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A New Rhabdochonid Species (Nematoda: Rhabdochonidae) Parasitizing *Cyprinion watsoni* (Cyprinidae) in Pakistan

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Abstract: During a survey for parasitic helminth fauna of freshwater fishes, a new species of rhabdochonid nematode *Rhabdochona* (*Rhabdochona*) *bifurcatum* n. sp. is described parasitizing the intestine of the fish host *Cyprinion watsoni* (Day, 1872) capture in the valley of district Bolan. The new nematode specimens differ in several diagnostic features compared with previously known species of the genus *Rhabdochona* Railliet, 1916. Unique to the new species is the presence of small spicule which is anteriorly bifurcated in to two unequal arms pointed at the tip. The present species is also characterized by having 8 prostomal teeth, 9 pairs of caudal papillae including 4 preanal and 5 postanal in males and eggs devoid of filaments or lateral floats in females. Excretory pore of both the sexes is postequatorial.

Key words: Nematode, *Rhabdochona* (*Rhabdochona*) *bifurcatum* n. sp., *Cyprinion watsoni* **(**Day)

INTRODUCTION

Species of *Rhabdochona* Railliet, 1916 (Nematoda:Rhabdochonidae) are parasitic to fishes infect the intestine mostly of freshwater fishes belonging to the families: Bagridae, Balitoridae, Catostomidae, Cichlidae, Cottidae, Cyprinidae, Gobidae, Ictaluridae, Pimelodidae, Poeciliidae, Schilbeidae, Scianeidae, Siluridae, and to a lesser extent of Salmonidae (Baylis, 1928; Weller, 1938; Gustafson, 1949; Chequette, 1950; Akhmerov, 1965; Petter, 1987; Sood, 1988; Sanchez-Alverez *et al*., 1998; Caspeta-Mandujanu *et al*., 2002; Mejia-Madrid *et al*., 2007; Moravec *et al*., 2009). In Pakistan, bagrid, balitorid, nemachilid, cyprinid and the schilbeid fishes seem to be the most important hosts for this nematode genus (Rehana and Bilqees, 1973; Zaidi and Khan, 1975; Siddiqi and Khattak, 1984; Ghazi *et al*., 2003; Asmatullah-Kakar and Bilqees, 2009).

In the present study a new species of *Rhabdochona* is observed collected from *Cyprinion watsoni* (Day) of River Bolan drainage system. The new species is named *R.* (*R.*) *bifurcatum* n. sp. The genus was revised by Saidov (1953 vide Moravec, 1975) according to which *R. bifurcatum* n. sp. belongs to the subgenus *Rhabdochona* Travassos *et al.*, 1928. Previously only one species of *Rhabdochona* was identified from *C. watsoni* from this region of Balochistan province (Asmatullah-Kakar *et al*., 2010).

MATERIALS AND METHODS

The fish examined for present investigation were collected from River Bolan, Balochistan during March, 2006. Forty nematodes including 18 male and 22 female were encountered from the intestine of 12 out of 16 fish host *Cyprinion watsoni* (Day). The parasites were fixed in 70% alcohol having few drops of glycerol and cleared in lacto-phenol. Mounting of worms was done on glass slide in pure glycerol. For enface view, method of Moravec (1975) was followed. Sketches of the parasite samples were drawn using camera Lucida attached to the light microscope. All measurements are given (length x width) in millimeters, with range followed by mean in brackets. The voucher specimens were submitted to depository of helminthes parasites in the Museum of Zoology Department University of Balochistan, Quetta, Pakistan.

RESULTS

Rhabdochona* (*Rhabdochona*) *bifurcatum, n. sp.
(Figs. 1-2)

Description

Description based on 5 male and 6 female

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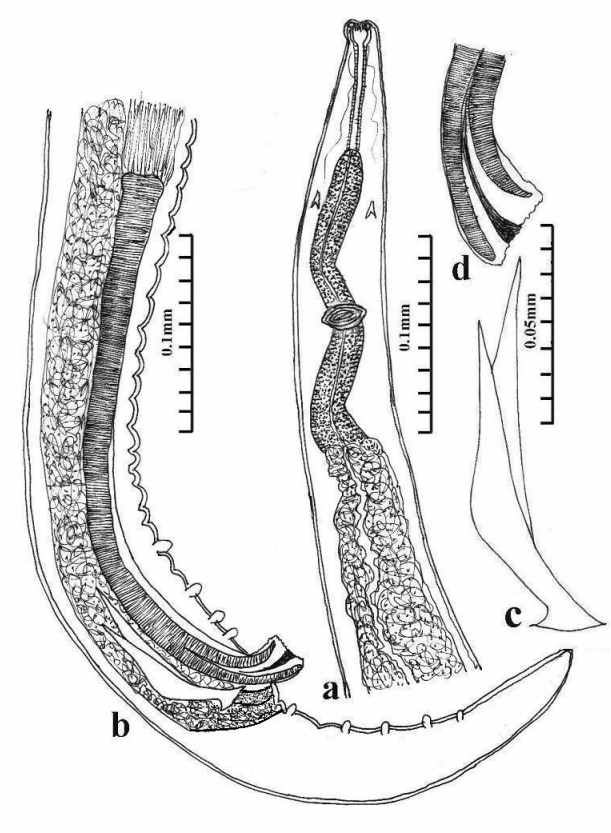

