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Occurrence of Two Spirurid Nematodes in Cyprinid Fishes from Nilwala River Basin, Sri Lanka

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Abstract- Characterization of the helminth parasites of wild fish has a considerable importance from the point of view of hygiene and public health, as well as from its taxonomic interest. To study the pathogenecity of parasites in the field of fisheries, require detail knowledge of the parasites inhabiting localities involved. Present study aimed to investigate the intestinal nematodes from the *Puntius* species inhabiting Nilwala river basin, Sri Lanka and to evaluate their population levels in host populations. Eight host fish species were surveyed and two spirurid nematodes, *Camallanus fotedari* from *Puntius dorsalis* and *P. titteya* and *Rhabdochona sarana* from *P. filamentosus* and *P. sarana*, were recovered. *C. fotedari* was found inhabiting in low infection levels in its two hosts, and it, however had a relatively high preference to *P. dorsalis* (20%) than *P. titteya* (4.2%). Of the two host species of *R. sarana*, high prevalence, mean intensity and abundance were reported in *P. filamentosus* (syn. *Dawkinsia singhala*) compared with those of in *P. sarana* (syn. *Systomus sarana*). Occurrence of these two spirurid nematode, *C. fotedari* and *R. sarana*, parasitizing in wild fresh water fishes is a new records to Sri Lanka.

Index Terms- First record, *Rhabdochona sarana*, *Camallanus fotedari*, Freshwater, *Puntius*, Sri Lanka

I. INTRODUCTION

Fish species occur in most of the tropical and subtropical countries of the world, including those of the south Asia, carry heavy infections of helminth parasites. Of them, parasitic nematodes represent an important group of fish parasites, many species of which are considerably parasites and may cause serious disease or death of fish. Though, parsitologists are now paying attention on pathogenecity of fish parasites, knowledge on the effect of parasitic nematodes on fish host is scarcer (Sood, 1988). Anemia, perforation of internal organs, muscle and skin or formations of tumour are the observed disease in fishes with nematode infections (Van Duijan, 1956). Therefore, to solve practical problems in fisheries and aquaculture, such as attempts to increase the productivity of fish farms, to enhance the stocks of valuable commercial fishes in the natural waters or to acclimatize fish in new localities, require detail knowledge of the parasites inhabiting the localities involved (Sood, 1988). Extensive surveys on parasitic fauna of wild freshwater fishes of Sri Lanka are meager, and only a few studies on fish parasitic fauna of Sri Lanka have been published (Nilakkarawasam, 1993 and 1998). The records of identified fish parasites of Sri Lanka comprise of a few intestinal parasites, few parasitic crustaceans and gill monogeneans, and species list of fish parasites recorded from inland fishes of Sri Lanka have been given in Nilakkarawasam, 1993. However, no information is available on fish parasite fauna of fishes of upstream river systems of Sri Lanka. Of the cyprinid fishes, species of the genus *Puntius* (Hamilton Buchanan, 1822) are abundant in streams and rivers of Sri Lanka, represent 16 (25.8%) of the 62-recorded freshwater dispersant species in Sri Lanka (Pethiyagoda, 1991). Present study aims to investigate the intestinal nematodes parasitizing in *Puntius* species inhabiting Nilwala river basin, Sri Lanka, and to compute population levels of parasites in host species.

