BIODIVERSITY AND DISTRIBUTION PATTERNS OF FREE-LIVING NEMATODE COMMUNITIES IN BA LAI RIVER, BEN TRE PROVINCE

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Abstract. Nematode communities in Ba Lai river, Ben Tre province were investigated in September of 2015 (eight stations from estuary to upstream). The results showed that the nematode communities have characterized by high density and biodiversity, providing useful information of nematode assemblages in freshwater habitas, in particular inland river. Also the results indicated that the distribution of nematode communities in Ba Lai river was strongly discriminated between in and outside of dam with two groups. The Ba Lai dam may be reasons for cause the nematodes distribution discontinuity. Present study is a pioneering attempt to record the impact of the dam on benthic - invertebrate in Viet Nam.

Keywords: Ba Lai dam, Ben Tre province, biodiversity, dam effect, distribution, nematode communities.

Classification numbers: 3.1.2; 3.4.2

1. INTRODUCTION

Currently, Mekong river is internationally recognised as the second most biodiversity river in the world (after the Amazon river) [1, 2]. There are current estimated at least 1,200 species of fish, possibly reaching 1,700 and high diversity is also exhibited by other aquatic animal and plant communities [3]. While studies and reviews on fish species diversity in Mekong river are increasingly common, to date, accuracy of other aquatic animal and plant groups diversity in the Mekong river estimation have not yet been revealed, despite the fact that 30% of the production from the fishery comes from non - fish sources [3]. Other aquatic animal have not been used directly as food and goods produced from natural resources but its (especially benthic fauna communities) have the potential to used as good biological monitoring for the ecological quality status of sediment [4, 5, 6]. Of all the benthic fauna groups, macrofauna (> 0.5 mm in size fraction) have been the organisms most frequently used as a longer - term biological monitoring

program in the Mekong river [7]. In recent years, meiofauna (ranged from 38 µm to less than 0.5 mm in size fraction) are have been used successfully for detecting sediment condition [8, 9, 10, 11]. The study by Ngo et al. (2016) is notable in that nematodes (domination numerically in the meiofauna communities) can be considered as a good candidate organisms for environmental monitoring [12]. However, in Vietnam, most of research on nematode communities were conducted in the coastal area: Ha Long Bay [13, 14], Nha Trang Bay [15], Mekong estuaries [12, 16], Can Gio and Ca Mau mangrove forest [17, 18]. Few studies have been carried out for nematode communities in freshwater habitats, such as Sai Gon river [19], Cau, Day, Cam and Nhue river [20]. Overall, basic information regarding the composition and biodiversity of nematode communities is still largely lacking for freshwater area, in particular inland river.

Ba Lai river is one of four branches of the Tien river which is the main northern distributary of the Mekong through Vietnam. In 2002, a irrigation dam (called Ba Lai dam) was built across the river to stop the infiltration of salt water and conserve fresh water for 88,500 hectares of farmland in Ben Tre province. Since then, several studies have warned that a lightly disturbed in Ba Lai's environmental sediment quality and river - estuarine has already been deposited. Ba Lai dam could be one of the major reasons for the disturbance and deposition of this river [21].

As mentioned above, the purpose of this research was to: (i) to survey of nematode assemblages in the Ba Lai river (from estuary to upstream) by assessing their composition, density and diversity and also (ii) determine effects of Ba Lai dam on the distribution of nematode communities.