Fig. 1. *Rhabdochona (rhabdochona) bifurcatum* n.sp. holotype male. A, anterior body region showing buccal capsule, mascular and glandular esophagus, nerve ring and deirids; b, posterior body region showing spicules, caudal papillae and talc; c, small spicule enlarged.

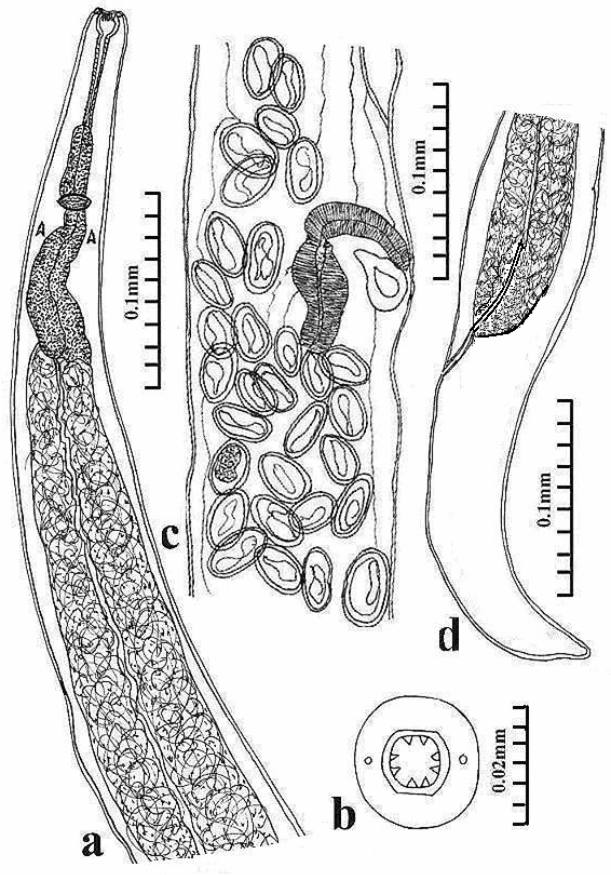

Fig. 2. *Rhabdochona (rhabdochona) bifurcatum* n.sp. allotype female. A, anterior body region showing buccal capsule, mascular, glandular esophagus and part of intestine, nerve ring and deirids are also seen b, Enface view of female showing prostomal teeth; c, Region of genital opening showing submarginal vulva, vagina and eggs; d, posterior region showing tails, and associated structures.

specimens. Small to medium-sized nematodes with smooth body except at the postero-ventral body regions including tail. Anterior and posterior body regions narrow, widest in the middle, anterior end flat, posterior pointed more so in male. Prostome armed with 8 teeth (2 dorsal, 2 ventral and 2 on each lateral side), pointed anteriorly. Buccal capsule funnel-shaped. Deirids bifurcated located near to anterior portion of glandular esophagus in male, but in female in the middle of glandular esophagus. Nerve ring almost in the middle of glandular esophagus in male and more anterior in female. Tail in male slightly curved ventrally while curved dorsally in female.

In male two spicules, dissimilar, unequal in shape and length. Spicular ratios 1:3.97-4.06. Large spicule wider at anterior and posterior end, finely striated, anterior end flat supported by numerous fibers, posterior end of striated portion is bifurcated and enclosed in a membranous envelope and medially supported by a thin cuticular streak. Small spicule long and delicate, anteriorly bifurcated, divided in to two unequal arms, sharply pointed at the tip, left portion wider in the middle, posterior tip is flat with pointed edges. Caudal papillae 9 pairs, including 4 preanal and 5 postanal. Excretory pore postequatorial.

Females larger than males, vulva and its opening sub-marginal, preequatorial. Vulva chitinus with opening in center leading antreriorly into muscular vagina which runs transversely for a short distance then directed backward joining the uterus posteriorly. Eggs numerous, elongate, devoid of globules or filaments. Excretory pore postequatorial.