II. MATERIALS AND METHODS

Puntius titteya Deraniyagala 1929, *P. nigrofasciatus* Gunther 1868 (synonyms: *Pethia nigrofasciatus* Pethiyagoda et al. 2012), *P. amphibious* Valenciennes 1842, *P. dorsalis* Jerdon 1849, *P. filamentosus* Valencienns 1844 (synonyms: *Dawkinsia singhala* Pethiyagoda et al. 2012), *P. sarana* Hamilton 1822 (synonyms: *Systomus sarana* Pethiyagoda et al. 2012), *P. chola* Hamilton 1822, *P. vittatus* Day 1865, and *P. cumingii* Gunther 1868 (synonyms: *Pethia cumingii* Pethiyagoda et al. 2012) were collected using field collecting gears and instruments from tributaries of Nilwala river basin, wet zone, southern part of Sri Lanka. Host fishes were collected from average age and size class for the population as 25 specimens for each species and identified using the diagrams and keys given in Pethiyagoda (1991), Munro (1955) and Deraniyagala (1952). Fish were euthanized by cervical dislocation and examined fresh (within 1 day from collection) for the parasites. Intestinal nematodes were recovered from intestine of host species under Stereo-zoom dissecting microscope (Wild 3MB) and the number of each host species infected and number of each nematode species in fish hosts were recorded. The recovered nematodes were washed in vertebrate saline solution, fixed with Berland's fluid (Berland, 1982) and preserved in 70% Alcohol. Temporarily mounted animals in glycerol were examined under a phase compound microscope (Olympus CH-2), identified and measured, male and female separately, using an ocular micrometer. Drawings were made with a aid of camera lucida (Olympus 1.25x) mounted on phase compound microscope (10x, 40x, or 100x objective lens, 10x ocular lens). Identification was carried out using consultation of original descriptions in the primary literature and synthetic keys given in Anderson et al, 1975; Moravec, 1975 and Sood, 1988. All measurements were given in micrometers.

Prevalence, Mean abundance and Mean intensity were computed as the common measurements of parasite population levels in host species. Prevalence refers to the percentage of individuals of the host infected by a particular species of parasite, while Mean abundance refers to the number of parasites of a given species per host examined, infected and uninfected. Mean intensity is the mean number of parasites of a given species per infected host (Bush et al, 1997).

III. RESULTS

Two species of Spirurid nematode (*Camallanus fotedari* and *Rhabdochona sarana*) were recovered from the survey (Figure 1), and population levels of the parasites computed in host fishes are depicted in Figure 2 and 3.

Camallanus fotedari Raina and Dhar, 1972
Synonyms: *Camallanus cotti* Fujita, 1927
Host: *P. dorsalis* and *P. titteya*

Description: Mouth terminal, slit like; buccal capsule consisting of two lateral chitinous valves, internally with 22-24 longitudinal rib like thickenings. From the point of junction of the valves, dorsally and ventrally, a trident-shaped chitinous process directed backward. A chitinous ring present at the junction of the valves and esophagus. Esophagus consisting of a short anterior muscular portion and a long posterior glandular portion enlarged posteriorily.

Male: (based on one specimen).
Length of body 2476, maximum width 116. Buccal capsule 77 x 81. Muscular esophagus 251, glandular esophagus 325. Nerve ring 137 from anterior end, caudal papillae 7+2+6=15 pairs. Post-anals disposed characteristically, as three pairs immediately post-cloacal in a group, two pairs slightly posterior to former and last sixth pair near tip of tail. Spicules unequal with pointed tips. Small spicule 109, large spicule 156 in length.

Female: (based on three specimens).
Length of body 4715 (2144-6929), maximum width 195 (114-263). Buccal capsule 116 x 119 (88-135 x 88-149). Nerve ring 195 (142-226) from anterior end. Muscular esophagus 372 (247-453), glandular esophagus 459 (263-585) in length. Vulva just post-equatorial, at 2284 (1042-3452) from posterior end. Tail with rounded tips, 860 (279-1340) in length. Egg size 25 x 19 (24-26 x 17-20).

Rhabdochona sarana Karve & Neik, 1951
Host: *P. filamentosus* (syn. *Dawkinsia singhala*) and *P. sarana* (syn. *Systomus sarana*)

Description: Anterior end of body narrow, rounded with indistinct cephalic papillae. Buccal capsule funnel shaped, supported by short longitudinal ridges projecting anteriorly as sharp teeth. Vestibule long and narrow. Esophagus distinctly divided into short muscular portion and long glandular portion. Body cuticle with transverse striations throughout its length.

