

## **NEW DISCOVERING ON INTERNAL CORRELATION OF NEMATODE COMMUNITIES' CHARACTERISTICS IN THE CO CHIEN ESTUARY, BEN TRE PROVINCE**

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### **ABSTRACT**

Insight on internal correlation of nematode communities' characteristics was first investigated along the Co Chien estuary in order to understand a link among their densities, composition, diversity, morphometric and biomass. Research results showed high value in the nematode densities and diversities in this estuary. Nematodes had medium size, individual biomass is rather small but total biomass in the communities is quite high due to their densities. The nematode size, shape and biomass have significant relationship with densities and feeding type structure in their communities along the Co Chien estuary.

*Keywords:* nematode morphometric, biomass, Co Chien estuary, Ben Tre province.

### **1. INTRODUCTION**

The Co Chien estuary was formed from the Co Chien River. This is one big branch of Mekong River which is the greatest river in the Southeast Asian. Some relevant publications of nematode and meiobenthos communities in the Mekong estuarine system [1,2,3,4]. However, investigate and analyse relationship among nematode communities such as morphometry and biomass distribution patterns in relation to other characteristics of nematode communities in the Co Chien estuary have not yet been published.

Investigating on characteristics of nematode communities such as densities, diversity and the trophic composition with nematode morphometric and biomass can find out their interesting relationship. This study will focus to discover current internal situation of nematode communities in the Co Chien estuary.

### **2. MATERIALS AND METHODS**

#### **2.1. Sampling location**

Current situation of nematode communities in the Co Chien estuary was investigated in the dry season, March 2009. Sampling stations were established at 4 stations with codes and coordinates as: ECC1 (9°48'47.10"N, 106°35'55.37"E), ECC2 (9°52'00.5"N, 106°32'53.7"E), ECC3(9°56'57.81"N, 106°26'16.16"E), ECC4(10° 0'24.45"N, 106°21'21.82"E) (Figure 1).

