NEMATODE DIVERSITY PATTERNS AT DIFFERENT SPATIAL SCALES IN THE TIEN YEN ESTUARY, QUANG NINH PROVINCE, VIETNAM

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There is a strong relationship between spatial scale and the processes that influences diversity; and this relationship has been a fascinating topic to ecologists. The level of diversity within a particular habitat has been defined as alpha-diversity or local diversity (Whittaker, 1972). A comparison of diversity (or the change in species diversity) between these habitats is considered as beta-diversity (habitat turnover diversity); and a measure of the overall diversity within a large region is gamma diversity or regional diversity (Whittaker, 1972).

Understanding the biodiversity patterns requires the identification of the drivers that generate these patterns and of the biodiversity components that respond to these drivers. The drivers of alpha- and beta- diversities are different, as alpha-diversity is generally associated with high abundance and high resource availability, whereas beta-diversity is more sensitive to the heterogeneity of the habitat. Therefore, it is known that diversity varies with spatial scales in which an increase in scale could promote an increase in diversity due to providing more resources to species (Crawley & Harral, 2001). The spatial patterns of nematode communities on different horizontal scales have been extensively investigated in different estuaries from temperate regions (Boucher & Lambshead, 1995; Adão *et al.*, 2009; Alvesa *et al.*, 2009). However the effect of spatial scales on diversity of nematode assemblage in tropical region has been poorly studied.

In this study we investigated the spatial patterns of nematode communities on different horizontal scales in the Tien Yen Estuary. More specifically, we wanted to address the following question: what is the contribution of different spatial scales on the total nematode biodiversity for this area?

I. MATERIALS AND METHODS

Study area and sampling mapping

The Tien Yen estuary is located in the Tien Yen Bay and connected with the East Sea and encompassed by a system of barrier islands. In total, five intertidal sampling stations were established in August, 2013 around Dong Rui island based on a random strategy (Figure 1 and Table 1). The spatial scale of sampling was about 10 kilometre in total (shortest distance between two stations: 1 km; largest: 2.5 km).

At each station, triplicate samples were randomly taken within a $1m^2$ area using Perspex hand cores of 3.6 cm diameter and 30 cm height. The cores were pushed down into the sediment up to 10 cm deep. All samples were preserved in 4% neutralized formaldehyde (heated to 60-70^oC), gently stirred and kept in plastic containers for further processing.

Table 1

The codes and coordinates of sampling stations in the Tien Yen Estuary

No	Stations	Code	Coordinates
1	Ba Che	TY 1	21°13’16.68’’N/107°22’11.59”E
2	Doi Thinh	TY 2	21°14’10.92’’N/107°22’53.40”E
3	Ong Cong	TY 3	21°15’17.65’’N/107°23’7.39”E
4	R/A	TY 4	21°16’4.01’’N/107°23’43.69”E
5	Ha Thu	TY 5	21°15’15.20’’N/107°25’4.50”E

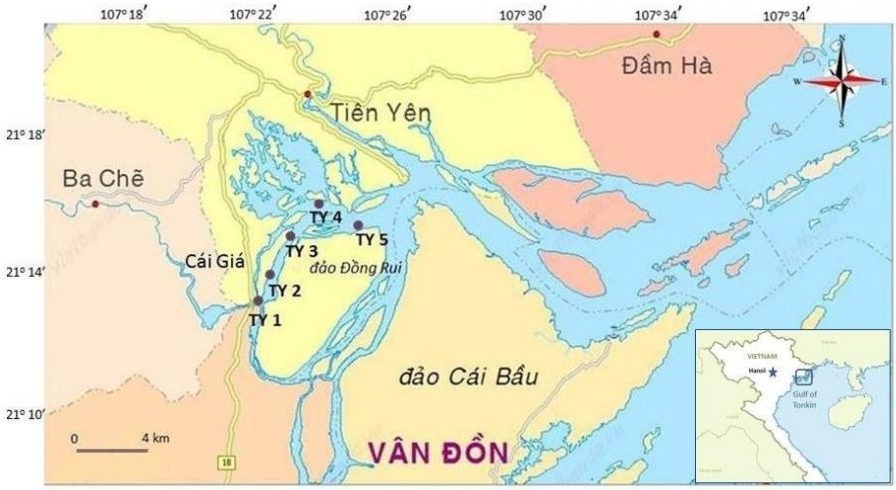

Figure 1: **The map of sampling stations in the Tien Yen Estuary**

Sample processing

Nematodes were extracted by decantation and Ludox extraction (d=1.18) which are based on the different density between nematodes and sediments. The extracted nematode was washed carefully with tap water, kept in FAA (Formalin Acid Acetic) solution in a suitable container and counted.

