and adjacent countries (ed. A.A. Balkema), vol. I, Rotterdam, pp. 541.
- Weller, T. H., 1938. Description of *Rhabdochona ovifilamenta* n. sp. (Nematoda: Thelaziidae) with a note on the life history. *J. Parasitol.*, **24**: 403–407.
- Yamaguti, S., 1961. *Systema helminthum. Vol. III. The nematodes of vertebrates.* Part I and 2 Inter-science Publishers. Pp.1–1261.
- Zaidi, D.A. and Khan, D., 1975. Nematode parasites from fishes of Pakistan. *Pakistan J. Zool.*, **7**: 51–73.

