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- Short communication

STUDY OF *NITELLA HYALINA* (CHARALES) BASED ON OOSPORE WALL ORNAMENTATION

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Charophytes are ancestors of land plants (Laurin-Lemay *et al.*, 2012) and multi-cellular, branched, macroscopic filaments from a few centimeters (cm) to several meters in length with colorless rhizoid (Urbanik and Kwiatkowski, 2019). The main filaments are organized into short nodes forming whorls of branches, and much longer internodal cells (Schubert, 2014). General morphology varies with environmental conditions such as temperature, depth of the water, light levels, and amount of wave action (Naz *et al.*, 2011). In reproductive structure, oospores are multilayered, pigmented and thick-walled female sex organ (Ahmadi *et al*., 2012), Differential deposition in this layer of the wall is frequently sculptured and forms specific oospores wall ornamentation (Ray *et al.*, 2001). On the different oospores characters, the ornamentation pattern is considered to be an important taxonomic marker because of its conservative nature (Urbaniak and Blaženčić, 2012; Casanova and Karol, 2008). *Nitella hyalina* (DC.) Ag. is a cosmopolitan species and occurs between 70° N and 40° S on all continents (Naz *et al.*, 2011), but seems always to be restricted to few locations (Krause, 1997). The aim of this study is to determine the type, size and ornamentation of the o pores of *Nitella hyalina* (DC.) Ag. using SEM.

Nitella hyalina (DC.) Ag. were collected by hand from a depth of 15-20 cm in July, 2020 from the river Padma near the Rajshahi city corporation area (24°22′0″ N, 88º 36′0″ E), in Bangladesh. Specimens were conserved under *Ex situ* conditions for the SEM study and identified by relevant cited literature (Naz *et al.* 2011, Wood and Imahori 1965). The oospores were collected from the living plants, choosing only matured oospores (dark colored or black). Oogonia were placed in a plastic pot containing 10-20 ml of distilled water (DW) about 15 days. As a result, the tube cells were removed from the oogonia. Oospores were washed several times in DW. The selected mature oospores were placed in a 1:9 solution of liquid detergent in water and kept for 12 hrs in an oven maintained at 50 °C. The oospores were again washed several times in DW. Then, oospores were centrifuged at 3000 rpm for 5 min with DW. Oospores were treated with glacial acetic acid (100%) for 10 min and centrifuged at 3000 rpm for 5 min and the acid was decanted off. The oospores were prepared by following (John and Moore, 1987) and discarded the unwanted material from the upper portion of the eppendorf tube. Glacial acetic acid (100%) was added for the second times and again centrifuged at 3000 rpm for 5 min and discard the unwanted material from the upper portion of the Eppendorf tube. Following washing and centrifuging the oospores were then passed through serial grades of alcohol (40% to absolute alcohol) for dehydration and finally stored in absolute alcohol (96%). Acetolysed and thoroughly cleaned mature oospores were mounted on specimen stubs having double-sided sticky tapes. The

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oospores were coated with gold-palladium by a Sputter-coater and observed with a JEOL JSM-6490 LA at an accelerated voltage of 15 kv. The SEM photographs were taken whenever desired at different magnifications. Morphological features of oospores of *Nitella hyalina* (DC.) Ag. were studied based on 20 oospores preserved in 70% ethanol in pyrex glass bottle for further study. Largest polar axis (LPA, length) and the largest equatorial diameter (LED, width) were measured and then isopolarity index (ISI= LPA/LED×100) was calculated. The number of ridge, width of fossa, distance from apical pole to LED (AND) and anisopolarity index (ANI=AND/LPA×100) were also examined as earlier described (Horn af Rantzien, 1956). The SEM photographs were taken at Centre for Advanced Research in Sciences (CARS) at University of Dhaka, Dhaka in Bangladesh. All statistical computations (Basic descriptive statistics and Regression Analysis) were performed with the use of IBM SPSS Statistics 20 version.

No work had been done earlier on the dimension of oospores at the population level in Bangladesh using SEM. The present investigation revealed that the oospores wall ornamentation was found to have a bright brown color with a prominent ridges and membrane minutely granule (Fig. 1a-c) similar to the findings of De Winton *et al*. (2007). However, the patterns are characterized by a shape, slightly roughened ornamentation due to the presence of numerous irregular pits and pores, the weakly developed striae and small ribbon-like structure found on the striae (Fig. 1a-c), but were often completely or was partially detached during cleaning. The projections on the striae were absent as previously reported for *Nitella hyalina* (Urbaniak and Blaženčić, 2012). Under LM, the fossa wall was finely granular, but under (SEM), it was fibrous,