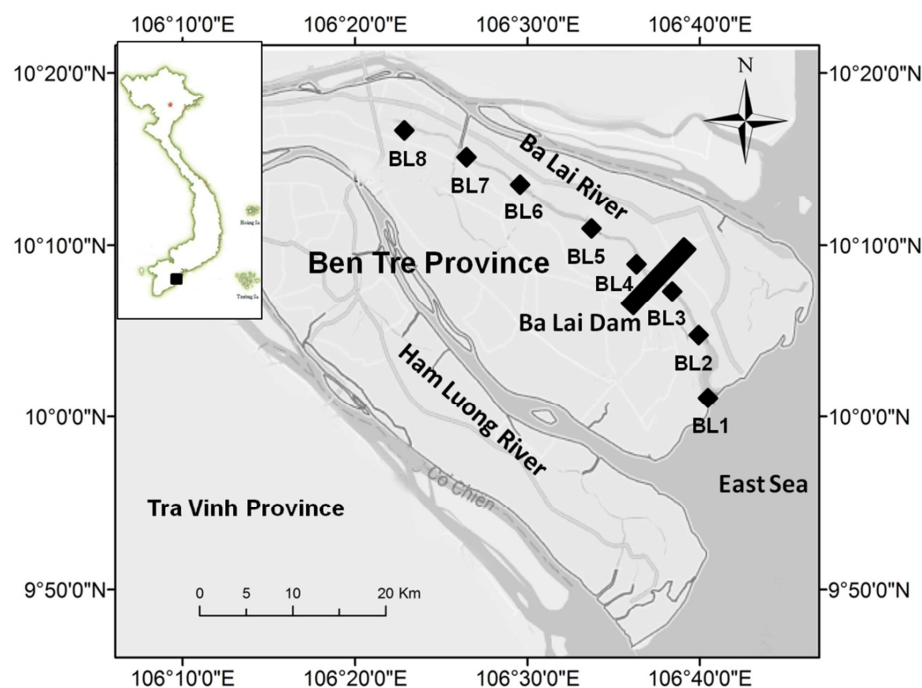## 2. MATERIALS AND METHODS

2.1. Sampling area and field operations

Figure 1. Sampling stations in Ba Lai river, Ben Tre province.

Table 1. Station characteristics.

Stations	Latitude	Longitude
BL1	10°1'52.61"N	106°41'23.65"E
BL2	10°5'19.96"N	106°41'6.25"E
BL3	10°8'28.69"N	106°37'58.45"E
BL4	10°8'48.09"N	106°37'37.86"E
BL5	10°11'37.71"N	106°34'10.46"E
BL6	10°13'28.04"N	106°30'24.00"E
BL7	10°15'47.23"N	106°26'36.73"E
BL8	10°17'16.27"N	106°23'20.41"E

In September of 2015, nematode samples were collected at eight low intertidal sampling stations (BL1, BL2, BL3, BL4, BL5, BL6, BL7 and BL8), located along the Ba Lai river, Ben Tre province (Fig. 1). Station characteristics are given in Table 1. Three replicates were taken at each station and samples were collected using cores of 3.5 cm in diameter (10 cm^2 surface area) and 30 cm in length. The cores were pushed down into the soft - bottom up to 10 cm deep. The samples were all fixed in a 7 % formaldehyde solution at 60 °C and gently stirred before transportation to the laboratory of Department of Environmental Management and Technology, Institute of Tropical Biology.

2.2. Laboratory techniques

In the laboratory, nematode samples were sieved through a 38 µm mesh and extracted by flotation technique with Ludox - TM50 solution (specific gravity of 1.18) [22]. Samples were discolored with 3 – 5 mL Rose Bengal solution (1 %). All individual numbers were counted under a stereomicroscope, two hundred nematodes were used for making slides with glycerine + ethanol solution [23] before identification. Nematodes were identified to genus level by using the following taxonomy literature: Platt & Warwick (1983, 1988) [24, 25], Warwick et al. (1988) [26], Zullini (2005) [27], Nguyen (2007) [28] as well as the NeMys online identification key [29].

2.3. Data analyses

Data of nematode communities was processed in Microsoft Excel 2007 to analyze the composition, species richness (S) and densities. The software Primer v.6.1.6 was used to measure the diversity indices such as Margalef diversity (d) [30], Shannon - Wiener diversity

(H'(log$_2$)) [31] and Hill indices (N$_1$, N$_2$ and N$_{inf}$) [32]. All the data per site were presented as average ± standard deviation.

The distribution of the nematode communities were further explored by using MDS analysis (non - metric multi - dimensional scaling) [33]. The data were transformed based Square root. The MDS was used to produce 2D graphs base on the Bray – Curtis similarity index in order to visualize similarity patterns. The SIMPROF method (SIMilarity PROfile) was used to test for significant different station groups.

