Research Article

Aspects of the Ecology of Tetraphyllid Cestodes from the Slender Bamboo Shark, *Chiloscyllium indicum* Gmelin, 1789 (Orectolobiformes: Hemiscylliidae) from Nellore Coast, Bay of Bengal, India

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Abstract | From the coastal belt of Nellore District, Bay of Bengal, Andhra Pradesh 109 specimens of *Chylloscyllium indicum* Gmelin, 1789 were collected in 2014-2015. Only, 28 (25.6%) specimens were parasitized with at least one or more tetraphyllid cestodes. A total of 125 tetraphyllid cestodes of the 3 genus *Acanthobotrium*, *Orectolobicestus* and *Yorkeria* were obtained from the spiral intestines of the host i.e *Acanthobotrium chiloscylli, Orectolobicestus chiloscylli* and *Yorkeria indica*. The monthly population dynamics and the seasonal dynamics of the parasites were analysed to determine the recruitment of parasites. The infracommunity of *C.indicum* was predominated by *Orectolobicestus chiloscylli* (75.2%), followed by *Acanthobothrium chiloscylli* (12.8%), *Yorkeria indica* (8.8%) and *Yorkeria parva* (3.2%). The frequency distribution of parasites and their distribution pattern was analysed. There is no core and secondary species in the parasitic community of the host and all the four species occupy the rank of satellite species. The effect of host size on the prevalence of infection were investigated and found that medium sized fish (30-51cm) were frequently infected. The impact of sex on the overall parasitization of cestodes was also analysed by Mann-whitney U test (Z = -0.59, P_1=0.277; P_2 = 0.555) revealed that there was no influence of host sex on the parasitization. Values of Jaccard's Index (JI) showed interspecific associations between each pair of parasite species. The interspecific association between *Y.indica - Y. parva* pair (JI= 0.50); *O.chiloscylli - A. chiloscylli* pair (JI= 0.19) and *A. chiloscylli - Y.indica* pair (JI= 0.154) explained common niche of these parasites i.e., spiral intestine within the host.

Keywords | *Chylloscyllium indicum*, Tetraphyllidea, *Orectolobicestus*, *Acanthobothrium, Yorkeria*

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INTRODUCTION

The Slender bamboo shark, *Chiloscyllium indicum* Gmelin, 1798 (Hemiscyllidae) is a small, sluggish inshore bottom dwelling shark found on sandy and muddy bottoms of coastal water throughout the Indo-west pacific ocean (Compagno et al., 2005; Froese et al., 2006). This species is likely to be threatened due to overfishing, disparaging fishing practices and habitat loss caused due to damage and devastation of coral reefs throughout much of its range. These fishes are regularly taken in inshore fisheries for fresh food in India, Sri Lanka and Thailand. It is accessed as "Near Threatened" on the IUCN Red list of Threatened species globally (Barratt et al., 2003). *Chiloscyllium indicum* serves as one of the favourite hosts for a wide range of trypanorhynchid and tetraphyllid cestodes. Cestode parasites are implicated as major causative agents for diseases in fishes as they affect the health status, growth, survival and reproduction of the hosts by reducing their food intake and provoking nutritional stress (Anderson and May, 1978; Petekevicius, 2007; Wenchaun, 2007). An extensive work has been contribute on systematics of cestode parasites of various species of *Chiloscyllium* by scientists all over the world (Southwell, 1927; Williams, 1968; Zaidi and Khan, 1976; Caira et al., 1999, 2007; Caira and Tracy, 2002; Caira and Healy, 2004; Li and Wang, 2006; Ruhnke et al., 2006; Caira, 2010; Cutmore et al., 2010; Desjardins and Caira, 2011; Purivirojkul and Boonsoong, 2012; Purivirojkul, 2013; Gracan et al., 2014) but a very few taxonomic reports from the various species of *Chiloscyllium* has been contributed from India (Subhapradha, 1955; Deshmukh, 1979; Shinde et al., 1986; Sarada et al., 1986a; 1986b; Gangadharam and Vijayalakshmi, 2004; Dongare et al., 2009). Also, there are only a few studies on ecology and niche partitioning of cestode parasites from elasmobranchs (Kennedy and Williams, 1989; Cislo and Caira, 1993; Curran and Caira, 1995; Friggens and Brown, 2005; Purivirojkul, 2013) from various parts of the world but there are no reports on the ecology of cestode parasites from the Indian sharks. Ecological concepts are the primary requisite in the study of parasites as they provide a better understanding about the host parasite relationship and their environment. Cestode parasites can be used as biological tags/biological indicators of host population (Mackenzie 1975, 1982, 1987, 1990). Elasmobranchs are characterized by having a spiral intestine and the cestode parasites and other intestinal helminth assemblages differ in composition from most of the teleosts. The parasites of elasmobranchs are highly host specific. The size and sex of the host serve as an important factor influencing the parasitic composition and are related to the behavioural, biological and physiological changes in the host. Also, the changes in feeding habitats and diet influence the parasite fauna. The main objective of the present work is to study the tetraphyllid parasitological occurrence in Nellore coast, Bay of Bengal and the impact of host size and sex on parasitization. The analyses of interspecific associations and the distribution of these parasites are discussed.