Male: (based on 10 specimens).
Shorter than female. Length of body 6325 (5095-7340), maximum width 111 (85-123). Length of prostom 16 (15-18), width 13 (11-15); vestibule including prostom 109 (98-128). Muscular esophagus 185 (140-220); glandular esophagus 2339 (1976-2690) in length. Nerve ring at distance of 121 (108-133); excretory pore 194 (178-213); deirids 53 (48-55) from anterior end. Preanal papillae: 6 pairs subventral and 2 pairs lateral; latter pairs located between 2nd and 3rd and then between 3rd and 4th (counted from cloaca). Six pairs of post-anal papillae present, first pair lateral, remaining subventral. All papillae rather elongated; cuticle around them inflated, forming large dome-shaped formations, which may be partly fused together in neighboring papillae (usually in post anal pairs). Larger spicule very slender, 203 (183-223) long. Small spicule 69 (60-78) long, relatively narrow, tapering to distal end. Tail 137 (118-160) long, with rounded tip (without any crown).

Female: (based on 10 specimens)
Body of female 14305 (8905-17524) long and 202 (145-263) wide. Length of prostom 23 (20-28), maximum width 17 (13-20). Vestibule including prostom 139 (110-163) long. Length of muscular esophagus 234 (210-298); of glandular esophagus 3699 (2952-4833). Distance of nerve ring from anterior extremity 151 (130-168), excretory pore 225 (205-245), deirids 60 (55-70). Tail relatively wide, 206 (158-273) long, with a rounded end having stumpy projections. Length of crown 9 (5-15), width 17 (15-20). Vulva post equatorial with slightly elevated lips, 6748 (3786-8238) from posterior extremity. Eggs smooth, oval, size 30 x 21 (20-38 x 18-35), without filaments.

Comparative measurement of *C. fotedari* and *R. sarana* recorded previously with present materials are given in Table 1 & 2.