Measurements

Male (n=5)

Body length 3.11-325 (3.19), greatest width 0.10-0.12 (0.108), prostome 0.004-0.005 (0.0045) x 0.006-0.007 (0.0064), mesostome 0.007-0.008 (0.0075) x 0.016-0.018 (0.0173), muscular esophagus 0.041-0.051 (0.046) x 0.004-0.006 (0.0049), glandular esophagus 0.14-0.16 (0.153) x 0.018-0.021(0.0196) in size. Distance of nerve ring, deirids and excretory pore 0.013-0.14 (0.0135), 0.081-0.092 (0.087) and 1.21-1.22 (1.209) respectively from the anterior end of body. Large spicule 0.278-0.325 (0.295) x 0.01-0.02 (0.017), small spicule 0.07-0.08 (0.075) x 0.016-0.018 (0.0167). Spicular ratio 1:3.97-4.06 (4.00). Tail 0.14-0.16 (0.149) mm long.

Female (n=6)

Body length 3.89-4.13 (3.98), greatest width 0.09-0.11 (0.10), prostome 0.005-0.007 (0.006) x 0.005-0.006 (0.0056), mesostome 0.006-0.007 (0.0116) x 0.007-0.008 (0.0076), muscular esophagus 0.043-0.045 (0.044) x 0.007-0.009 (0.0081), glandular esophagus 0.118-0.125 (0.121) x 0.022-0.024 (0.023) in size. Distance of nerve ring, deirids and excretory pore 0.087-0.095 (0.091), 0.011-0.012 (0.019) and 2.8-2.9 (2.86) respectively from the anterior end of body. Vulva 0.03-0.04 (0.036) x 0.02-0.025 (0.022), anterior vulvar lip 0.024-0.025 (0.0243) x 0.007-0.01 (0.008), posterior vulvar lip 0.03-0.04 (0.032) x 0.011-0.012 (0.0116). Distance of vulva 1.82-1.93 (1.88) from the posterior end of body. Vagina 0.13-0.15 (0.136) x 0.03-0.04 (0.036). Mature eggs 0.021-0.046 (0.034) x 0.012-0.025 (0.019) in size. Tail 0.24-0.25 (0. 246) mm long.

Taxonomic summary

Type host:	*Cyprinion watsoni* (Cyprinidae)
Site of infection:	Intestine
Type locality:	River Bolan, Balochistan
Number of specimens:	40 nematodes including 18 male and 22 female from 12 fishes, maximum 5 male and 6 female from a single host, 16 hosts were examined.
Holotype male:	ZBU-N54
Allotype female:	ZBU-N55

Etymology

The species name *R.* (*R.*) *bifurcatum* refers to bifurcated proximal part of the small spicule.

DISCUSSION

The species of *Rhabdochona* described from Pakistan only 6 of the 20 possess 8 teeth in the prostome: *R. sarana* (Karve and Naik, 1951) Akaram and Khatoon, 2001 (description based on female only); *R. kharani* Asmatullah *et al*., 2006; *R. watsoniai* Asmatullah-Kakar and Bilqees, 2007a (description based on male only); *R. uvaginus* Asmatullah-Kakar and Bilqees, 2007b; *R. bolani* Asmatullah-Kakar *et al*., 2008 (description based on male only); and *R. mujibi* Asmatullah-Kakar and Bilqees, 2009. The present new species *Rhabdochona bifurcatum* n.sp. is the seventh member of genus with this combination of characters.

Moreover, females of above named species have similarities with present species in having smooth eggs but differ from it in the morphology of vagina, position of excretory (except *R. mujibi* and *R. bolani*) and genital openings. Description of female of *R. watsoniai* and *R. bolani* is not known.

The *R. bifurcatum* n. sp. can be distinguished from these reported Pakistani species and those described from other parts of the world by the metrical arrangement, number of caudal papillae and spicular ratios. Another unique character which set apart the new species is the bifurcated deirids not found in above mentioned species.

The present new species *R. bifurcatum* n. sp. has 9 pairs of caudal papillae, these are 4 preanal and 5 postanal, spicular ratios is 1:3.97-4.06. *R. kharani* has 17-18 pairs of caudal papillae, 10-11 preanal, 1 adanal, 6-7 postanal, spicular ratios is 1:3.6-3.8. *R. watsoniai* has 17 pairs of caudal papillae, 12 preanal, 7 postanal, spicular ratios is 1:4.13 (description based on single specimen only);

R. uvaginus has 13 pairs of caudal papillae, 8 preanal, 5 potanal, spicular ratios is 1:2.30; *R. bolani* possess 17 pairs of caudal papillae, 12 of which are preanal and 5 postanal, spicular ratios is 1:5.49-5.91. In *R. mujibi* caudal papillae are 15 pairs including 9 preanal and 6 postanal, spicular ratios is 1:4.40-4.48. Description of male of *R. sarana is* unknown.