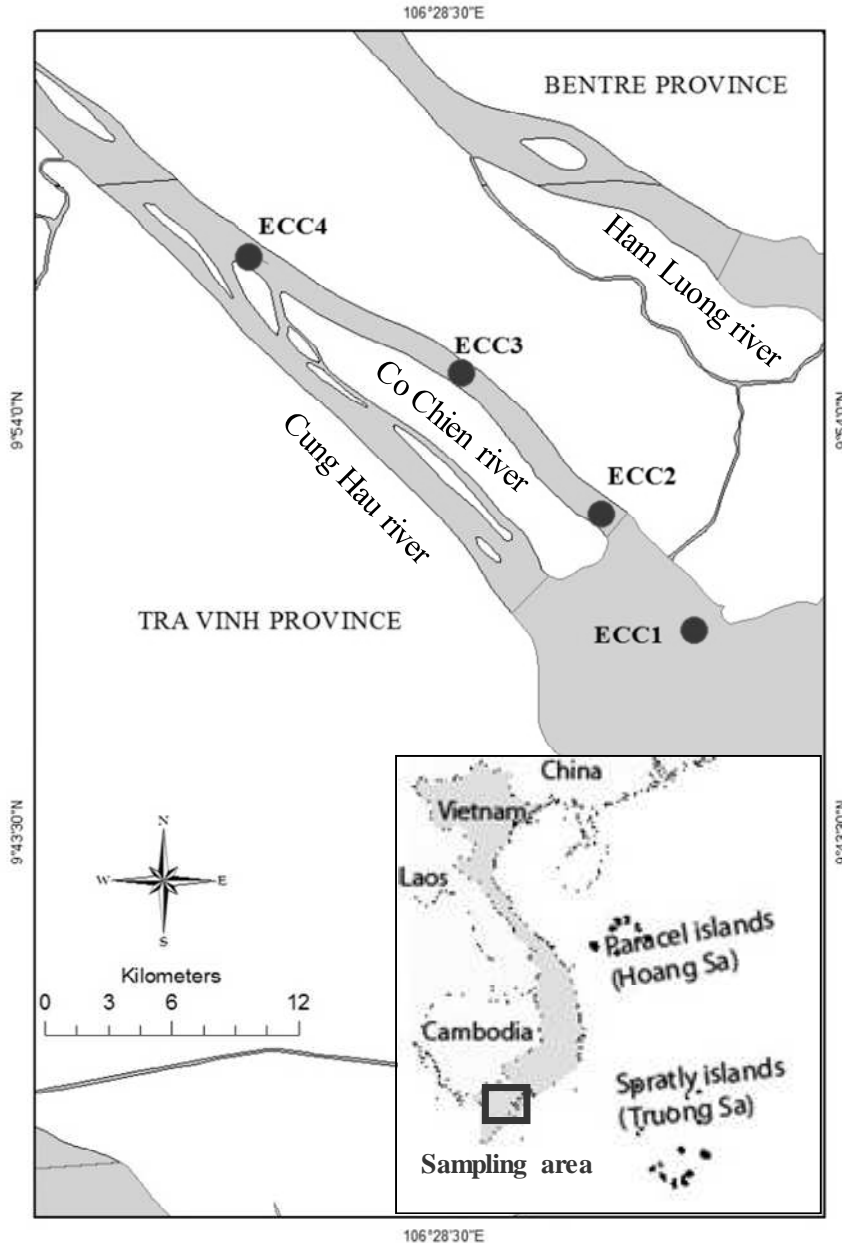


Figure 1. Sampling stations for nematode biomass in the Co Chien estuary.

## 2.2. Materials for sampling and experiment

*Materials need for sampling, extraction and decantation nematode samples:* 3.5 cm diameter plastic core, 5l beaker with handle, 38 µm and 1mm sieve, wash bottle, tall 500 ml beaker, square counting dish, petri dishes, handling needle, stereo microscope, staining block with cover,

fire proof tube, heating plate, oven at 35 - 40 °C, dessicator or airtight pot (weckpot), pipette, small pots, short very fine glass pipette, slides, lamens, rope ruler.

*Chemical solutions apply for experiment process:* formalin 4 %, ethanol 96 %, pure glycerin, distilled water, paraffin.

### **2.3. Sampling methods and analysis**

#### *Specimen collection and measurement method*

In each station, triplicate of nematode samples were collected in 10cm deep of sediment surface by plastic core 3.5 cm diameter. All samples are fixed in hot formalin 10 % at field before transferring to laboratory. 200 nematode specimens were picked out randomly for making slides. Nematodes were identified by using a high magnification microscope Leica (Type III) and Olympus BX41 using different documents [5, 6, 7] and the NEMYS database of the Marine Biology Section, Ghent University, Belgium [8].

Diversity of nematode communities was measured using the Shannon-Wiener diversity -  $H'(\log_2)$  [9] which is calculated from the proportional abundances  $p_i$  of each species (abundance of the species ( $N_i$ ) per total abundances ( $N_t$ )):  $H' = -\sum (p_i * \log(p_i))$  (in which  $p_i = N_i/N_t =$  relative abundance of the  $i^{\text{th}}$  species or genus.

These nematode specimens were measured in terms of the length (excluding filiform tail) and width at the maximum points by the software Leica Application Suite integrated with Microscope Leica. Individual nematode wet biomass was calculated following to Andrassy's formula (Andrassy [11]): Biomass ( $\mu\text{g WW}$ ) =  $L \times W^2 / 1\ 600\ 000$ , with L the nematode length ( $\mu\text{m}$ ) and W the nematode width ( $\mu\text{m}$ ). The dry biomass was estimated to be 25 % of the wet biomass (Wieser [12]) and expressed in  $\mu\text{g}$ .

#### *Data analysis*

The nematodes were identified into four feeding categories, based on the structure of the buccal cavity according to Wieser [10]: Type 1A: selective deposit-feeders, genera with very small-unarmed buccal cavity, presumed to feed selectively on small particles such as bacteria; Type 1B: non-selective deposit-feeders, genera with unarmed buccal cavity of moderate size, which feed less selectively because also larger particles, such as diatoms can be ingested; Type 2A: epistratum (epigrowth) feeders, genera with a medium size buccal cavity, provided with small teeth that are used to attack food particles or to scrape them of solid surface; Type 2B: predators or omnivores, genera with wide buccal cavity with large teeth or other powerful structure that are used to destroy relatively large food organisms.

All nematode data such as the length ( $L(\mu\text{m})$ ), the width ( $W(\mu\text{m})$ ), ratio of length and width ( $L/W$ ), individual nematode biomass ( $\mu\text{g}$ ), total nematode biomass ( $\mu\text{g}/10\ \text{cm}^2$ ) were analyzed in relation to other characteristics of nematode communities such as densities, percentage of feeding types and trophic index-ITD (total square percentage of feeding types according to Heip et al. [13]). The significant difference of nematode variables between stations was detected by one way ANOVA analysis or non-parametric Kruskal-Wallis ANOVA by rank test if the ANOVA assumptions were not satisfied.