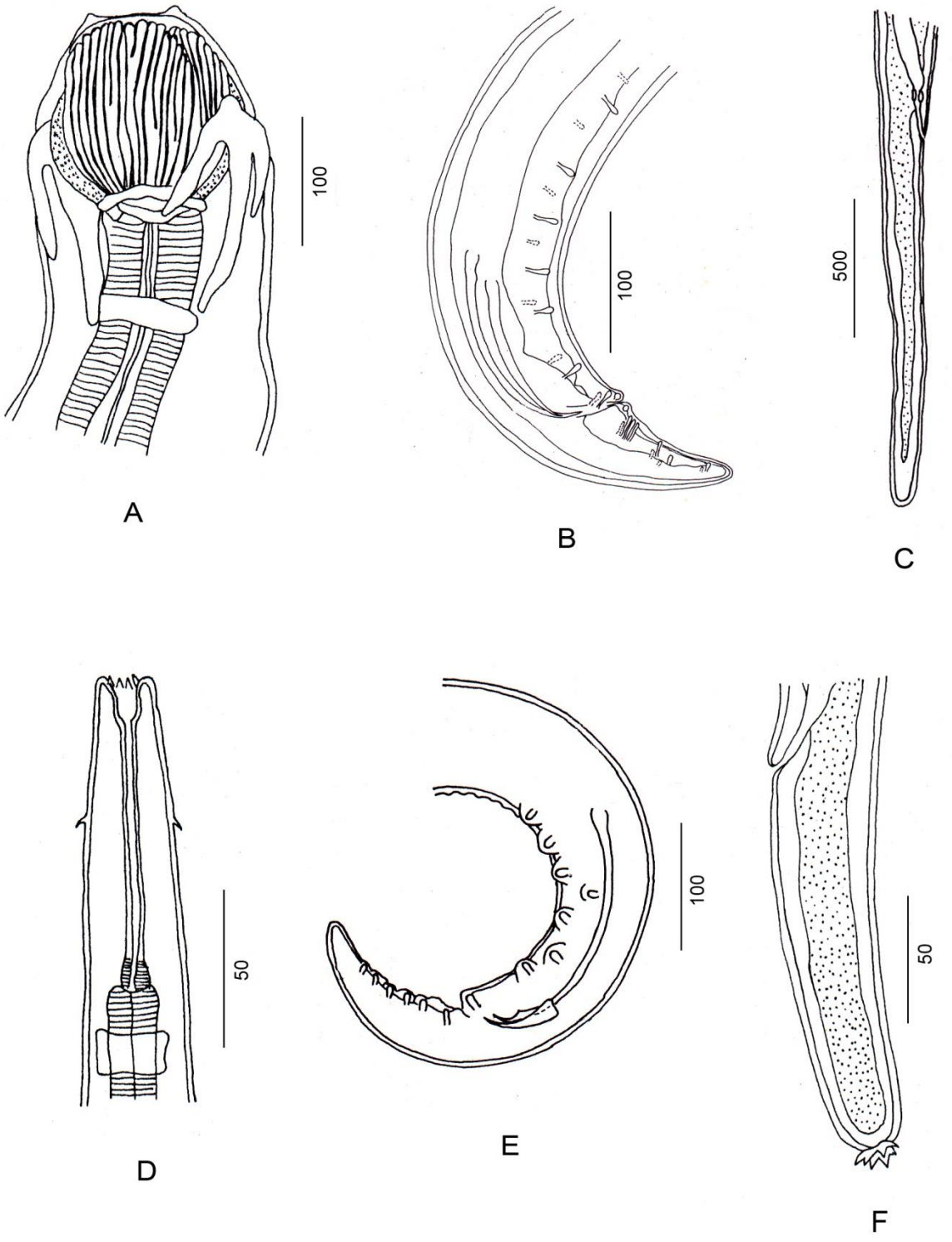

Figure 1: *Camallanus fotedari*: A- head region of male, B- tail region of male, C- tail region of female; *Rhabdochona sarana*: D- head region of male, E- tail region of male, F- tail region of female. (Values with scale bars are in micrometers)