To subsample nematodes in samples, about 200 nematodes (or all if lower numbers occurred) were picked out randomly and transferred gradually to glycerine following the method of De Grisse (1969). The nematodes were arranged into a small drop of glycerine surrounding by a bee wax ring on a slide and covered with a cover glass.

Nematode taxonomic classification was classified according to Lorenzen (1994) and De Ley and Blaxter (2004). Nematodes were identified up to family, genus and species level following pictorial keys (Warwick *et al.*, 1998) and Nemys Database.

Data analysis

The statistical analyses such as ANOVA, MDS (Non-metric Multi-Dimensional Scaling analysis), Cluster analysis and ANOSIM (One way ANalysis Of SIMilarity) were done by the software PRIMER 6 and PERMANOVA+, an add-on package for PRIMER 6.

The biodiversity was measured at the three levels introduced by Whittaker (1972): alpha, beta, and gamma diversity. In this study, the gamma diversity was additively partitioned in one

alpha (within replicates) and two beta fractions (between replicates (β1) and between stations (β2)) to investigate the contribution of different samples and stations to the overall nematode biodiversity. The PARTITION v3.0 program with the reshuffling algorithm of the program to test whether the observed diversity components (alpha and beta) at each special scale could be obtained by a random distribution of individuals among samples at all hierarchical levels. The additive partitioning was also calculated using the Shannon-Wiener index (unequally weighting samples). A sample's weight is the number of individual that contains as a proportion of the total number of individuals in the dataset, thus lager samples gave more weight in determining alpha- and beta diversity.

II. RESULTS

1. Characterization of the environment

Table 2

Data on chemical-physical parameters in the Tien Yen Estuary

No	Code	Temp ^{o}C	pH	Turb (mg/l)	DO (mg/l)	NaCl (%)
1	**TY 1**	30.4	6.54	32	5	2.4
2	**TY 2**	30.8	6.89	21	3.9	2.4
3	**TY 3**	29.6	7.24	28	4.5	2.8
4	**TY 4**	30.1	7.27	31	2.6	2.9
5	**TY 5**	31.2	7.32	34	2.84	2.9

The seddimentation data of five stations of the Tien Yen Estuary was shown in table 2. The temperatures were marginally ranged between 29.6 and 31.2^{o}C. Turbidity (Turb) and dissolved oxygen (DO) varied slightly between stations, while salinity (NaCl) and pH increased from more inland (TY1 and TY2) to near the mouth (TY3, TY4 and TY5). Grain size fractions (Table 3) had similar profiles between stations which were dominated by silts except that TY1 was characterized by sands.

Table 3

Grain size fraction data (in %) based on the Udden/Wenthworth grade scale.

Median (µm)	TY1	TY2	TY3	TY4	TY5
>2000	0.37	5.01	0.13	0.03	0.37
1000-2000	0.56	6.79	1.59	0.27	0.37
500-1000	1.77	4.71	6.20	0.99	1.14
250-500	7.30	5.73	10.64	3.88	2.70
125-250	40.90	3.38	11.46	6.69	4.00
63-125	15.65	3.87	1.74	5.52	1.76
<63	33.44	70.51	68.23	82.63	89.66

2. Nematode community patterns

The nematode densities of all 15 samples from five stations are shown in Figure 2. The nematode densities per sample varied between 92 ind./ 10cm^2 in TY3.1 and 2330 ind./ 10cm^2 in TY2.3. The averaged nematode densities per station were highest in station TY2 with 1925 ± 356 ind./10cm^2, followed by station TY1 (916 ± 222 ind./ 10cm^2), TY5 (747 ± 434 ind./10cm^2), and TY4 (626 ± 636 ind./10cm^2) while the lowest density was found in station TY3 with only

254 ± 154 ind./10cm^2. Based on ANOVA, there were only significant differences in densities between station TY2 and the stations TY3, TY4 and TY5.

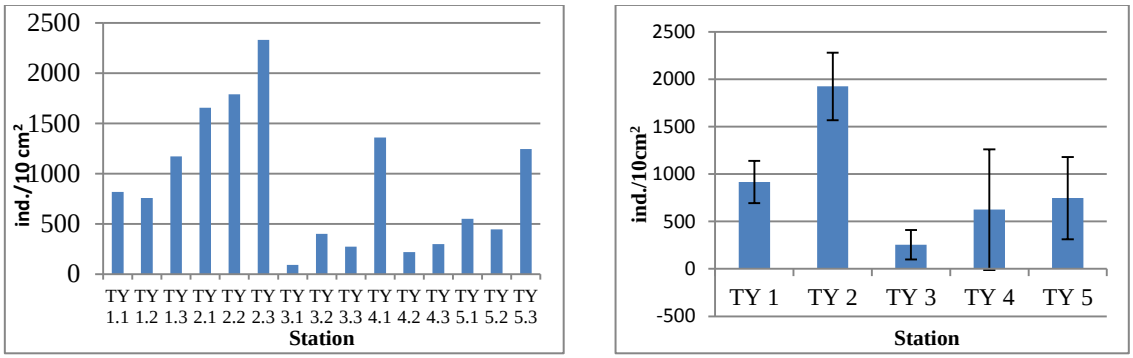

Figure 2: **Nematode densities (ind./10cm^2) of fifteen samples (A) and averaged nematode densities (mean +/- SD ind./10cm^2) of five stations (B) in the Tien Yen Estuary**