MATERIAL AND METHODS

Study Area

The coastal belt of Nellore District (4° 27' N, 80° 02' E), Bay of Bengal is selected as study area to collect the fish samples as there are no records of the parasites from the fishes of this coast (Figure 1).

The study has been designed for a period of one year 2014-2015 in which the spiral intestines of 109 *C.indicum* were examined for the tetraphyllid cestodes. Monthly 8-15 fishes were sexed, measured for its length and weight individually before dissecting them by a mid-ventral incision to confiscate the spiral intestines. These intestines were then transferred into petridishes filled with physiological saline to remove excess mucus and then dissected along the ventral blood vessel and the intestinal mucosa with a longitudinal incision. Spirals were unrolled as a flat sheet and the gut contents were collected, washed and decanted to remove excess mucous. The gut contents were finally observed under the stereozoom microscope (LM-52-3621). The cestodes were found adhered to the surface of gut wall, some deeply embedded in the intestinal mucosa with the scolex. Parasites were collected intact with scolex and washed in saline solution.

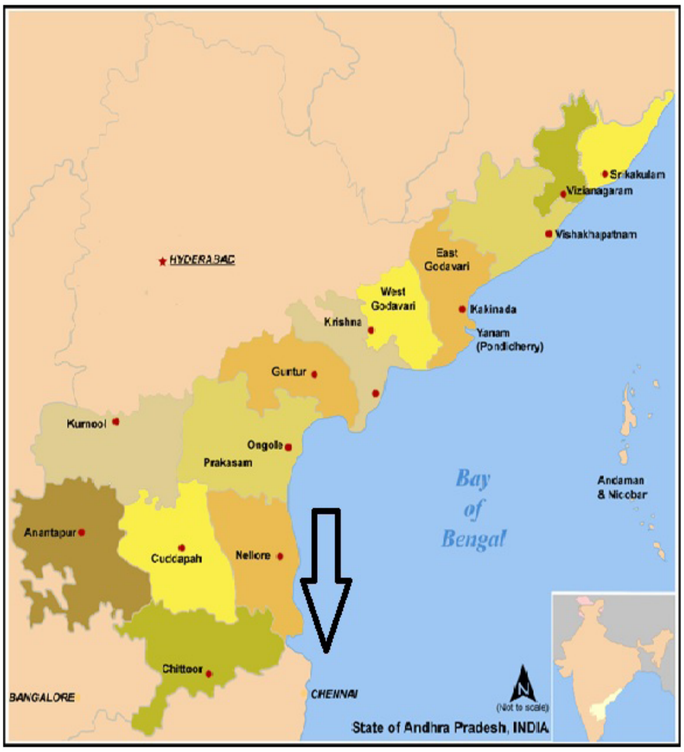

Figure 1: Locationof study area, Nellore Coast, Bay of Bengal

Conventional techniques were employed for the preparation of permanent slides (Hiware et al., 2003; Madhavi et al., 2007). FAA (Formalin, alcohol and acetic acid (10: 85: 05) was used as the fixative for whole mount preparations. Parasite specimens were micro photographed and measured in Euromex Bioblue BB-4260 microscope. Monthly population dynamics of the parasites were conducted. Seasonal influence on the rate of infection was also calculated by chi-square test to show the significance between season and prevalence of parasitization. *C. indicum* measured 19.9-59.9 cm (mean= 34.8±8.83) in total length. The standard length of male (34.9±9.48 cm, n=52) and female (34.7±8.27 cm, n=57) fish in the sample were significantly different (t= 0.887). The classification of Species was done according to Bush and Holmes (1986) and Bush et al. (1997, 2001) as central/core species (if prevalence >66.6%), Secondary species (prevalence between 33.3% and 66.6%) and satellite species (prevalence <33.3%) of total number of fish analysed. The dispersion pattern of parasite species was estimated by Dispersion index (DI) and were classified as aggregated (DI>1.96), regular (DI<-1.96) and random (DI < 1.96). The relationship between host length and parasitic burden was determined by Pearson's linear correlation coefficient, r. Impact of host sex on the prevalence and abundance of parasites was computed by Mann-Whitney U-test (Vassarstat.net/utest.html). The degree of association between species is measured by Jaccard's Index (JI) whose value ranges between '0 to 1' and as the value approaches