The significant differences in the univariate measures between sampling stations were tested by the parametric test one - way ANOVA or the non - parametric Kruskal - Wallis test, using the software STATISTICA 7.0. In order to test the assumption of homogeneity of variances, the Levene's test was used. Tukey HSD multiple comparison tests were used when significant differences were detected (p < 0.05). Data were log - transformed prior to analysis when assumptions were not fulfilled.

3. RESULTS AND DISCUSSION

3.1. The composition of nematode communities in Ba Lai river

The nematode assemblages in Ba Lai river, Ben Tre province consisted of 118 genera belonging to 45 families, 12 orders such as Araeolaimida, Chromadorida, Desmodorida, Desmoscolecida, Dorylaimida, Enoplida, Monhysterida, Mononchida, Plectida, Rhabditida, Triplonchida and Tylenchida. During field sampling period, most individuals belong to three dominant orders: Monhysterida (39.50% of total abundance), Araeolaimida (22.65 %) and Enoplida (15.77 %). The subdominant orders were Chromadorida, Plectida, Desmodorida, Mononchida and Dorylaimida (6.71, 4.84, 3.57, 2.92 and 2.79 %, respectively). The percentage of the remaining orders ranged from 0.05 % to 0.65 % (Fig. 2).
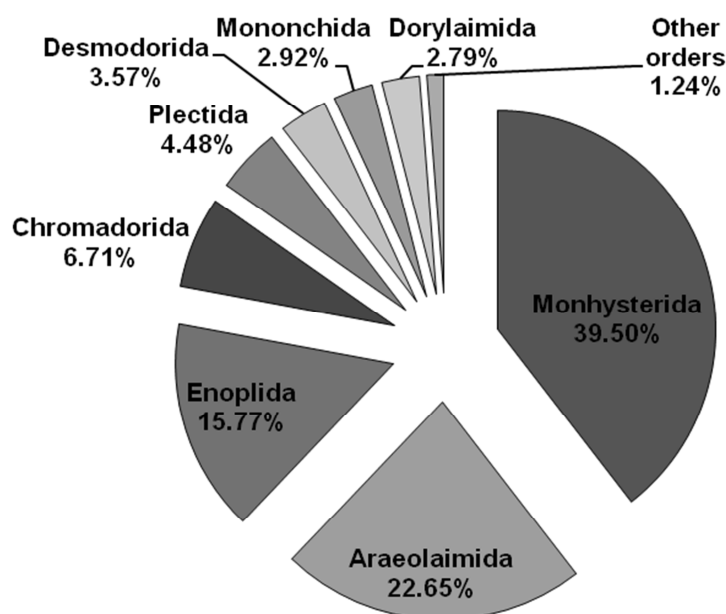

Figure 2. Nematode orders composition.