In order to investigate the relationship between nematode size, shape and biomass with other nematode characters, the software STATISTICA 7.0 was used to calculate the Spearman

Rank Correlation analysis (r coefficients) and multiple regressions. In the case of multiple regressions,  $R^2$  was adjusted for the number of independent variables ( $R^2$  adj.). Beside linear regression, some other models of regression such as exponential or logarithmic models were applied occasionally in order to explain better the variation in nematode morphometry.

### 3. RESULTS AND DISCUSSION

#### 3.1. Nematode communities' characteristics in the Co Chien estuary

Total of 21115 nematode specimens were identified into 105 nematode genera distributing along the Co Chien estuary. Those stations in the mouth estuary (ECC1, ECC2) showed lower value to compare to in land stations (ECC3, ECC4). One way ANOVA test showed significant different in densities ( $p = 0,00001$ ) (see Table 1 and Figure 2a). In contrast, number of genera expressed significant higher in land comparing to mouth station (one way ANOVA,  $p = 0,0003$ ) (Table 1 and Figure 2b).

Genera *Daptonema*, *Terschellingia*, *Dichromadora*, *Halalaimus*, *Parodontophora*, *Theristus*, *Thalassomonhystera*, *Desmodora* were found dominant distribution in this estuary.

Results of trophic index (ITD), diversity index (H') and percentage of feeding types were showed in the Table 1.

*Table 1.* Value of some measured characteristic of nematode communities (S- genera number, N – densities, ITD – trophic index, and nematode feeding type (1A, 1B, 2A, 2B)) in the Co Chien estuary.

<b>Samples</b>	<b>S</b>	<b>N</b>	<b>ITD</b>	<b>H'</b>	<b>%1A</b>	<b>%1B</b>	<b>%2A</b>	<b>%2B</b>
ECC1.1	39	1138	0,30	4,15	0,13	0,37	0,35	0,14
ECC1.2	34	827	0,33	4,25	0,18	0,47	0,27	0,08
ECC1.3	34	1025	0,31	4,14	0,13	0,40	0,35	0,12
ECC2.1	21	662	0,33	3,17	0,18	0,25	0,47	0,10
ECC2.2	22	404	0,27	3,28	0,34	0,17	0,19	0,30
ECC2.3	26	272	0,29	3,12	0,11	0,24	0,39	0,25
ECC3.1	21	3892	0,36	3,50	0,16	0,51	0,27	0,07
ECC3.2	23	3181	0,30	3,52	0,41	0,30	0,19	0,10
ECC3.3	21	3009	0,39	3,06	0,08	0,48	0,38	0,06
ECC4.1	23	1873	0,31	3,60	0,29	0,42	0,23	0,06
ECC4.2	17	2285	0,36	3,44	0,27	0,51	0,17	0,05
ECC4.3	17	2547	0,32	3,39	0,35	0,39	0,22	0,04

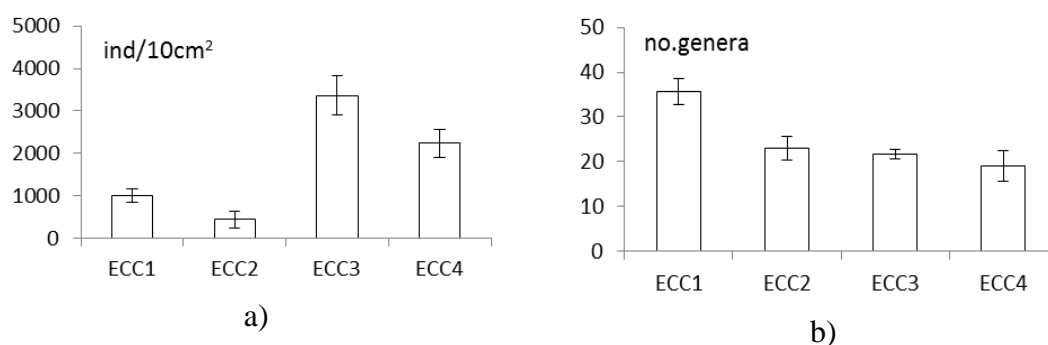


Figure 2. Average and standard deviation of nematode densities (a) and genera number (b) along the Co Chien estuary.

