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Local and Temporal Distributions and a new Dispersal Phenomenon of the Freshwater Mussel *Nitia Teretiuscula* (Bivalvia: Unionidae) in the River Nile at Sohag Governorate, Egypt

Fathey E. Soliman, Tarek G. Ismail, Alaa Y. Moustafa, Olfat Abdelsaleheen[*]

Zoology Department, Faculty of Science, Sohag University, EGYPT.

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Abstract: The unionid freshwater mussel *Nitia teretiuscula* (Philippi, 1847) was collected from the River Nile at Sohag Governorate. Both the spatial and temporal distributions were investigated using a systematic sampling technique. In addition, the inter-relationships between the abundance of the studied species and some measured environmental factors was examined. The abundance of the mussel fluctuated during most of the year and showed clear favoring for the shallow area near the bank of the river. The organic content of the sediment was the most effective environmental factor on the mussel distribution. The present species showed also a new dispersal phenomenon that is recorded for the first time in family Unionidae. The individuals release many gelatinous structures, named embryophores, that possess many cone-shaped arms that help in carrying early stages away by water currents.

Keywords: *Nitia teretiuscula*, River Nile, embryophore, local and temporal distribution, dispersal phenomenon.

1 Introduction

Family Unionidae is one of the largest freshwater bivalves that are usually found with high local densities [1,2]. Adult unionid bivalves live partially buried in sediments [3] and have slow active movement to few meters [4]. Unionids have a characteristic reproductive behavior including internal fertilization which occurs in female's gill chambers. The latter form brood chambers, known as marsupia, brood the developing glochidia [5] and consider as the source of calcium for the glochidial-shell formation [6].

The dispersal is critical for freshwater mussels, it plays a crucial role in the distribution of these mussels and might result in, increasing or decreasing their bed densities [1]. These densities reflect the mussels significant role in freshwater ecosystems through, improving habitat, transporting nutrients and energy to the benthic organisms by filtering activity [7].

Larval dispersal of unionids occurs by three strategies, first, glochidia larvae adhere to the gills or fins of fishes after releasing into the water [8]. Second, it acts as a parasite on some amphibians [9]. While, the third strategy, larvae of a few unionid species may directly develop inside the parent gills [10]. The glochidium penetrates the epithelial cells of the host and forms a cyst [11]. The parasitic period ranging from days to months and considered as larval dispersal strategy through the aquatic ecosystems, especially when passive dispersal is poor as a result of limited water currents [2,12]. The higher dispersal abilities of mussels, and hence distribution, show stronger association with the presence of host fish or local environmental factors than do the mussels with low dispersal abilities [2].

The spatio-temporal distribution pattern of unionids and the timing of emergence on a local scale have been limited by several factors, such as increases in temperature, reproductive activities, day length, flooding events, substrate structure and level of physical disturbance [13,14].

The present work aimed to study the local and temporal distribution of *N. teretiuscula* and its dispersal in the River Nile and to examine the inter-relationships between the abundance of the species and some prevailed environmental factors.

2 Material and Methods

2.1 Identification of Studied Species

[*]Corresponding author e-mail: olfat.tharwat@science.sohag.edu.eg

The present studied species, *Nitia teretiuscula* was identified by Dr Kevin S. Cummings, Illinois Natural History Survey, USA and through the mussel project (http://mussel-project.uwsp.edu) which was hosted by The University of Wisconsin-Stevens Point and funded by The National Science Foundation.

2.2 Study Site

A site was selected for investigation of the freshwater mussel, *Nitia teretiuscula*. This site is located at the western bank of the River Nile at Bani-Helal village, EL-Maragha district (26° 43′ 30″ N and 31° 35′ 42″ E) about 25 Km to the north of Sohag city (Fig.1). The river bank at the collecting site is an agricultural area contains few trees and short grasses. The water currents at the studied area differ seasonally and ranged from slow to moderate. The water plant, *Paspalum distichum*, disperses in large parts of the studied area, near the river bank and grow up above the water surface with different lengths that range from about 0.5 to 2 ms. Moderate numbers of the Nile common fish, *Oreochromis niloticus*, were observed moving around the studied area and hiding among stems of *Paspalum distichum.* The studied area subjects to habitat loss and destruction through anthropogenic activities as fishing that was clearly noticed in the area. In addition to that, the area faces pollution via drainage of irrigation water loaded with fertilizers and pesticides from the nearby cultivated areas.