Table 1: Monthly changes in overall prevalence, Mean intensity, SD and mean abundance during the study period (March, 2014-February, 2015) in *C.indicum*

Months examined	No. of examined fishes (a)	No. of infected fishes (b)	No. of parasites (c)	Prevalence = b/a*100	MI±SD	MA±SD	Index of infection
March, 14	9	1	3	11.1	3.0±2.1	0.3±0.2	0.04
April,14	10	1	4	10.0	4.0±2.8	0.4±0.3	0.04
May,14	10	2	7	20.0	3.5±2.5	0.7±0.5	0.14
June,14	8	1	7	12.5	7.0±4.9	0.9±0.6	0.11
July,14	9	1	8	11.1	8.0±5.7	0.9±0.6	0.10
August,14	11	3	17	27.3	5.7±4.0	1.5±1.1	0.42
September,14	8	3	14	37.5	4.7±3.3	1.8±1.2	0.66
October,14	8	3	10	37.5	3.3±2.4	1.3±0.9	0.47
November,14	9	4	16	44.4	4.0±2.8	1.8±1.3	0.79
December,14	8	5	23	62.5	4.6±3.3	2.9±2.0	1.80
January,15	9	2	9	22.2	4.5±3.2	1.0±0.7	0.22
February,15	10	2	7	20.0	3.5±2.5	0.7±0.5	0.14

Table 2: Seasonal changes in overall prevalence, mean intensity, mean abundance and Index of infection of cestode parasites of *C. indicum*

Seasons	No. of examined fishes (a)	No. of infected fishes (b)	No. of parasites (c)	Prevalence = b/a*100	MI	MA	Index of infection	**χ_2 Value** (at 5% level of significance and 2 degrees of freedom)
Summer	37	5	21	13.5	4.2	0.6	0.076	
Rainy	36	10	49	27.8	4.9	1.4	0.378	0.1161
Winter	36	13	55	36.1	4.2	1.5	0.55	

Table 3: Distribution patterns and diversity parameters of parasitic species of *Chiloscyllium indicum* (n=109)

Name of the parasite	Infected fishes	No. of parasites	Prevalence	Mean intensity	Mean abundance	Index of infection	Range	D.I	Location	Nature of infection *	Nature of species **
Orectolobicestus chiloscylli	18	94	16.5	5.2	0.9	0.14	2-8	0.75	Spiral intestine	Frequent	Satellite
Acanthobothrium chiloscylli	12	16	11.5	1.3	0.1	0.016	1-3	0.128	Spiral intestine	Frequent	Satellite
Yorkeria indica	6	11	5.5	1.8	0.1	0.005	1-3	0.088	Spiral intestine	Rare	Satellite
Yorkeria parva	2	4	1.8	2	0.04	0.0006	1-2	0.032	Spiral intestine	Sporadic	Satellite

***Common=** 30-50%; **Frequent=** 10-30%; **Rare=** 4-10%; **Sporadic=** <4%; ****Core sp.=**>66%; **Secondary sp.**=between 66-33%; **Satellite sp.=** <33%

to 1, indicates the presence of high association between species. All the statistical tests were conducted using excel in MS-Office and statistical significance level adopted was p≤ 0.05.