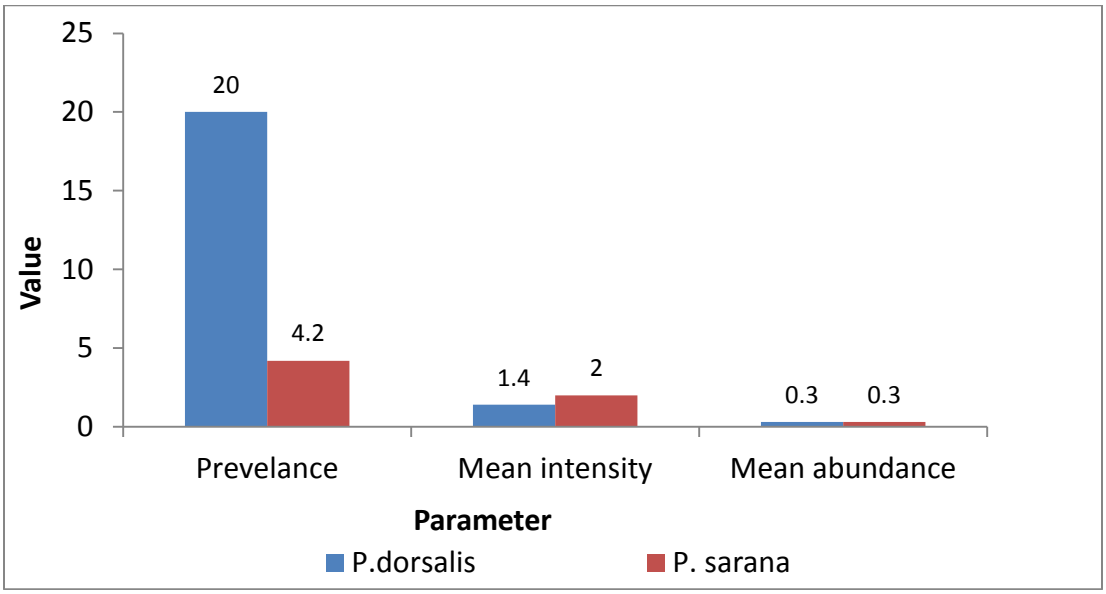

Figure 2: Ecological data of *C. fotedari* in its host populations

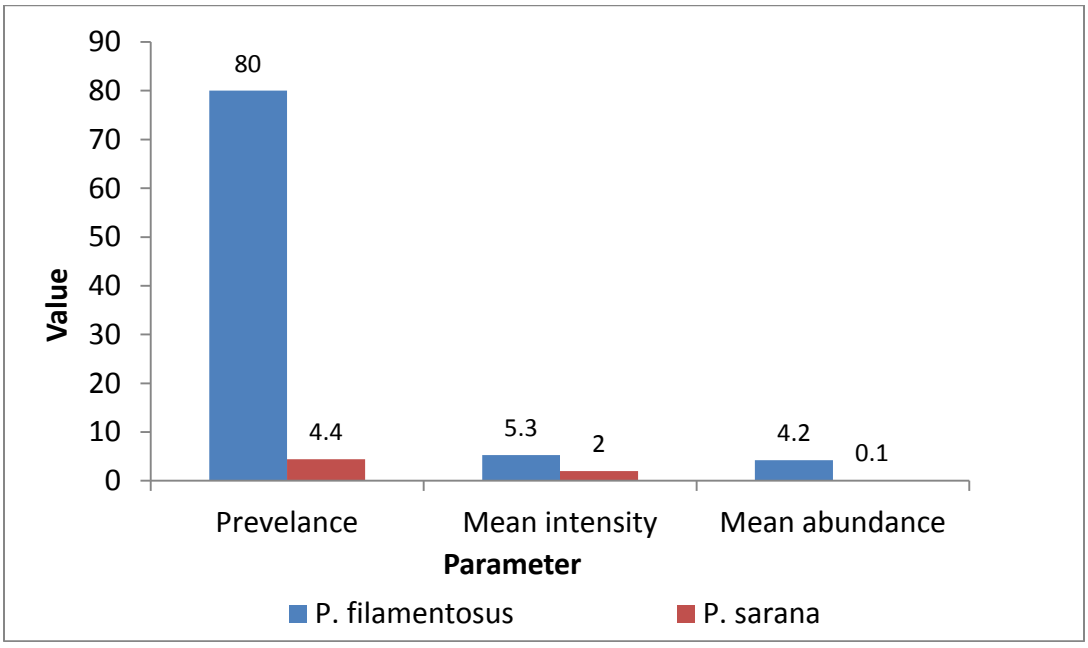

Figure 3: Ecological data of *R. sarana* in its host populations

Table 1: Comparative measurements of *C. fotedari* (male and female) with present material

	Raina and Dhar, 1972		**Present material**	
	Male	**Female**	**Male**	**Female**
Body length	2030 - 4950	1980 - 11920	2476	4715 (2143 - 6928)
Body width	90 - 280	120 - 520	116	194 (114 - 262)
Buccal capsule length	60 - 160	70 - 130	77	116 (88 - 130)
Buccal capsule width	90 - 100	50 - 160	81	119 (88 - 148)
Muscular esophagus	280 - 450	160 - 660	251	372 (246 - 452)
Glandular esophagus	290 - 490	280 - 870	325	459 (282 - 584)
Tail length	-	280 - 1450	-	860 (299 - 1339)
Vulva from anterior	-	2170 - 6820	-	2431 (1101 - 3476)
Small spicule	100 – 110	-	109	-
Large spicule	130 - 160	-	156	-
Caudal papillae	(6–8) + 2 + 6 = 14-16 pairs	-	7 + 2 + 6 = 15 pairs	-

Table 2: Comparative measurements of *R. Chodukini* (female and male) and *R. Sarana* (female only) with present material