3.2. Nematodes density and diversity

Overall, the average density (inds/10cm^2) of three stations outside the dam (BL1, 2 and 3) were considerably higher than the remaining stations (BL4, 5, 6, 7 and 8) appeared inside the dam. More specifically, BL1 was expressed as the highest density (4,580.33 ± 1,532.95) followed by BL2 (3,435.33 ± 469.98) and BL3 (1,465.67 ± 393.76). By contrast, all stations inside the dam were showed low density with a mean annual density ranged from 83.00 ± 16.98 (BL5) to 355.67 ± 43.41 (BL4) (Fig. 3A).
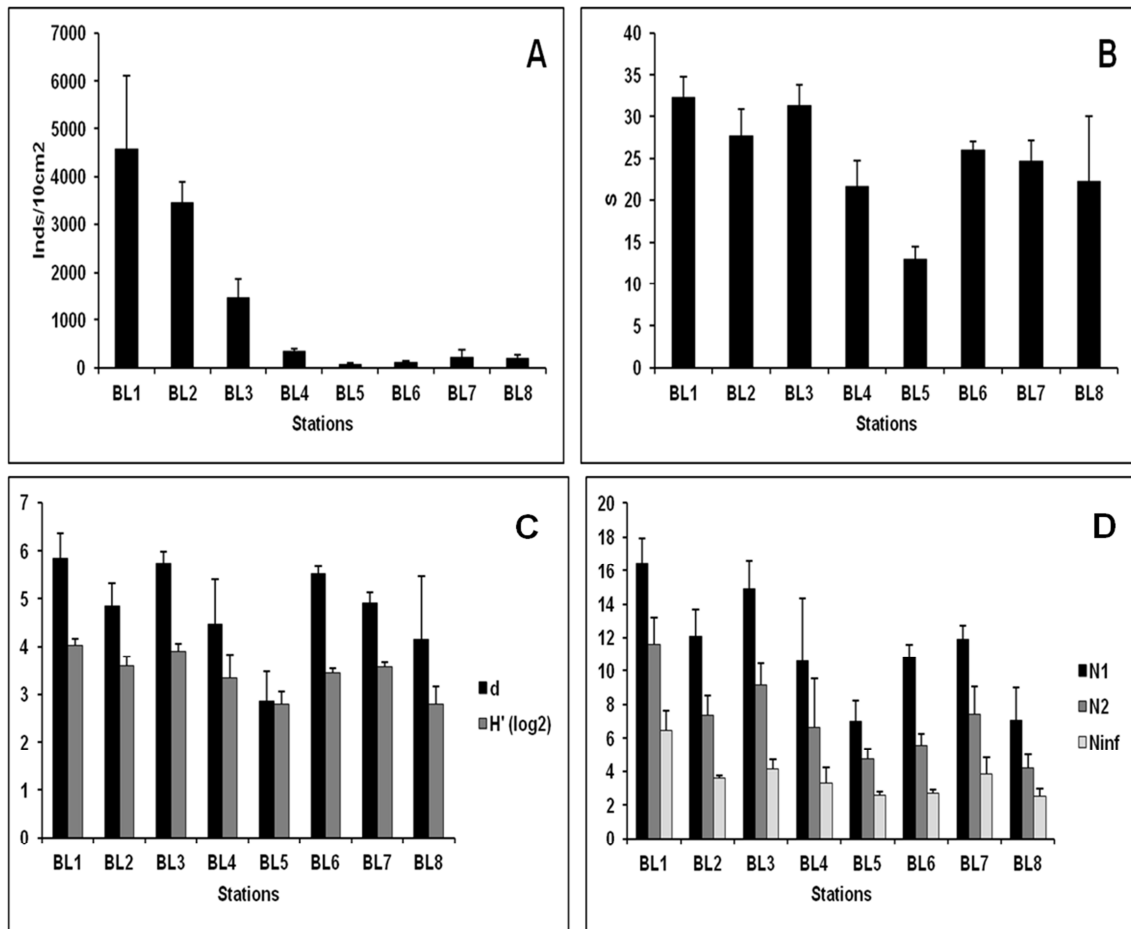

Figure 3. Characteristics of nematode communities. (A) Densities, (B) Species richness (S), (C) Margalef diversity (d), Shannon - Wiener diversity (H'(log$_2$)), (D) Hill indices (N1, N2 and Ninf).

As the same to results of densities, the average diversity indices such as genera richness (S), Margalef diversity (d), Shanon - Wiener (H') and Hill indices (N_1, N_2 and N_{inf}) of stations outside the dam were often higher than the stations where appeared inside the dam. More specifically, the species richness (S) ranged from 13.00 ± 1.41 to 32.33 ± 2.52. BL1, 3, 2 were the station that presented the highest species richness (32.33 ± 2.52; 31.33 ± 2.52; 27.67 ± 3.21, respectively), whereas lowest values was found in BL5 (13.00 ± 1.41) (Fig. 3B). The Margalef index measured from 2.86 ± 0.61 to 5.86 ± 0.49 and the Shanon - Wiener index ranged from 2.79 ± 0.37 to 4.03 ± 0.49. The Hill indices, indicated average values ranging

between 6.99 ± 1.25 - 16.43 ± 1.49 for N_1, between 4.18 ± 0.78 - 11.64 ± 1.57 for N_2, and between 2.50 ± 0.46 - 6.45 ± 1.16 for N_{inf}. In general, station BL1 was always expressed as the highest diversity values, whereas station BL5 (d, H', N_1), BL8 (N_2, N_{inf}) showed lowest diversity index through field sampling period (Fig. 3C,D).