Cluster analysis and Multi-dimensional scaling (MDS)

The SIMPROF dendrogram which identified groups based on significant differences showed that all stations separated significantly (Figure 3). TY3 separated from the group of all other stations which further separated TY1 and TY2 from TY4 and TY5.

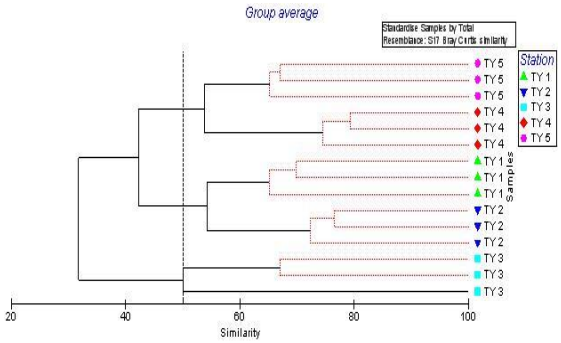

Figure 3: **The SIMPROF analysis of nematode communities at species level**

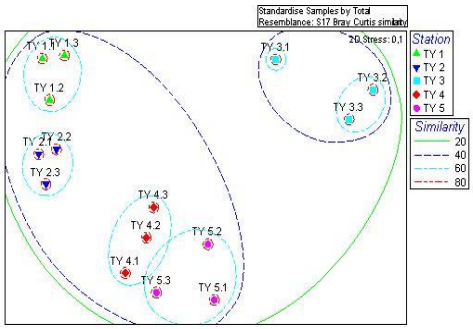

Figure 4: **The 2D-MDS plot for the nematode community patterns based on standardized densities data with overlying clusters at four similarity levels**

The 2D-MDS graphs depicted in Figure 4 displays the (dis) similarities between samples of different stations in the Tien Yen Estuary. All the samples of station TY3 were clearly separated from all other samples. ANOSIM analysis showed the significant difference between stations (R= 0.953, p= 0.001).

The increase in number of species was investigated in relation to the number of replicates per station as well as in relation to an increasing number of stations (Figure 5). For each of the scales (station and area) the species richness did not reach the asymptote, indicating high nematode richness in the area. Only station TY2 (with highest density of nematodes) showed an asymptote based on three replicates. This suggests that three replicates and five stations may be not enough to identify the full set of species in the area. Therefore, the true diversity can be higher than what was observed based on the present sampling strategy.

3. Different spatial scales of species richness

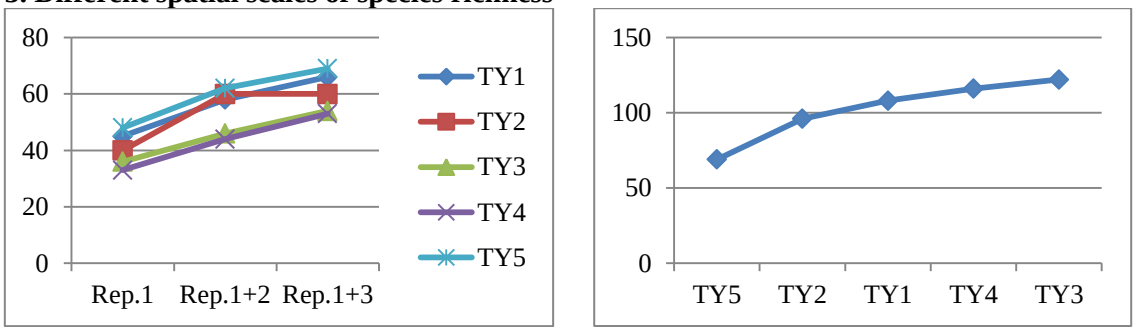

Figure 5. **Increase in number of nematode species at different spatial scale: per replicates within 5 station (left side) and per station for whole area (right side)**

4. The biodiversity of nematode assemblage at different levels (alpha, beta and gamma)

The biodiversity of the Tien Yen Estuary was measured at three levels (alpha, beta, and gamma diversity). Additive partitioning of species richness revealed that the beta fraction related to the difference between stations (β2) was the most important contributor to the total gamma diversity (50.5%). In addition, α-diversity was significantly lower than expected compared to the null model, while β-diversity was consistently higher than expected. Conversely, if additive partitioning was based on the Shannon-Wiener index, the alpha diversity was explaining the most important fraction (86.3%) (Figure 6).