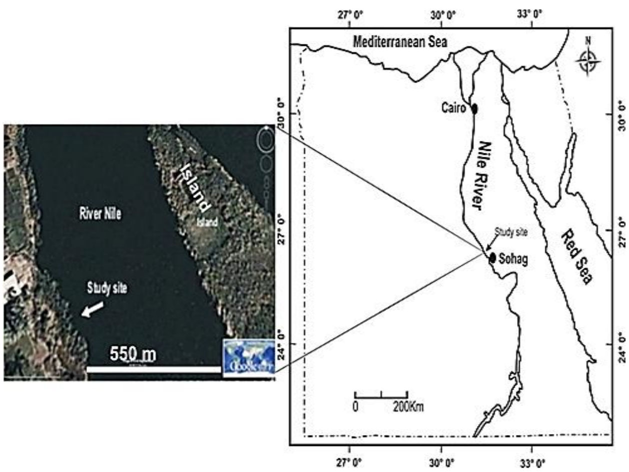

Fig. 1: Egypt map showing the location of studied site. To the left a Google earth map showing the Nile River bank where the specimens were collected.

2.3 Sampling Plan

To evaluate the abundance, distribution and population parameters of freshwater mussel in the study site, a sampling quadrate technique was applied to collect freshwater mussels during the period, January, 2014 to December, 2014. Five lines were set perpendicular to the river bank at 5- meter intervals (labelled A, B, C, D and E), and another 3 lines parallel to the bank at 1- meter intervals representing three water depths, shallow, middle and deep lines (Fig. 2).

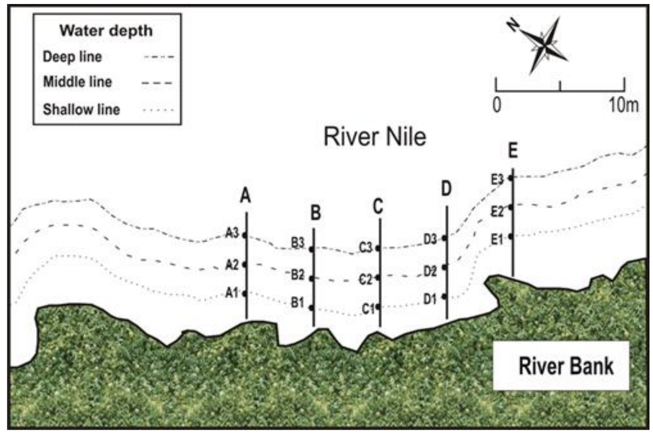

Fig. 2: A contour map of the studied site at the western bank of the River Nile showing the sampling plan.

The cross points (15 points, A1 to A3, B1 to B3, C1 to C3, D1 to D3 and E1 to E3] were determined as the sampling points. The water depth (distance from water surface to bottom) was monthly measured, since it depends on changes in water elevations (rise or fall) that depend on the water flow coming from High Dam. Monthly sampling was carried out during the daytime using a 25×25×10 cm iron quadrate (total volume= 6250 cm^3). In the field, contents of each quadrate were sieved using a 0.2 mm mesh sieve to collect large and small mussels above this size. The specimens of each quadrate were immersed in a small water tank during counting and observations' recording, then they were returned to the same sampling point from which they were collected. On the other hand, some large individuals were collected and carried to the laboratory for rearing purposes.

2.4 Environmental Factors

Air, water and surface sediment temperatures and hydrogen ion concentration (pH) were recorded monthly during period of investigation using thermometers and a digital pH meter (HANNA model 211). Also, sediment sampling was carried out seasonally, since large changes in sediment structure were not expected because of slow water currents in the collecting area. In addition to that, the studied area was characterized by the presence of a water plant in most months of the year which makes the sediment sampling difficult. Therefore, three sediment samples were carried out during January, April and October 2014. Surface sediment samples were collected using polyvinyl chloride (PVC) tube of about 5 cm diameter and 10 cm height.