	***R. chodukini* Osmanov, 1957**		***R. sarana* Karve and Naik, 1951**	**Present material**	
	Female	**Male**	**Female**	**Female**	**Male**
Body length	11720 - 13910	7500 - 8430	8551 - 12270	14305	6325
Body width	190 - 218	122 - 136	140 - 200	202	111
Prostom length	21 - 27	18	22 - 25	23	16
Prostom width	18	15	17 - 20	17	13
Vestibule including prostom	126 - 156	135 - 147	115 - 125	139	109
Muscular esophagus	342 - 384	258 - 288	260 - 290	234	185
Glandular esophagus	3670 - 4830	3020 - 3170	2440 - 3140	3699	2339
Nerve ring from anterior	195 - 222	204 - 210	130 - 150	151	121
Excretory pore from anterior	273 - 306	291 - 300	170 - 300	225	194
Deirids from anterior	84 - 120	102	-	60	53
Tail length	246 - 303	210 - 255	110 - 150	206	137
Crown length	4 - 6	-	5	9	-
Large spicule	-	411 - 420	-	-	203
Small spicule	-	120	-	-	69

IV. DISCUSSION

Of the nine *Puntius* species investigated, four fish species were parasitized with two nematode species, *Camallanus fotedari* and *Rhabdochona sarana*. *C. fotedari* was recovered from *P. dorsalis* and *P. titteya*, while *R. sarana* was recovered from *P. filamentosus* and *P. sarana*. Two species did not found coexisting in any infected fish host. Considering the importance of characterization of the parasites in wild freshwater fishes, present paper illustrates the morphological characters and describes the morphometric characters of the two nematodes recovered from *Puntius* species in Sri Lanka. Those will enable the parasitologists to identify the species and compare the conspecific species recovered from other studies.

The presence of main characters which Railliet and Henry (1915) used to define the genus *Camallanus* made it easy to place the nematode of family Camallanidae to the genus *Camallanus* Railliet and Henry, 1915. Using the key to the species of *Camallanus* reported from fishes in South Asia (Sood, 1988) these worms could be easily identified as *C. fotedari*, as they posses similar characters of *C. fotedari* described from *Nemacheilus kashmirensis* Hora. Measurements given to describe the *C. fotedari* Raina and Dhar, 1972, were nicely fit with those of present material, confirming the identification of the species (Table 1). All measurement of, both male and female, *C. fotedari* from *P. dorsalis* and *P. titteya* from Sri Lanka lie in between the measurements range of *C. fotedari* described from Srinagar, Kashmir. Moreover, present specimens are similar in all aspects to *C. fotedari* recorded from *Nemacheilus kashmirensis* Hora in Srinager, Kashmir.

Although six *Camallanus* species (including *Zeylanema* species, as suggested by Sood, 1988) have been recorded from Sri Lankan fresh water fishes (Kulasiri and Fernando, 1956, Fernando and Furtado, 1963), occurrence of *C. fotedari* in *P. dorsalis* and *P. titteya* species is a new record to Sri Lanka. Moreover, in Sri Lanka, only *Camallanus anabantis* has been previously recorded from a *Puntius* species; *P. filamentosus*. In India *Camallanus sweeti* has been recorded from *Puntius ticto*. As sited in Sood (1988), Campana-Rouget and others (1976) reported the *C. fotedari* in Guppies; *Lebistes reticulatus* and *Danio rero*, maintained in aquaria in France. It is evident that, *C. fortedari* seems a cosmopolitan species, which is probably spread throughout with the trade of guppies (*Poecilia* species). They may have entered to the Sri Lanka with guppy trade and now established even in the endemic species, *P. titteya*. As a widely distributed species even in non-cyprinid host, the guppies, inability of parasite to infect other *Puntius* species inhabiting in Nilwala river basin may relate to their food preference. *C. fotedari* also has low infection levels in both of its host, however, it has a little high preference to *P. dorsalis* (20%) than *P. titteya* (4.2%). The low values in mean intensity and abundance in both species may be due to its high dispersion in different host species. Parasite may have a very common intermediate host (copepod) to have a wide distribution in the world.