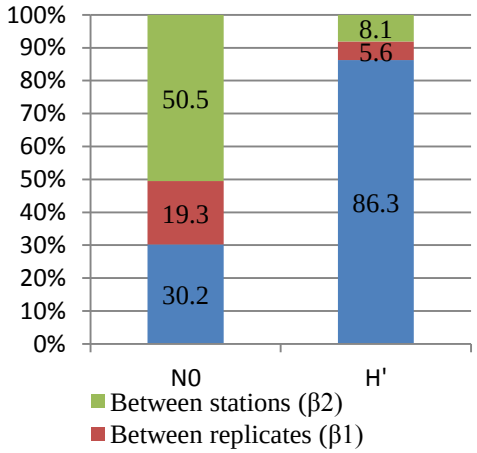

Figure 6. **Stacked columns depicting the composition of the gamma diversity and the proportion of alpha diversity and different beta diversity levels (within stations and between stations)**

DISCUSION

The spatial distributions of nematode composition visualized by the Multivariate analysis (MDS) showed a separation between stations. Particularly, station TY3 which is characterized by a younger mangrove forest and lowest nematode density clearly separated from all other samples. Higher order grouping of the stations indicated that different zones might be informally identified along the estuary: group 1, including station TY1 and TY2 which are located more inland; group 2, including only station TY3; and group 3, including TY4 and TY5 which is near the mouth. Notably, the salinity of the Tien Yen Estuary which slightly increased

from inland to near the mouth may explain the differences between stations. Thus, it can be noticed that the small change in environment can make a significant change in the nematode communities. However, more studies should be carried out to understand the environment characters to figure out their influences on nematode communities.

Although replicate or alpha diversity was generally lower than expected; species turnover along the estuary lead to much higher values for regional or gamma diversity. In the Tien Yen Estuary, despite the small differences in salinity and sediment characteristics, the beta diversity between different stations was high. The fact that mangroves may provide different environmental niches, for example the process of leaf litter decay (Gee & Somerfield, 1997), a succession of species associated with the decomposition, or the different levels of attraction for nematodes of different species of mangroves (Torres-Pratts & Schizas, 2007), can explain the high beta diversity between stations in Tien Yen. Therefore, it can be concluded that quantifying beta diversity as variation is crucial in detecting the effect of patchiness, and providing a comprehensive view of natural or human-driven changes in the multivariate structure of nematode assemblages.

In addition, the total nematode species biodiversity of the Tien Yen Estuary based on three replicates per station and five stations in the area is not yet fully estimated as illustrated by the cumulative curves at two different scales (replicate and station). The additive partitioning analysis indicates that the larger scale (between stations) explained most of the total biodiversity of the area. So even at small local scales of only 10 km in a mangrove intertidal area, more samples per station, and more stations per estuary will ultimately lead to a higher biodiversity estimates.

III. CONCLUSION

Despite the small distances and the similar environment (mangrove system and intertidal mudflat), the nematode assemblages in the Tien Yen Estuary were characterized by high density and the significant differences between stations. It indicates that the small changes in environmental characters and the patchiness of mangrove forest may play the important roles in regulating nematode community structures. The additive partitioning analysis showed that the larger scale (between stations) explained most of the total biodiversity of the area. The link between spatial scales, environmental parameters and nematode community structure need to be further investigated.

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MÔ HÌNH ĐA DẠNG TUYẾN TRÙNG VỚI PHẠM VI KHÔNG GIAN KHÁC NHAU TẠI CỬA SÔNG TIÊN YÊN, TỈNH QUẢNG NINH, VIỆT NAM

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TÓM TẮT

Các mẫu tuyến trùng biển tại cửa sông Tiên Yên được thu vào tháng 8 năm 2013 để tìm hiểu về đa dạng sinh học của chúng với 5 điểm cắt khác nhau dọc theo 10 km cửa sông. Kết quả phân tích định lượng cho thấy mật độ của tuyến trùng dao động lớn từ 92 đến 2330 cá thể/10cm^2. Phân tích MDS cho thấy tất cả các mẫu tại TY3 nằm riêng biệt so với các mẫu tại các địa điểm khác. Sự tăng số lượng các loài khi tăng số lượng mẫu thu chỉ ra rằng sự đa dạng loài tuyến trùng tại cửa sông Tiên Yên chưa được đánh giá đúng và có thể còn cao hơn nếu tăng số lượng lần lặp và tăng số địa điểm thu mẫu. Kết quả áp dụng phương pháp cộng từng phần độ giàu có của loài chỉ ra rằng đa dạng sinh học beta giữa các địa điểm thu mẫu khác nhau quyết định phần lớn đa dạng sinh học gamma của khu vực nghiên cứu.