In each season, 15 sediment samples from the 15 sampling points were brought back to the laboratory. The sediment samples were dried at 100° C for about 2 hours and crushed. From each sediment sample, 10 g were taken and sieved with six sieves having different diameters (0.6, 0.5, 0.4, 0.2, 0.1, 0.01 mm) to obtain the different sizes of sediment fractions [15]. The sediment fractions were carefully collected on separate sheets of paper and their weights were recorded. The weight of each sediment fraction was expressed as a percentage of the total sediment dry weight [15], as follows:

The Percentage of the sediment fraction = $\frac{\text{weight of the dried sediment fraction}}{\text{weight of dried sediment sample (10g)}}$ x100

2.5 Determination of the Percentage of Sediment Organic Content

To determine the seasonally organic content in the sediment, the previous samples of sediment were used and the procedure of Williams [1987] was applied. The previous dry sediment samples were grinded in a pestle and mortar. Then the sediment samples were poured in a preweighted crucible and their weights recorded. The sediment samples were heated to the red case in the crucibles over a Bunsen flame for 15 minutes to incinerate all the organic material. The carbon deposits were wiped off. After 10 minutes the crucibles were reweighted, and their weights were subtracted. The weights of burned sediments were subtracted from the weight of the sediments before burning to give the weight of organic content, as following:

The Percentage of organic content in dry sediment=$\frac{\textbf{weight loss in burnt sediment}}{\textbf{weight of dry sediment}}$ X 100

2.6 Laboratory Investigation

During rearing mussels for reproductive investigation [16], the females were observed releasing large numbers of mucous structure (embryophores). The embryophores were microscopically examined, counted and photographed using 8 Mega Pixels Digital Camera (Canon, power shot A590) attached to a microscope (Optika, B-500Tph). Also, some of the embryophores were fixed in 4% glutaraldehyde for Scanning Electron microscopy. These specimens were examined under JSM 5400 LV scanning electron microscope (SEM) at the Faculty of Science, Assuit University.

2.7 Statistical Analysis

Statistically, ANOVA test and Person's correlation coefficient were applied to investigate the effect and the relationships of the measured environmental factors on/with population density. Also, the relationship between the number of arms and number of embryos was examined by the linear regression analysis using the logarithmic equation, log Y=log a + b log X, where Y and X are the number of embryos and number of arms, respectively. The degree of association between variables was calculated by the determination coefficient (R2).

3 Results and Discussion

3.1 Results

3.1.1 Air, Water and Surface Sediment Temperatures

Figure (3) shows the monthly changes in air, water and sediment temperatures at the studied site. Generally, the fluctuations in air, water and sediment temperatures during the sampling period showed almost the same trend. Air temperatures recorded the highest values followed by water and sediment, respectively. The values of the three measured temperatures start to increase gradually from January, reaching their highest peak in June, followed by a gradual decrease till December.

3.1.2 The Hydrogen Ion Concentration

The values of the hydrogen ion concentration fluctuated during the sampling period and ranged between 7.1 and 8 in January and June, (Fig. 3). The pattern of the pH curve coincided with those of the three measured temperature curves. Generally, the three measured temperatures showed no effect on temporal distribution of *N. teretiuscula* (air temp., F=0.16, P=0.45, water temp., F=1.8, P=0.20, sediment temp., F=2.8, P=0.11), however, pH was significantly do (F=17.9, P=0.001).

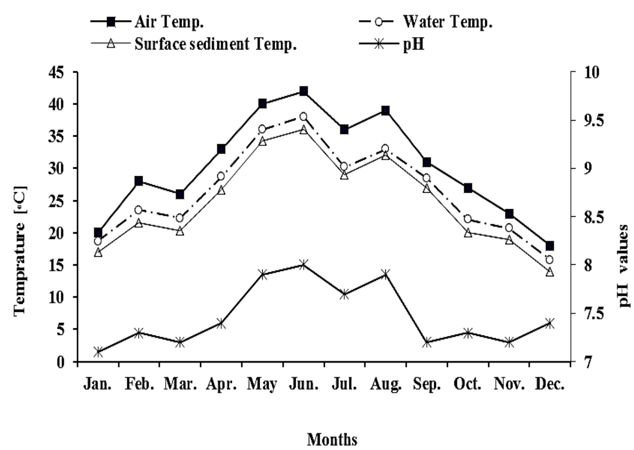

Fig. 3: Monthly changes in the values of the air, water and surface sediment temperatures and pH at the studied site. Temp. = temperature.