RESULTS

Monthly population dynamics of tetraphyllid cestode parasites were graphically presented in terms of month-wise prevalence, mean intensity, mean abundance and index of infection (Figure 2). The prevalence of infection was high in the months of September, October, and November and reached to the peak in the month of December with a mean intensity (4.6±3.3) and lowest in the month of April with a mean intensity of 4.0±2.8 (Table 1). Mean intensity was high in the months of June, July and August. However, the mean abundance and index of infection was high in the months of November and December. Influence of Seasons on the tetraphyllid cestode infection was analyzed and a profound effect of seasons on prevalence and rate of parasitization was observed as evidenced by the calculated $\chi 2$ value ($\chi 2$=0.1161 at 5% level of significance and 2 degrees of freedom). Prevalence of infection was high during winter season and lowest during the summer season (Table 2, Figure 3). Recruitment of the parasites may take place after summer and reach their peak periods in the winter months.

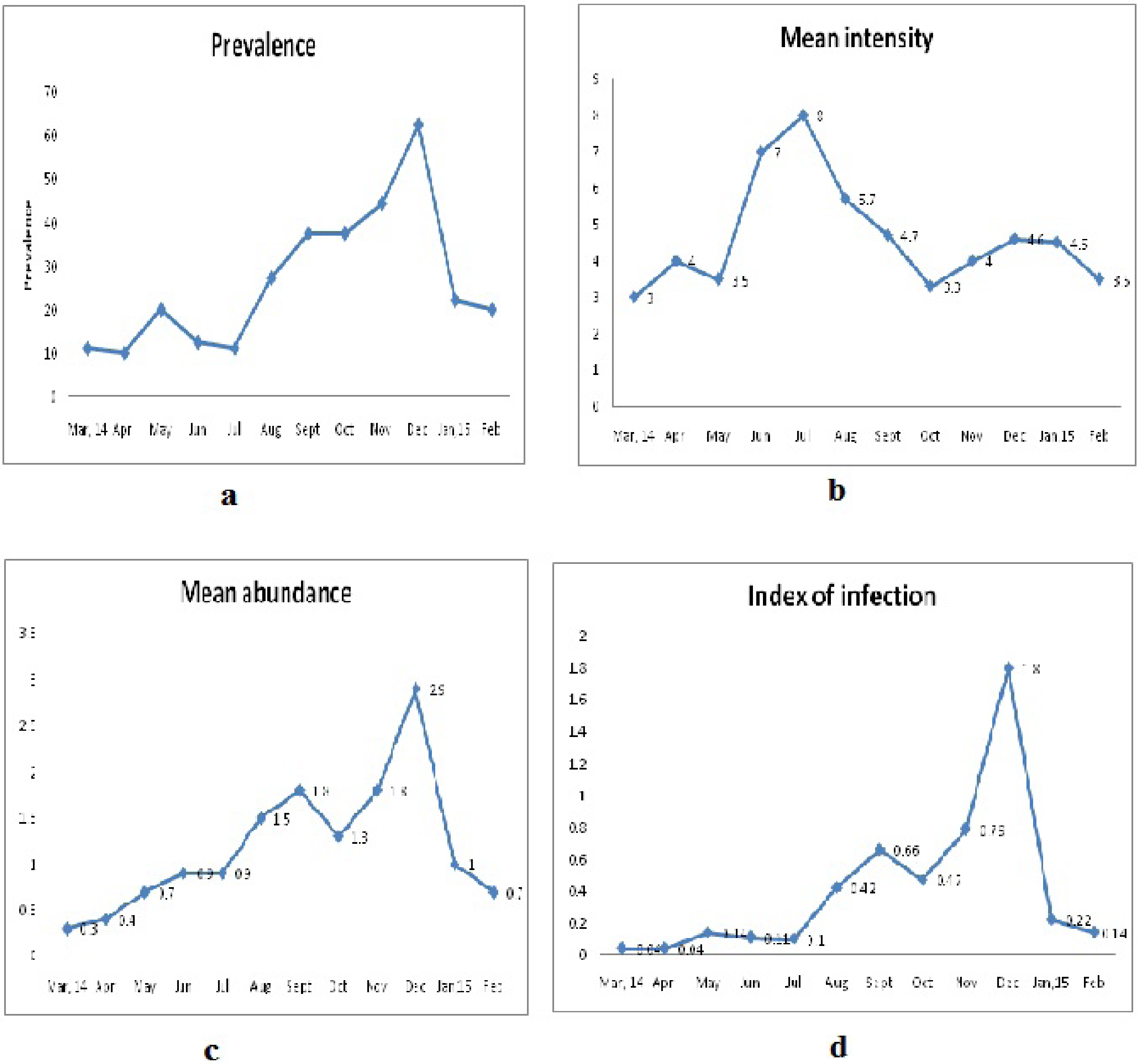

Figure 2: Monthly population dynamice of cestode parasite of *C. indicum*
a) Prevelence; **b**) Mean intesity; **c**) Mean abundance; **d**) Index of infection

Distribution and Structure of Infracommunity of Tetraphyllids in Host Samples