The results of an ANOVA/ Kruskal – Wallis showed significant differences between stations for densities and all indices. A Post Hoc comparison showed significant differences between sampling stations were found, such as BL1 with other stations (except for BL2), BL2 with the remaining stations within densities (Table 2).

Table 2. The results of an ANOVA / Kruskal – Wallis and Post hoc comparison / Multiple comparison for characteristics of nematode communities.

ANOVA		
Variables	p value	Post hoc test
Densities	< 0.001	BL1 ≠ BL3, 4, 5, 6, 7, 8; BL2 ≠ BL3, 4, 5, 6, 7, 8
S	< 0.001	BL1 ≠ BL4, 5; BL5 ≠ BL2, 3, 6, 7
d	0.003	BL5 ≠ BL1, 3, 6
N_1	< 0.001	BL1 ≠ BL4, 5, 6, 8; BL3 ≠ BL5, 8
N_2	< 0.001	BL1 ≠ BL4, 5, 6, 8; BL3 ≠ BL8
N_{inf}	< 0.001	BL1 ≠ BL2, 3, 4, 5, 6, 7, 8
Kruskal – Wallis		
Variables	p value	Multiple comparison of mean rank for all stations
H'	0.014	BL1 ≠ BL8

Although the studies on nematode communities have been widely conducted in Vietnam, the knowledge on nematodes in freshwater habitats remains limited. Further more, the study by Ngo et al. (2016) have demonstrated that nematode assemblages seems to give a good indication of the environmental monitoring [12]. Therefore, the results of this study provides a base line for further research on using nematodes for determining the ecological quality status of sediment in Ba Lai river gradually disturbed.

Comparing our results with other studies has been difficult, mainly because of uncertainties in exploring nematode communities in freshwater habitats. Therefore, it can be compared with other comparable studies in the river estuary around the world. In general, the nematode communities in Ba Lai river, Ben Tre province have been recorded in high density and diversity. More specifically, the results showed nematode densities ranging from 83.00 ± 16.98 to 355.67 ± 43.41 (stations inside the dam) and from $1,465.67 \pm 393.76$ to $4,580.33 \pm 1,532.95$ (inds/$10cm^2$) (stations outside the dam). The density of stations where appeared outside the dam were higher than observed in several studies such as by Wu et al. (2010) in Beigan River Basin (ranged from 75 to 2,474 inds/10 cm^2) [34] , by Ngo et al. (2010) in Mekong estuarine system (683.7 ± 374.4 to $2,099 \pm 100$ in Ba Lai estuary and from 454 ± 289.9 to $3,137.7 \pm 337.1$ inds/$10cm^2$ in other estuaries) [16], by Alves et al. (2009) in the Mira estuary (12.44 ± 3.91 - $2,234 \pm 400$ inds/$10cm^2$) and in the Mondego estuary (38.93 ± 5.29 – $1,323.10 \pm 389.52$ inds/$10cm^2$) [35]. More recently, the density of stations intside the dam were within the ranges

found by Ngo et al. (2017) in Sai Gon river (ranged between 13.3 ± 2.9 to 408.7 ± 142.5 in the dry season) but our estimates were lower than the nematode densities in the wet season (from 58 ± 41.9 to $1,649.7 \pm 1,462$ inds/10cm^2) [19]. Meiofaunal density are often increasing with increasing salinity [36]. It could be one of the major reasons why the density of stations outside the dam were considerably higher than the remaining stations appeared inside the dam.