3.1.3 Analysis of the Sediment Constituents

Analysis of the sediment structure in the three seasons (winter, spring and summer) reflects minor variations, therefore, their results were pooled and shown in Figure 4.

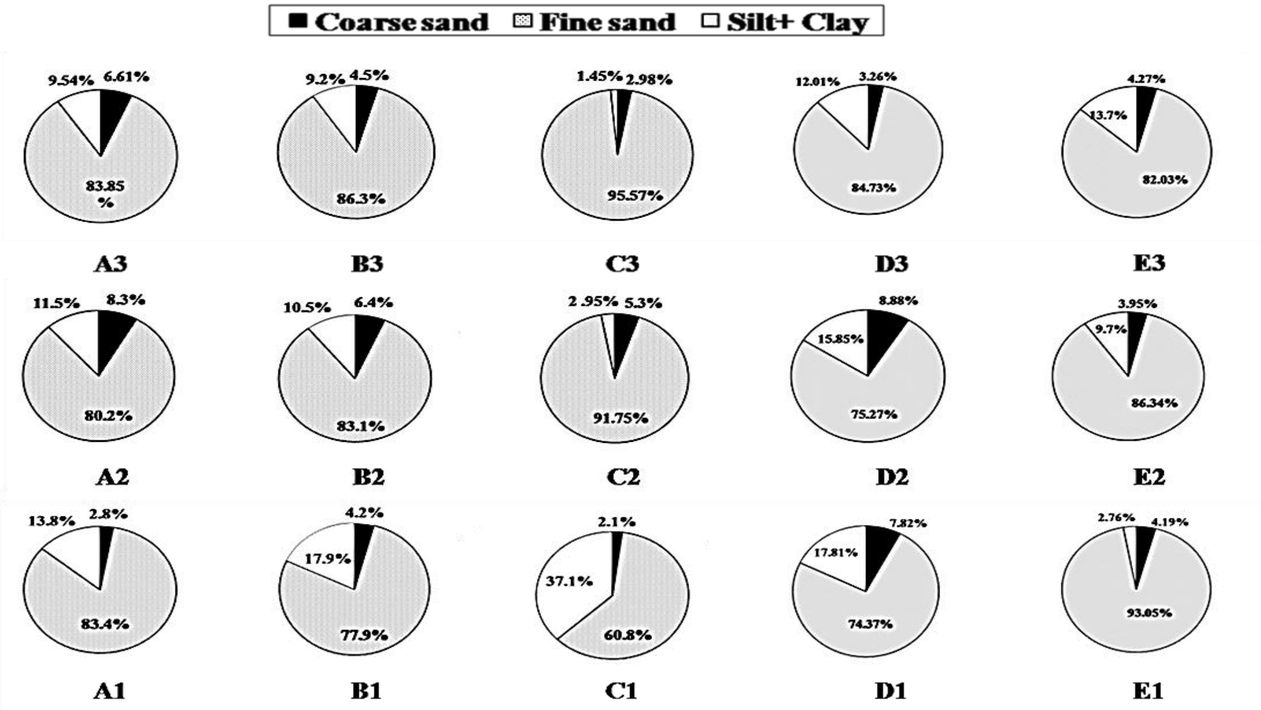

Fig. 4: The mean relative percentage of the sediment constituents at each point of the studied site. Data are pooled for the winter, spring and autumn 2014.

Generally, the mean percentages of coarse and fine sand were higher at the middle line, while their lowest values recorded at the shallow line. On the other hand, the mean percentages of the silt and clay were higher at the shallow line and the lowest was at the deep line. As for perpendicular lines (A-E), the highest mean percentages of coarse and fine sand were recorded at line (D) and that for fine sand at line (E). The lowest recorded mean percentages were for coarse sand at line (C), then fine sand at line (D) and silt and clay at line (E).

3.1.4 Local and Seasonal Variations of the Organic Content

Figure (5) shows the variations of organic contents during winter, spring and summer seasons at the parallel sampling points of river bank. The sampling points on the shallowest line, A1, B1, C1, D1 and E1 showed a very clear decrease from A1, B1, C1 and D1, while D1 was an exception where the percentage of the organic content was higher and nearly similar to that of A1. At the middle line, the sampling points B2 and C2 showed slightly higher percentages of the organic content than D2 and E2 and slightly lower than that at A2. The trend of the percentages of organic content at the deep sampling line was lower than that of the shallow and middle ones. There was a slight increase in the percentage of organic content from A3 to D3 followed by a decrease at E3.