Of the 109 *C.indicum* examined, only four species of the order Tetraphyllidea i.e., *Acanthobotrium chiloscylli* (Gangadharam and Vijayalakshmi, 2005), *Orectolobicestus chiloscylli* (Subhapradha, 1955; Ruhnke et al., 2006), *Yorkeria indica* (Sharada et al., 1984) and *Yorkeria parva* (Southwell, 1927) were explored from the host (Table 3) and only 28 (25.6%) fish were recurrently parasitized with one or more than one parasite species. *Orectolobicestus chiloscylli* (n=94) triumphed the majority of the components of the infra community as specified by Berger-parkers dominance index (0.75) followed by *Acanthobothrium chiloscylli* (0.128), *Yorkeria indica* (0.088) and *Yorkeria parva* (0.032) (Table 3).

O.chyloscylli showed highest prevalence (16.5%) and mean intensity (5.22). Only, 18 (16.5%) hosts were infected with single parasitic species and only, 10 (9.17%) were infected with two parasitic species (Table 4). The ratio of variance to mean values give the index of dispersion (DI) and all the four parasitic species showed random or non-dispersed type of distribution patterns due to lesser DI values (Table 5). There are no core and secondary species in the parasitic community. All the four species conquered the position of satellite species. Host standard length functions as a vital factor in determining the parasitic burden in a host. The size of the sampled host ranged from 19.9-59.9 cm (mean= 34.8±8.83) in total length. The fishes were categorised into 4 groups: Group-1 (19-29cm); Group-2 (30-40cm); Group-3 (41-51cm) and Group-4 (52-62cm).

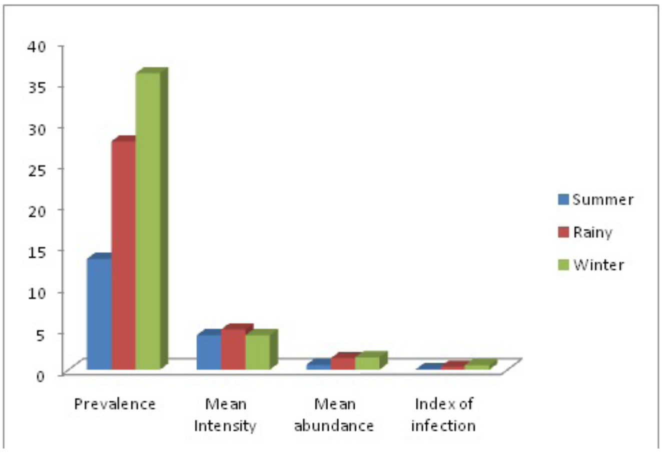

Figure 3: Seasonal dynamice of cestode parasitization in *C. indicum*

Table 4: Frequency distribution of number of parasitic species per individual in *C. indicum*