The nematode communities in Ba Lai river are not only characterized by high density, but by a high diversity with 118 genera belonging to 45 families, 12 orders. The nematode diversity expressed as a number of genera were lower than in the eight estuaries of the Mekong river, with 135 genera [16] or the 120 genera in five European estuaries [36]. Howere, the nematode diversity expressed as a number of families, our estimates were higher than Mekong estuarine and European estuaries system (with 35 families for each locations). Addition, the number of genera and families of nematode assemblages in the Ba Lai river were higher than the genera reported in the Mondego and Mira estuaries, with 45 genera belonging to 19 families [37], but also in Sai Gon river (88 genera, 42 families, 10 orders in dry and 102 genera, 45 families, 10 orders in wet season) as published by Ngo et al. (2017) [19].

Regarding nematode diversity indices, the Shannon diversity of nematode communities in Ba Lai river (2.79 ± 0.37 to 4.03 ± 0.49) were considerably higher than observed in Sai Gon river, with ranged from 0.62 ± 0.67 to 3.43 ± 0.17 [19]. However, the Margalef and Shanon - wiener index in this study were very similar to the more recently observed by Ngo et al. (2010) in Mekong estuarine system, with the Margalef index ranged from 2.64 ± 0.64 to 5.99 ± 1.06 and the Shanon - Wiener index varied between 1.83 ± 0.59 to 4 ± 0.17 [16].

3.3. Exploring the distribution of nematode communities in Ba Lai river by using SIMPROF and MDS

The SIMPROF based on relative nematode abundances provided two statistically significant groups at the level of 30%, in which one is further subdivided at the level of 40% in three subgroups. Also one outlying stations were identified, not classified in one of the three subgroups (Fig. 4A). The first group includes all stations appeared inside the dam (except for BL4.2). The second main group consisted of three subgroups. The subgroup A including the stations BL1.1, 1.2, 1.3 and the subgroup B including the stations BL2.1, 2.2, 2.3; while the subgroup C including the stations BL3.1, 3.2, 3.3. All stations of second group were distributed outside the dam. Figure 4B shows the MDS results with the four groups encircled as they were identified by SIMPROF.