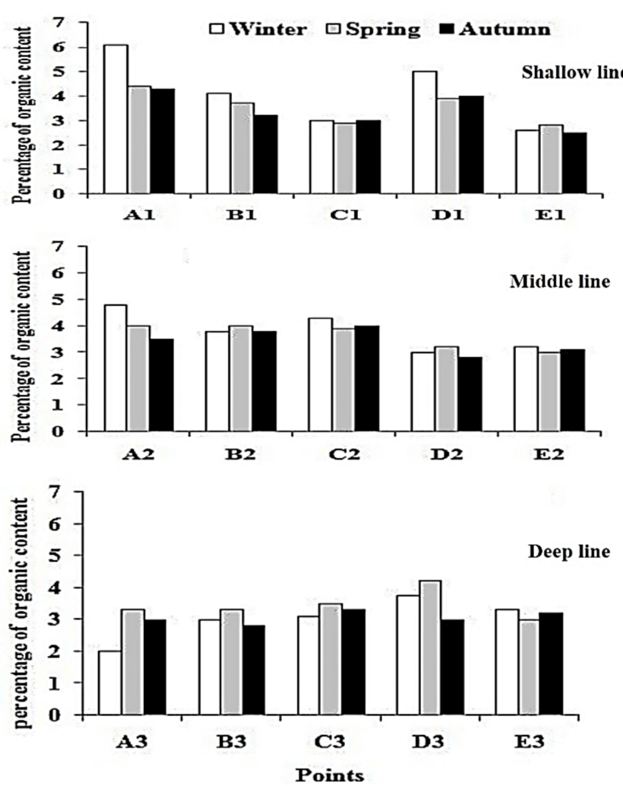

Fig. 5: Comparison of the Sediment Organic Content at each of the Three River Bank-Parallel Lines at the Sampling Site.

3.1.5 Local and Temporal Fluctuation in the Density of Nitia Teretiuscula.

During the one-year study period, a total of 242 individuals/9375 m3 of *N. teretiuscula* were collected. The mussel density showed local and temporal fluctuations during the collecting period (Fig. 6). Generally, the mussel was significantly favoring the shallow (146 inds.) and the middle (83 inds.) bank-parallel lines during most of the year (G test=26.06, $p<0.03$). In January, the mussel was inhabiting the shallow and the middle points of lines A, B, C and E. In February, it was found in the shallow points of lines, C and E, and inhabiting the middle points of lines A, D and E. In March, it was observed inhabiting the middle points of lines B, C, D and E, in addition to a single shallow point at line E. In April, only a single shallow point at line E and a single deep point at line B had individuals. On the other hand, no individuals were collected during May, June, July and August, due to the high cover with the water plant, *Paspalum distichum* that make collecting difficult. This was accompanied with River Nile flood and high-water level in July and August. In September, the mussel was inhabiting all the three parallel points of line A, a single shallow point at line C and a single deep point at line D. In October, it was inhabiting all the three points of line A, the shallow and the deep points of line C, and a single shallow point at line D. In November, it was found inhabiting the shallow and the middle points of lines A, C and D, and a single shallow point at line E. In December, the mussel was found inhabiting all three points of line A, the shallow and the middle points of lines D and E and a single middle point at line C. The highest number of *N. teretiuscula* was 39 individuals/6250 cm3 and recorded in September, at the sampling point A1, while the lowest number was 1/6250 cm3 and recorded in January at the sampling points B2 and C2, February at points A2, D2 and E1, April at point B3, September at points A2, C1, October in point C3, November in point C2 and December at points A1, E1 and E2.

3.1.6 Dispersal in Early Stages (Larval Stage) of Nitia Teretiuscula.

During May, June and August, the laboratory-reared specimens of *N. teretiuscula* were observed expelling large numbers of light brown-colored mucous structures from the exhalant siphons. These mucous structures were expelled to a distance of about 3 cm off the parent mussels. Each structure has many coned arms (Plate 1A, B). In the present work, each mucous structure will be referred to as embryophore. The arms of each embryophore were equal in length. However, among different embryophores, the arm's lengths were varying. The arms of each embryophore were arranged in two levels, where every arm in the upper level lies above the angle between the two arms of the lower level (Plate 1A, B). Each arm has 3-5 rayed supporting structures that are arranged in vertical rows parallel to each other. The ends of the arms are quite tapered (Plate 1D, F).