Sl. No.	No. of parasitic species	No. of infected fishes	% of frequency
1	1	18	16.5
2	2	10	9.17
3	3	0	0

n = 109; **Σx** = 2; **X** = 2/109= 0.018; **Range** = 1-2

Pearson's linear correlation coefficient, r was applied to find the relationship between host standard length and parasitic burden and the calculated values of r = 0.313 depicts a meager positive correlation between host size and parasitization. Medium sized fishes (Group-2 and group-3) represented higher infection rate compared to smaller and larger fishes (Group-1 and group-4). However, the parasitization of *A. chiloscylli* (r= -0.36), *Y.indica* (r= -0.57) and *Y.parva* (r= 0) showed negative correlation which depicts that increase in host size and age may reduce the level of parasitism (Table 6). Of the total sample of 109 *C.indicum*, 57 were females (55%) and 52 were males (48%) of which only 13 (22.8%) females and 15 (28.8%) were infected by these cestode parasites. The influence of host sex on the parasitic burden was analyzed by Mann-Whitney U-test. The calculated Z (U) value, i.e., Z = -0.59, P_1=0.277; P_2 = 0.555 revealed that there are no differences in the levels of parasitism in males and females hosts. However, the calculated Z (U) value, i.e., Z = 0.58, P_1=0.281; P_2 = 0.5619 of *Acanthobothrium chiloscylli* shows that there is impact of sex on parasitization and females are more infected than males. The negative values of Z (U) for the remaining three species shows that sex of the host has negligible influence on the parasitization (Table 7). Jaccard's Index (JI) is applied to scrutinize the interspecific association between each pair of parasite species. The interspecific association between *Y.indica* - *Y. parva* pair (JI= 0.50); *O.chiloscylli* - *A. chiloscylli* pair (JI= 0.19) and *A. chiloscylli* - *Y.indica* pair (JI= 0.154) shows that these parasites share a common niche i.e., spiral intestine within the host. However, *O.chiloscylli* - *Y.indica* pair (JI=0.045) showed a negligible values (Table 8).

DISCUSSION

The monthly population dynamics depicts the recruitment of parasites month wise. Winter months (October, November and December) showed highest prevalence while the summer months (March, April, June and July) showed lowest prevalence. The present study is in full coherence with the views of Akhter et al. (1997), Banu et al. (1993), Chandra et al. (1997) and Lizama et al. (2006) who opined that prevalence of diseases in fish is elevated during winter due to the drastic fall in water temperature which reduces the immune response in fish and thus makes them more vulnerable to infection.

Table 5: Mean (X), Variance (s^2) and Dispersion index (s^2/X) of parasite species in *C.indicum*

Name of the parasite	2014-2015 (n =109)			
	No. of parasites collected	Mean (X)	Variance (s^2)	Dispersion index (s^2/X)
Orectolobicestus chiloscylli	94	5.22	2.88	0.55
Acanthobothrium chiloscylli	16	1.36	0.386	0.28
Yorkeria indica	11	1.82	0.566	0.309
Yorkeria parva	4	2	0	0

Table 6: Parasitic abundance in the different size classes of *C.indicum*

Sl. No.	Groups	Size	*O. chiloscylli*	*A. chiloscylli*	*Y. indica*	*Y. parva*	Total no. of parasites
1	Group-1	19-29 cm	15	6	3	2	26
2	Group-2	30-40 cm	37	6	5	2	50
3	Group-3	41-51 cm	36	4	3	0	43
4	Group-4	52-62 cm	6	0	0	0	6
Correlation coefficient, R			0.33	-0.36	-0.57	0	0.313

Table 7: Diversity parameters of parasitic species in males and females and values of Mann-Whitney U-test to evaluate rate of host sex and parasitic abundance in *C. indicum*