Due to presence of main characters which Moravec (1975) used to separate the genus *Rhabdochona* Raillet 1916, from other genera, the nematode recovered from *P. filamentosus* and *P. sarana* was belongs to genus *Rhabdochona*. The genus *Rhabdochona* that seems to be bound to fresh water only is widely distributed and contains a large number of species. Members of this genus are parasite of a number of fish species (largely in Cypriniformes but also in others), which are often of economic importance (Moravec, 1975). According to key provided by Morvec (1975) to Eurasian species of *Rhabdochona* these specimens should be either *R. chodukini* Osmanov, 1957 or *R. sarana* Karve & Naik, 1951. In this synthetic key, two species are differentiated only by the length of the female tail and by their locality. Our specimens are more closer to *R. chodukini* recorded from *Barbus brachycephalus & B. capito conocephalus*, and *R. sarana* recorded from *P. sarana*. However, Karve and Naik, 1951, have described only the female specimens of *R. sarana*. Males are not known. Therefore, measurements of our specimens were compared with the measurements of *R. chodukini* (both male and female) from *Barbus brachycephalus* and *Barbus capito conocephalus* from Tadzhikistan and those of the female *R. sarana* (Table 2).

Though, comparative measurement of female *R. chodukini* and *R. sarana* with present material did not give clear evidence to differentiate species as *R. chodukini* or *R. sarana*, males of present material gave the clear differentiate characters from *R. chodukini*. Of the anal papillae described for male *Rhabdochona chodukini*, the second pair of post anal papillae remains lateral and sub-ventral (counted from cloaca). In present specimen not the second pair, but first pair is lateral and remains sub-ventral. Of the pre-anal papillae of *R. chodukini*, 6 pairs remain sub-ventral and 2 pairs remain lateral. These lateral pairs are located between second and third and then at level of fifth sub-ventral pair. In present specimens also, 6 pairs are sub-ventral and 2 pairs remain lateral. But, lateral pairs are located between second and third and then between third and forth. Because of these characters of males, present specimens were different from *R. chodukini*. The dissimilarities with *R. chodukini*, especially on male, and the similarity of host with *R. sarana* made me to identify our specimens as *R. sarana*. Therefore, present paper describes the male's characters that can be easily used for identifying the *R. sarana* from *R. Chodukini*. *Rhabdochona sarana*, which is previously reported in *P. sarana* in India, was found in both *P. sarana* and *P. filamentosus* in present study, and high preference was reported not in *P. sarana* but in *P. filamentosus*. Probably it can also be present in other *Puntius* species as well. *R. sarana* in *P. filamentosus* has high prevalence infecting 80% of the population and about 5 individuals were present in infected host as shown in Figure 3. In *P. sarana*, its infection values are very low (prevalence 4.4%, mean intensity 2.0) compared to *P. filamentosus*. These common measurements of parasite population revealed that, of the *Puntius* species in Nilwala river basin of Sri Lanka, *P. filamentosus* were liable to infect *R. sarana* infecting 5 parasites per individuals of the fish population and may cause damage or deterious effect on their population. There was only one previous record of *Rhabdochona* species from Sri Lanka by Nilakarawasam (1993) in *Etroplus suratensis* Bloch. Hence, this is the first instance, *R. sarana* parasitizing in fishes of Sri Lanka.

In conclusion, occurrence of these two species, *Camallanus fotedari* and *Rhabdochona sarana* in *Puntius* species inhabiting Nilwala river basin is a new record to Sri Lanka. The restriction of present study to *Puntius* species in upstream tributaries of Nilwala river basin warrants the further investigations. It would be interesting to investigate whether the down streams have higher population numbers, whether there are other parasites on similar fish species in the other areas or any absence of the investigated species in them. Also, it would be interesting to investigate this two species coexist in single host and investigation of the parasite fauna of the non *Puntius* species in same areas would throw light on their host specificities.

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