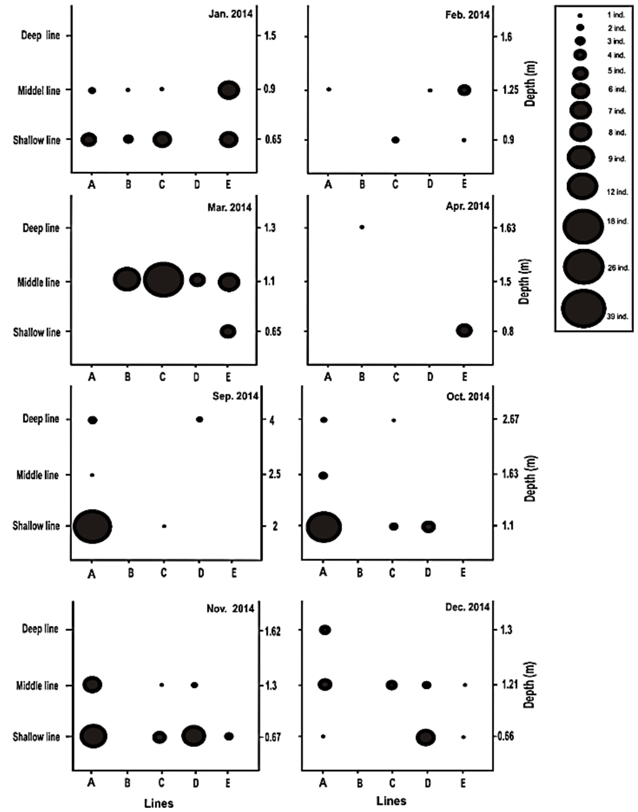

Fig.6: Local and temporal fluctuations in the density of *Nitia teretiuscula* at the studied site. Ind. = individuals.

Few numbers of glochidia larvae were found on the middle of the upper arms level (Plate 1A, B, E). The embryos were also in two levels above each other that twisted by 90o, so those in the upper level lie on the angle between that on the lower level. Table (1) shows the arm's numbers and lengths of investigated embryophores and the corresponding embryos' numbers and levels of their arrangement. The data showed that, the arms' numbers were even and ranged between 8-20 arm/embryophore and their lengths ranged from 1.23 to 1.59mm. On the other hand, the embryos' numbers were odd or even and ranged between 4 to 10/embryophore (Table 1). Also, the data revealed that, embryophores with a similar number of arms have nearly similar arm lengths. In all of the measured embryophores, the number of arms were always higher than that of the carried embryos.

The relationship between the log number of embryophore arms and log number of carried embryos was examined using regression analysis (Fig. 7). The data showed a significant positive correlation between the two variables ($r= 0.87$, $df= 55$, $P> 0.001$). Also, the analysis showed that the slope, value (b) was 0.911, which was significantly illustrating that, the relationship is positively allometric ($F= 173.36$, $df= 55$, $P> 0.0001$). This revealed that, the increase in number of arms is superior to the increase in the number of the carried embryos. The coefficient of determination (R2) value was 0.76, reflecting that the application of the relationship between the number of arms and the number of embryos is suitable.