### 3.2. Morphometry and biomass of nematode assemblages

Morphometry and biomass of nematode assemblages in the Co Chien estuary were described specifically in Table 2. The biomass of nematodes depends on the shape of nematode since it was measured from the length and width. The nematode shapes at 4 stations along the Co Chien estuary seem small and short. The lengths ranged from 166.1  $\mu\text{m}$  to 3857.1  $\mu\text{m}$  and their means varied from 538.4 to 989.9  $\mu\text{m}$  per replicate sample. The nematode width ranged from 7.1  $\mu\text{m}$  to 159  $\mu\text{m}$ , and their mean from 19  $\mu\text{m}$  to 28.6  $\mu\text{m}$  on average. The nematode morphometry (length plotted versus width) of all measured nematodes in the study area are shown in Figure 3.

Nematode length and width in the Co Chien estuary had a quite large range. This result was overlapped with the range of nematode observed by Soetaert et al. [14] although in this study nematodes were up to 5000  $\mu\text{m}$  long.

The ratio of length and width of the Co Chien estuary varied from 3.86 to 183.56. According to Schratzberger et al. [15], the L/W ratio is a measure of a nematode's body shape with long/thin animals having high ratios, and stout animals' low ratios. In addition, Soetaert et al. [16] and Schratzberger et al. [15] also mentioned that three morphologies have developed over evolutionary time-scales: stout, slender and long/thin basing on the following criteria: stout with a L/W ratio < 18, slender with a L/W ratio of 18 - 72 and long/thin with a L/W ratio > 72. Therefore, nematode communities along the Co Chien estuary was represented 66.71 % slender shape, 26.3 % stout shape and 5.03 % long and thin shape. These results differ from Schratzberger et al. [15] with 82 % slender shape, 6 % stout animals and 12 % long/thin animals.

Table 2. Morphometry and biomass of nematode communities in the Co Chien estuary.

Samples	L	W	L/W	ind.bio	Tot.bio
ECC1.1	555,63	26,96	20,61	0,07	90,00
ECC1.2	538,36	21,44	25,11	0,04	40,83
ECC1.3	600,87	25,04	24,00	0,08	91,56
ECC2.1	840,43	20,77	40,46	0,06	48,36
ECC2.2	852,10	18,29	46,60	0,08	36,64

ECC2.3	989,86	20,39	48,55	0,07	21,12
ECC3.1	641,29	28,59	22,43	0,16	650,04
ECC3.2	681,11	23,20	29,36	0,10	329,20
ECC3.3	583,29	27,93	20,88	0,11	351,29
ECC4.1	699,21	25,10	27,86	0,11	203,07
ECC4.2	548,32	19,03	28,82	0,03	92,69
ECC4.3	662,36	21,35	31,02	0,08	231,58

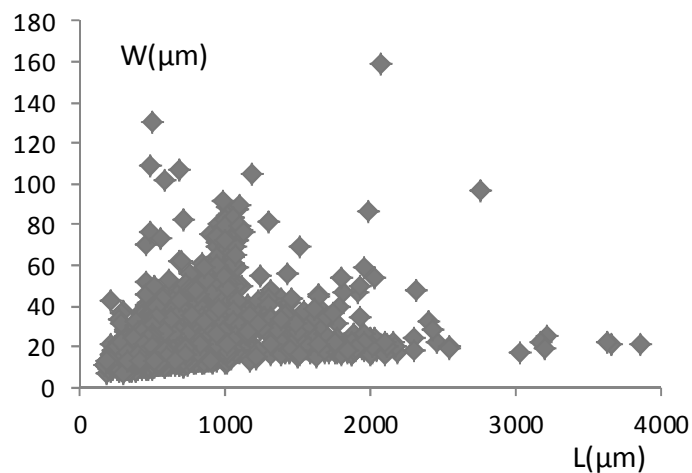


Figure 3. The length and width along the Co Chien River.