Host name	Chiloscyllium indicum (N_m = 52; N_f = 57)								Mann –Whitney U test (Z)		
Parasite	N_{mi}	N_{fi}	P_m	P_f	MI_m	MI_f	MA_m	MA_f	Z (U)	P_1 (significance level)	P_2 (significance level)
Orectolobicestus chiloscylli	9	9	8.25	8.25	5.5	4.8	0.45	0.40	-0.19	0.4247	0.8493
Acanthobothrium chiloscylli	4	8	3.66	7.34	1.25	1.37	0.045	0.10	0.58	0.281	0.5619
Yorkeria indica	5	1	4.58	0.92	2	1	0.09	0.009	-0.72	0.2358	0.4715
Yorkeria parva	2	0	1.83	0	2	0	0.03	0	-0.34	0.3669	0.7339

*N_m = Number of males examined; N_f = Number of females examined; N_{mi} = Number of males infected; N_{fi} = Number of females infected; P_m & P_f = Prevalence of males and females respectively; MI_m & MI_f = Mean intensity of males and females; MA_m & MA_f = mean abundance of males and females respectively.

Table 8: Values of Jaccard's Index (JI) to estimate interspecific association between each pair of parasite species of *C.indicum*

Name of parasites	*O. chiloscylli*	*A. chiloscylli*	*Y. indica*	*Y. parva*
O. chiloscylli	-	0.19	0.045	0
A. chiloscylli	0.19	-	0.154	0
Y. indica	0.045	0.154	-	0.5
Y. parva	0	0	0.5	-

Dominance of the tetraphyllids in parasite community has been detected in the present study which might be due to varied food habits of *C.indicum* whose feeding items include invertebrates, crustaceans and small fishes which might serve as intermediate hosts of parasites. The present study also holds upright with the opinions of Shotter (1973), Hanek and Fernando, (1978a, b); Fernandez (1985); Valtonen et al. (1990); Roubal (1990), Takemoto and Pavanelli (2000), Robert et al., (2008) and Anu prasanna and Vijayalakshmi (2013) who discoursed that hosts with intermediate lengths showed high levels of parasitism. Infection was less in the younger fishes and older fishes and might be due to the fact that fish attain parasites in the youth phase and eliminates in the adult phase due to the development of immunological competence. Takemoto and Pavanelli (1994) and Machado et al. (1994) were of the opinion that some parasites show a significant increase in levels of parasitism with increase in size and age and in the present study, only, *O.chiloscylli* (r=0.33) have shown the positive correlation.

Host sex is also one of the crucial factors in depicting the parasitization. The distinction of biological, physiological and behavioral factors between males and females may produce slight variations in the parasitization (Thomas, 1964). This might be due to the reproductive stress in the hosts which support or not the acquirement of the parasites. The present study was in full agreement with the observations of Takemoto and Pavanelli (1994, 2000) and Lizama et al. (2005) who were of the view that there is no influence of host sex on overall parasitization. However, only, *Acanthobothrium chiloscylli* showed the impact of sex on parasitization and females are more infected than males and the remaining three species showed negligible influence of host sex on the parasitization.

There are many factors which interfere in the parasitic community. They are main causes for the competition among the species (presence of one species inhibits or impair the presence of the other) or form associations. Struggle for space and food, reproductive barriers to obstruct hybridization among taxonomically close species, vulnerability difference in hosts, low immunity of host with regard to parasite, similarity and difference of hosts and need of parasite species for similar conditions are essential to study the interspecific associations among species (Bush and Holmes, 1986; Holmes, 1990). In the present study three assosciations were associated and they these parasites share a common niche i.e., spiral intestine within the host.

AUTHOR'S CONTRIBUTION

Srinivasa Kalyan C and Hemalatha M had collected the host samples from the sampling sites, carried out dissections and collected parasites for further processing, involved in data compilation and literature survey while Anuprasanna Vankara who is also the corresponding author prepared the initial manuscript, framed and formatted to get a final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to the work.

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