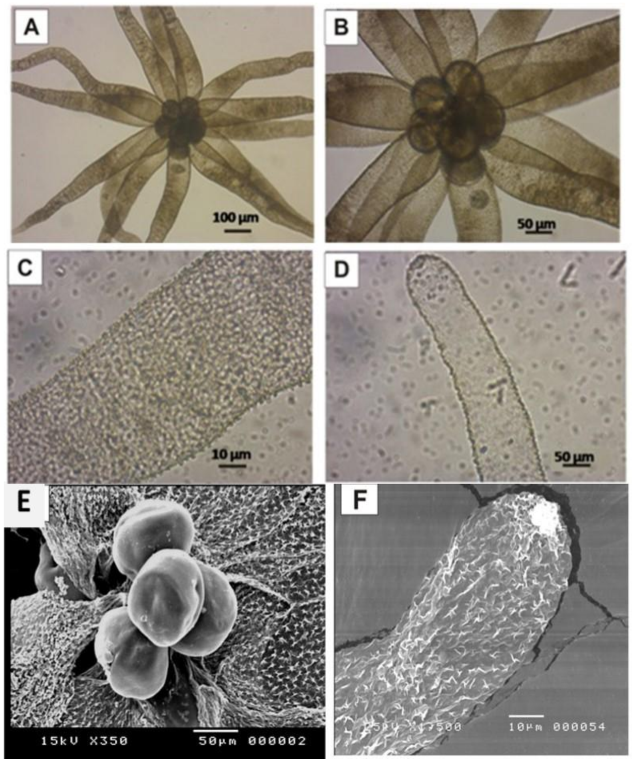

Plate 1 (A-D). Light photographs showing an embryophore of *N. teretiuscula*. A and B: Complete structure of embryophore, showing the arms arranged in two levels and embryos in the middle of the upper arms level. C: Enlarged portion of the surface of an arm showing numerous projections. D: The free end of an arm. (E-F). Scanning electron micrographs showing an embryophore of *N. teretiuscula.* E: Embryos arranged in two levels above the upper arms level. F: The free end of an arm with numerous projections.

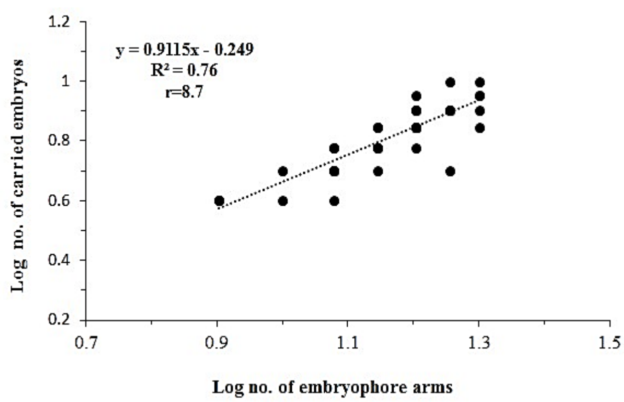

Fig.7: The relationship between the log number of embryophore arms and the log number of carried embryos. r= Spearman's correlation coefficient. The relationship between the log number of embryophore arms and the log number of carried embryos. r= Spearman's correlation coefficient.

Table 1. Variation in both the number of embryophore's arms and the carried embryos in *N. teretiuscula.*

specimen No.	No. of Arms	Length of arms (mm)	No. of Embryos	Arms & Embryos arr.
1, 14	20	1.59	9	2
2, 8, 18, 19, 28, 49, 50	12	1.35	5	2
3, 5, 6, 7, 9, 11, 27, 33, 39,43	16	1.47	7	2
4, 24, 32, 47, 56	14	1.41	6	2
10, 16, 42, 44, 45	16	1.47	8	2
12, 26, 30, 38, 51, 55	18	1.53	8	2
13	10	1.29	4	2
15, 48, 53	12	1.35	6	2
17	14	1.41	5	2
20	16	1.47	6	2
21	20	1.59	8	2
22	20	1.59	10	2
23, 25, 40, 52	14	1.41	7	2
29	16	1.47	9	2
31	12	1.35	4	2
34, 37, 41, 46	8	1.23	4	2
35	18	1.53	5	2
36	10	1.29	5	2
54	18	1.53	10	2

arr.=arrangement

3.2 Discussion

Family Unionidae is one of the most bivalve species-rich families [17,18], however, in the River Nile of Egypt, one endemic and nine widely distributed species were recorded representing 1% of the total recorded unionid species in North Africa [19].

The temporal distribution of *N. teretiuscula* in the studied area was fluctuated during most of the year, but it showed high abundance during early spring and early summer. The present studied species showed two spawning periods, one occurred in March and the other in August [16].