Results also showed that individual nematode biomass had values from 0.041  $\mu\text{g}$  to 0.167  $\mu\text{g}$  on average along the Co Chien estuary. The range of individual biomass was overlapped with the ranges for individual dry weight of nematodes which was reported by Soetaert et al. [16] with values from 0.038  $\mu\text{g}$  to 0.205  $\mu\text{g}$  and by Soetaert et al. [14] with values from 0.067  $\mu\text{g}$  to 0.18  $\mu\text{g}$ , and from a tropical lagoon reported by Boufahja et al. [17] with values from 0.0525 to 0.2625  $\mu\text{g}$ . However, nematode individual biomass in the Co Chien estuary was lower than that in a temperate estuary reported by Smol [18] ( $0.38 \pm 0.02 \mu\text{g}$  to  $0.46 \pm 0.02 \mu\text{g}$ ).

The total biomass of Mekong nematodes showed rather high values from 9.08  $\mu\text{g}$  dwt/10cm<sup>2</sup> to 706.3  $\mu\text{g}$  dwt/10 cm<sup>2</sup> in comparison with some other studies. Dye and Furstemberg [19], Van Damme et al. [20] and Tita et al. [21].

Along the Co Chien estuary, the nematode length and ratio L/W showed both significant differences between station ECC2 and remaining stations when tested by one way ANOVA analysis followed by a pair wise comparison test (HSD) (length:  $F_{(8,3)} = 15.206$ ,  $p = 0.001$ ; ratio L/W:  $F_{(8,3)} = 18.545$ ,  $p = 0.000583$ ). The log transformed total biomass of nematodes was significantly different between ECC2 and ECC3, ECC4; ECC1 and ECC3 ( $F_{(8,3)} = 18.61$ ,  $p = 0.000576$ ). There is no significant difference between stations for the nematode width and individual nematode biomass ( $p_w = 0.07$ ,  $p_{\text{ind.bio.}} = 0.118$ , Kruskal-Wallis ANOVA by rank test).

### 3.3. Correlation between nematode morphometric data and other characteristics of nematode communities

Results showed that along the Co Chien estuary, nematode length was negative significantly correlated with the percentage of feeding 1B but again positive correlated with the feeding type 2B. The ratio between L/W showed a significant negative correlation with the ITD and percentage of feeding type 1B whereas it was positive correlated with 2B. The width of nematode was not found significant correlated with any measured data of nematodes. Both individual biomass and total biomass had a significant positive correlation with nematode densities (Table 3 & 4, Figure 4).

*Table 3.* The correlation between nematode morphometric data with nematode community characteristics along the Co Chien estuary (n = 12).

Nematode morphometry		ITD	N	% 1A	% 1B	% 2A	% 2B
Av.Length	r	-0.5712	-0.4686	0.0831	<b>-0.8198</b>	0.2828	<b>0.6963</b>
	p	0.052	0.124	0.797	<b>0.001</b>	0.373	<b>0.012</b>
L/W	r	<b>0.6051</b>	-0.5551	0.2534	<b>-0.8313</b>	0.0716	<b>0.7392</b>
	p	<b>0.037</b>	0.061	0.427	<b>0.001</b>	0.825	<b>0.006</b>
Individual bio.	r	0.1581	<b>0.6312</b>	-0.0645	0.1382	-0.0013	-0.1060
	p	0.624	<b>0.028</b>	0.842	0.668	0.997	0.743
Total biomass/10cm <sup>2</sup>	r	0.4921	<b>0.9086</b>	0.0163	0.4731	-0.1877	-0.4574
	p	0.104	<b>0.000</b>	0.960	0.120	0.559	0.135

*Table 4.* The significant regression of percentage of feeding type 1B (% 1B), 2B (%2B) with nematode lengths and total biomass (Total bio.), individual biomass (Individual bio.) and densities (N) of nematode communities along the estuary ECC.

Regression test	R <sup>2</sup>	F	df	p
% 1B & L	0.672	20.49	1,10	0.0011
% 2B & L	0.55	5.5	1,1,9	0.027
Total bio. & N	0.825	47.32	1,10	0.00004
Individual bio. & N	0.63	7.688	1,1,9	0.011