Species of the genus reported from other regions also have 8 prostomal teeth similar to that of *R. bifurcatum* n. sp. These include *R. paski* Baylis, 1928; *R. congolensis* Campana-Rouget, 1961; *R. versterae* Boomker and Petter, 1993 (from Africa); *R. chodukini* Osmanov, 1957; *R. sulaki* Saidov, 1953 (Ex-USSR); *R. coronacauda* Belouss, 1965; *R. chabaudi* Mawson, 1956 (Europe); *R. ovifilamenta* Weller, 1938; *R. beatriceinsleyae* Holloway and Klewer, 1969 (North America); *R. fotedari* Katoch and Kalia, 1993; *R. garuai* Agrawal, 1965; *R. moraveci* Duggal and Kaur, 1987; *R. barusi* Majumdar and De, 1971; and *R. sarana* Karve and Naik, 1951 (8-10 teeth) from south Asia. Males of these nematodes differ from species under study in the relevant length ratio and structure of spicules and in number, arrangements of caudal papillae. These species also differ in lacking postequatorial excretory pore.

The caudal end of *R. paski* is provided with 13 pairs of papillae including 7 preanal and 6 postanal, spicular ratios is 1:1.86-2.08; in *R. congolensis* caudal papillae are 21-22 pairs, 15-16 preanal, 6 postanal, spicular ratios is 1:2.37 (Description based on single male); in *R. versterae* caudal papillae are 18-21, pairs, preanal 12-15, postanal 6, spicular ratios is 1:2.11-2.74. In *R. chodukini* caudal papillae are 14 pairs, 8 preanal, 6 postanal, spicular ratios is 1:3.42-3.50. The species *R. sulaki* has 15-16 pairs of caudal papillae. These are 10 preanal and 5-6 postanal, spicular ratios is 1:5.0. *R. coronacauda* possess 13 pairs of caudal papillae, 7 of which preanal and 6 postanal, spicular ratios is 1:2.02. In *R. chabaudi* caudal papillae consists of 8 preanal and 6 postanal pairs, spicular ratios is 1:4.08-4.09. In *R. ovifilamenta*, caudal papillae are 14 pairs, 9 preanal, 5 postanal, spicular ratios is 1:4.6-5.7; in *R. beatriceinsleyae* caudal papillae are 11 pairs, 6 preanal, 5 postanal, spicular ratios is 1:4.0-5.4. In the following south Asian nematode species *R. fotedari* caudal papillae are 13 pairs, 8 preanal, 5 postanal, spicular ratios is 1:3.16-3.24; in *R. garuai* caudal papillae are 15-20 these are 11-14 preanal, 4-6 postanal, spicular ratios is 1:2.9-3.4; in *R. moraveci* caudal papillae are 9 pairs (similar to species under discussion) including 6 preanal and 3 postanal, spicular ratios is 1:2.5-3.8; in *R. barusi* caudal papillae are 22 pairs including 12 preanal, 1 adanal and 9 postanal, spicular ratios is 1:5.5 (Description based on single male). Description of males of *R. sarana* is unknown.

The present species has smooth eggs similar to *R. paski*, *R. chodukini*; *R. fotedari*, *R. garuai*, *R. coronacauda*, *R. chabaudi*, *R. versterae*, *R. sarana* and *R. moraveci* but differ from it distinctly in egg sizes. In *R. paski* eggs are 0.045-0.047 x 0.025-0.027 mm in diameter; in *R. chodukini* 0.033-0.036 x 0.021-0.024; in *R. fotedari* 0.034 x 0.021; in *R. garuai* 0.021-0.037 x 0.015-0.02; in *R. coronacauda* 0.036-0.039 x 0.021-0.023; in *R. chabaudi* 0.032 x 0.018; in *R. versterae* 0.035-0.037 x 0.021-0.022; in *R. sarana* 0.0275 x 0.0175-0.02; and in *R. moraveci* 0.03-0.032 x 0.013-0.018.

The species under discussion also differ from *R. ovifilamenta*, *R. beatriceinsleyae* and *R. sulaki* in egg size and lacking filaments on it. In *R. ovifilamenta* eggs (averaging) are 0.034 x 0.019; in *R. beatriceinsleyae* 0.043-0.049 x 0.021-0.026. The eggs of *R. sulaki* are 0.030-0.039 x 0.015-0.021 in diameter. In *R. barusi* eggs are provided with cuticular floats and 0.045 x 0.037 in diameter compared with the egg size (0.021-0.046 x 0.012-0.025) of *R. bifurcatum* n. sp. While in *R. congolensis* eggs were not described.

In view of the differentiating diagnostic features above discussed the present species is regarded as new with the specific name *R.* (*R.*) *bifurcatum* n. sp.

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