In the present investigation, individuals of *N. teretiuscula* were found preferring the peripherals of the studied area (Lines A and D) than other parts. The present work revealed also, the presence of significantly more individuals of *N. teretiuscula* in points of shallow and middle lines that characterized by substrate rich in fine sand followed by silt and clay. Moreover, points of shallow line were more preferentially inhabited than middle ones and this might attribute to presence of more silt and clay in these points. This clearly reflects that, stability of substrate structure in the studied area of River Nile affect *N. teretiuscula* distribution. Members of family Unionidae found to have positive relationships with sandy substrate [20,21] or sandy-mud substrates [22], which allow water currents to flow freely within it and assure a steady food supply for mussels [23,24,25]. Mud can constitute a serious problem to mussel individuals, where mud fills the gills and interferes with respiration. Also, the light weight and inflated bivalves of *N. teretiuscula* prevented them from sinking deep into the mud [26].

Unfortunately, distribution of *N. teretiuscula* was generally recorded along the River Nile basin [19]. The present species was widespread across Nile basin from White Nile to the Delta Region in Egypt, in addition to some parts in West Africa [19]. Therefore, it was not surprised to find no relationships between the present species and measured temperatures. However, the presence of significant effects of pH made this factor arose beside the substrate structure as important factors in determining the distribution of the present species. pH as an environmental factor reflects water quality which is an important consideration for mussel conservation. The mining, drainage of irrigation water and contributions of factories in water pollution are resources of water pollution across Nile basin, therefore, more studies are needed to determine the extent to which water quality is a limiting factor for mussel distribution [27]. Vegetation, also, arises as another factor that can limit both density and distribution of freshwater mussels, where dense plants and weeds reduce ability of mussels to burrow down in sediments [21]. Vegetation may stabilize sediment, presenting a suitable habitat for mussels, and providing perfect refuges for fishes that, needed for dispersal of mussel's larvae [28].

In the present work, organic content of the sediment seems important for distribution of *N. teretiuscula*, where individuals were found in points with high organic contents near river bank as a result of deposition and erosion of agricultural area. The sediment organic matter constitutes a prospective food source, however, high levels of sediment organic matter reported to be unsuitable for some species of unionids [29].

Mussels mostly are sessile organisms and movement of adult unionid are restricted to few centimeters and therefore they have limited dispersal. For freshwater mussels, dispersal mode is more important [30,31], and the absence of mussels from some areas does not necessarily indicate unsuitable environmental conditions but can be caused by dispersal limitations [2].

To overcome their limited dispersal, unionid mussels have developed numerous strategies to use certain species of fish as hosts for their larvae which grow and mature as a parasite on these fish [32,33]. All the previous studies that have been dealt with dispersal of unionid mussels showed their dependence on fish host and developing a variety of strategies to attach the host [34,35].
In the present studied area, only the common Nile fish, *Oreochromis niloticus*, was observed and may play as a target host for glochidia larvae of the present species. Therefore, the species distribution and abundance may be influenced by the dynamics of mussel/host-fish relationships [28] that was not investigated in the present work. As a consequence, the host fish and its mobility are important ecological factors affecting mussel dispersal [12,36].

However, the presence of only one kind of fish suggest limited dispersal for the present species, especially when the number of fish in the area is low and suffer from high fishing activity. This may force *N. teretiuscula* to seek another dispersal method that is mainly depend on fish host. The present species, *N. teretiuscula* displays a new dispersal phenomenon that appeared during metamorphosis to juveniles, and which is considered as a new dispersal method in family Unionidae. Females of *N. teretiuscula* release large numbers of light brown-colored mucous structures (embryophores) carrying glochidia. The release of embryophores of *N. teretiuscula* was observed only in the laboratory in May, June and August 2014 during their rearing. Releasing embryophores may be attributed to variation in water temperature between field and lab. Supporting our finding, some authors reported that gravid mussel females are sensitive to anything that interferes with respiration [37]. Therefore, the releasing of many unionoid's glochidia larvae was affected by sudden changes in water temperature (thermal shock) which can induce immediate shedding of glochidia [38].

In the present work, the positive relationship between number of embryophore arms and number of attached embryos was an interesting phenomenon and suggests that the increase in number of embryophores' arms will increase the number of embryos. Further studies are recommended to emphasis the phenomenon of embryophores in the present species and in Unionidae as a whole.

4 Conclusions

Specimens of *N. teretiuscula* were found burrow in sandy-mud substrate near the river bank. pH, substrate structure and organic content were the most effective environmental factors on the distribution of the present species. Vegetation arises as another factor which can limit both density and distribution of *N. teretiuscula*. In the present study, *N.*

teretiuscula showed a new dispersal method not recorded before in family Unionidae.

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