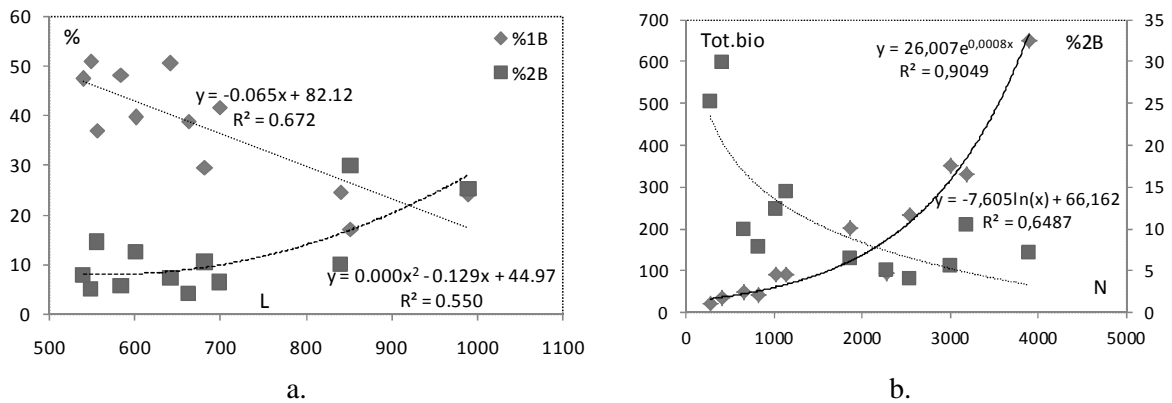


Figure 4. The correlation between nematode length and percentage of feeding type 1B and 2B (a), the densities with 2B and total biomass (b) along the estuary ECC.

The length and L/W ratio's still correlated positive with the predators/omnivores but there was also a negative correlation with the non-selective deposit feeders (1B), instead of the selective deposit feeders (1A). In this view, we can assume that the predators/omnivores tend to have slender or longer bodies while non-selective or selective deposit feeders tend to be shorter. This may be a special adaptation by developing in opposite ways to maintain the natural predator – prey interaction. The predators have longer bodies to move faster and to prey better on smaller nematodes.

According to Soetaert et al. (2002)[16], the longer and more mobile nematodes penetrate much deeper into the sediment than their plump and presumably rather immobile relatives. The vulnerability of individuals to a predator attack is often inversely related to the size of the animals. The authors also mentioned that predators must have a larger mouth cavity to swallow a fat nematode compared to a thin nematode and therefore prey nematodes with thicker bodies will survive better because when predators attack the head, tail or from the side, thick nematodes may not break as easily as very thin ones.

However, our results were contrasting with the study of Tita et al (1999)[21] on three intertidal assemblages of St Lawrence Estuary, Quebec, Canada. The authors found nematode morphotypes (body width/body length ratio = w/l ratio) associated with feeding groups. Small W/L ratios mean high L/W ratio was typical for microvores, while greater W/L ratios were typical for epigrowth feeders and predators.

The nematode individual and total biomass did not have any correlation with feeding types but they both had a positive relationship with total densities. This kind of relationships is enforcing each other since nematodes become bigger when densities increase.

#### 4. CONCLUSION

In conclusion, nematode communities in the Co Chien estuary was discovered with high densities, high diversity. Their morphometry and biomass were characterized with medium sizes in both length and width. The individual biomass was lower in general but total biomass was relatively high. The nematode size, shape and biomass have significant relationship with densities and feeding type structure in their communities along the Co Chien estuary.



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### **TÓM TẮT**

#### **KHÁM PHÁ MỚI MỐI TƯƠNG QUAN NỘI TẠI GIỮA CÁC ĐẶC ĐIỂM TRONG QUẦN XÃ TUYẾN TRÙNG Ở VÙNG CỬA SÔNG CỎ CHIÊN, TỈNH BẾN TRE**

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Khám phá mới mối tương quan nội tại của quần xã tuyến trùng sống tự do ở vùng cửa sông Cỏ Chiên. Nghiên cứu này nhằm hiểu rõ sự liên hệ giữa mật độ phân bố, thành phần, tính đa dạng, hình thái và sinh khối. Kết quả nghiên cứu cho thấy, quần xã tuyến trùng ở đây có tính đa dạng cao, mật độ phân bố lớn, kích thước cỡ trung bình và sinh khối cá thể thấp nhưng tổng sinh khối khá cao. Bằng phương pháp phân tích tương quan và hồi quy giữa kích thước, hình dạng và sinh khối của quần xã tuyến trùng với các tính chất khác trong quần xã của chúng như: mật độ cá thể, kiểu dinh dưỡng cho thấy có sự chi phối rất chặt chẽ từ mật độ phân bố và các kiểu cấu trúc dinh dưỡng trong quần xã.

*Từ khóa:* quần xã tuyến trùng, cửa sông Cỏ Chiên, tỉnh Bến Tre.