3.4. The Ba Lai dam may be reasons for cause the nematode distribution discontinuity

In recent years, many studies have demonstrated that the negative impacts of dams on the environment such as the hydrological system, river dynamics diversion and also alteration in bio - geochemical cycling [38, 39]. Addition, dam construction and operation can make a substantial contribution to ecosystem hazards, things like loss of forests and wildlife habitats, loss of aquatic diversity, including upstream and downstream fisheries, interruption of fish migration and loss of the services of downstream wetlands, riverine and estuarine ecosystems [40, 41]. Ecologically, dams have the ability to change river's physicochemical characteristics that are the major reasons for change in the aquatic communities structure [42]. Clearly, nematode communities distribution was strongly discriminated between in and outside of dam. Unfortunate, there are lack of information about environmental conditions in - and outside of the dam in this study.
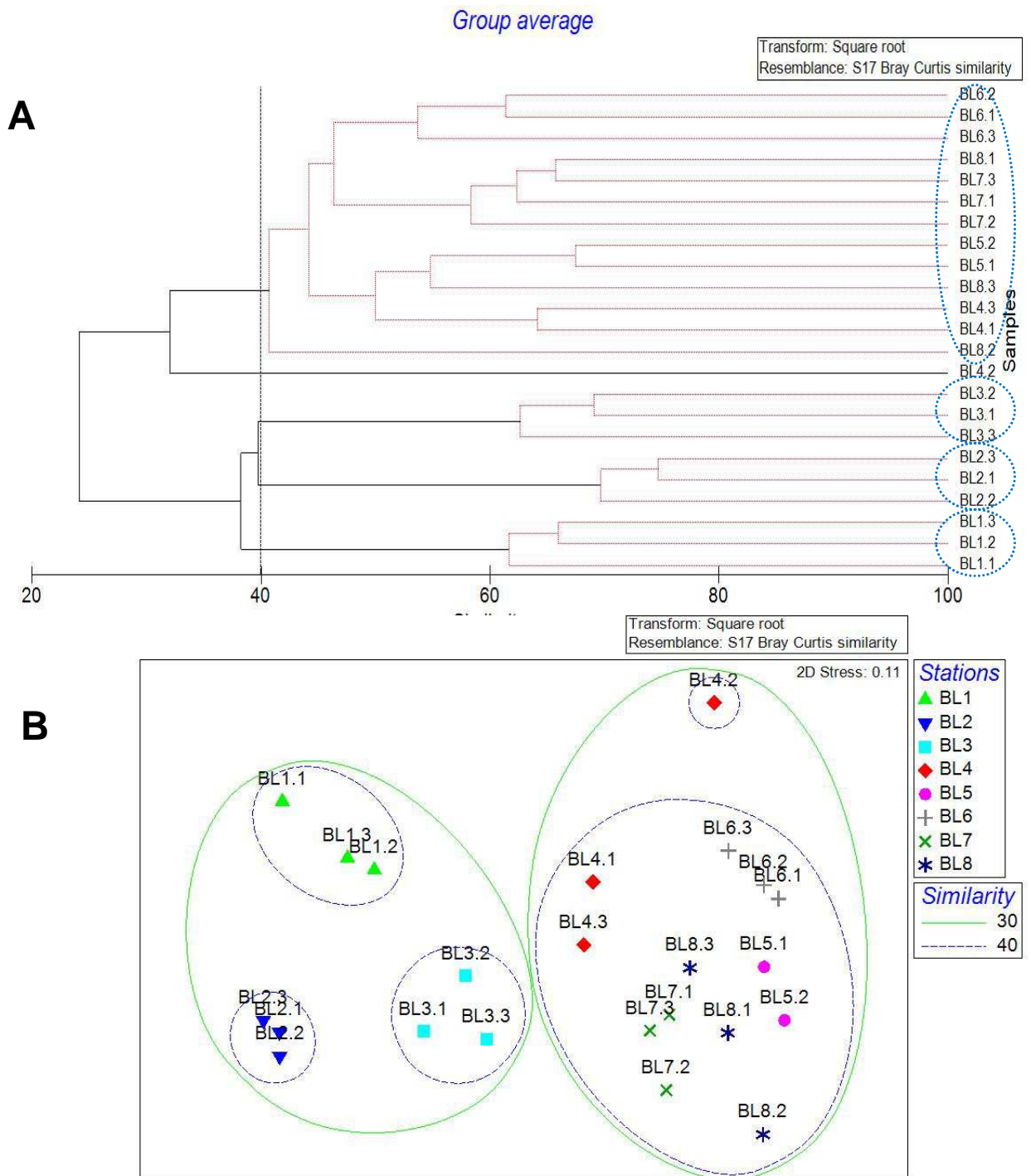
Figure 4. Patterns of nematode communities distribution. (A) The SIMPROF analysis of nematode assemblages in Ba Lai river, (B) The MDS based on Square root transformed densities with overlying clusters at two similarity levels.

Together, these findings show that the Ba Lai dam may lead to nematode distribution discontinuity. Similar results have been reported by Mueller et al. (2011), patterns of macroinvertebrate communities structure strongly discriminated between in and outside of dam, which probably results from different flow conditions in - and outside of dam [42]. More precise estimates of the impacts of Ba Lai dam will become possible when we have a stduy compared the abiotic characteristics in and outside of dam as well as their effects on the other aquatic groups such as periphyton, aquatic macrophytes, macroinvertebrate and fish.

4. CONCLUSION

The nematode communities in Ba Lai river, Ben Tre provinve are not only characterized by high density, but by a high diversity. Supporting the base line for further research on using nematodes for determining the ecological quality status of sediment in Ba Lai river gradually disturbed. Also the nematode communities distribution was strongly discriminated between in and outside of dam with two groups. More specifically, the first group includes all stations appeared inside the dam and the second main group (distributed outside the dam) consisted of three subgroups. The Ba Lai dam may be reasons for cause the nematode distribution discontinuity. Present study is a pioneering attempt to record the impact of the dam on benthic - invertebrate in Vietnam.

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