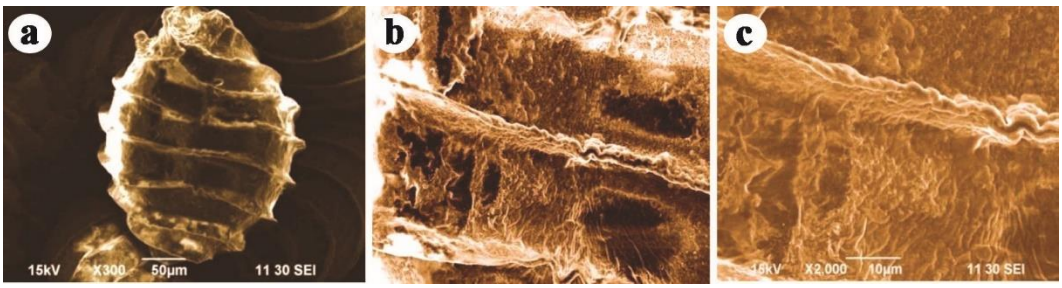


Fig. 1(a-c). A general view of the oospores showing well developed striae and minutely fibrous fossa wall.

as previously reported (Casanova and Karol, 2008; De Winton *et al*., 2007; Sakayama *et al.*, 2005). The oospores were ellipsoidal (ISI index 103.818-187.208) and had an ovoid to ellipsoidal shape (ANI index 64.676-53.487). LPA ranged from 64.65 - 97.78, with an average of 80.949±7.477 µm, LED range appeared to 41.12 to 76.28 with an average of 66.614±8.214 µm. There were 8 ridges on the oospores surface; the mean width of fossa was 8.909±1.607 µm, as they can be from 6.83 to 13.16 µm; the coefficient of variation ranged from 9.237% for oospores length to 18.041% for width of fossa. The mean of apical pole (AND) to LED was 40.9033.273 µm, range of the same was found to be 34.325 to 48.89 and coefficient of variation was 8.002% (Table 1). Ahmadi *et al*., 2012, described that the mean value of LPA, LED, ISI-index, width of fossa and number of ridges are respectively 269.13±1, 208.06±3, 1.29±1, 31.53±1, 8±0.7, which assumes that our samples were small in size than them. The mean value of the Macedonian populations of *Nitella hyalina* (DC.) Ag. LPA, LED, number of ridges, width of fossa, ISI index was 330±17.8, 294±8.8, 6±0.5, 45±6.2, 1.16±0.05, respectively. The findings detected from Balkan Charophytes were dissimilar to our populations (Urbaniak and Blaženčić, 2012) which is also similar to our findings. The Pearson's correlation between LED and LPA was given

Table 1. Descriptive statistics of the oospores of *Nitella hyalina* (DC.) Ag.

Features	Mean	SD	Median	Min.	Max.	V (%)
LPA	80.950	7.477	81.120	64.650	97.780	9.237
LED	66.614	8.214	86.275	41.120	76.280	12.331
No. of ridges	8	00	8	8	8	0.00
Width of fossa	8.910	1.607	8.595	6.830	13.160	18.041
ISI index	122.926	16.530	119.368	103.818	187.208	13.447
AND	40.903	3.273	41.278	34.325	48.890	8.002
ANI index	50.605	1.500	50.039	64.676	53.490	2.964

AND-distance from apical pole to LED; ANI Index-Anisopolarity Index AND/LPAx100; ISI=Isopolarity Index LPA/LED*100; LED-Largest Equatorial Diameter; LPA-Largest Polar Axis; Max.-maximum; Min.-minimum; SD-Standard Deviation; V-Variation coefficient.

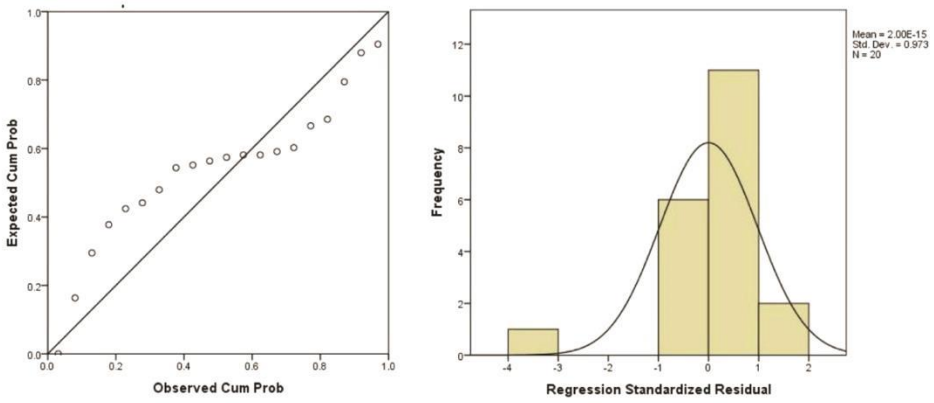
Table 2. Pearson's correlation between LED and LPA.

r	t-test	p.value	95% CI
0.633	3.472	0.003	(0.265, 0.840)

Predictors: (Constant), LPA, Dependent Variable: LED

Table 3. Simple linear regression analysis of LED on LPA.

Model	Estimate	Standard error	t-test	p.value	R-square
Intercept	10.30	16.288	0.632	0.535	0.401
LPA	0.696	0.200	3.471	0.003	
F-value	12.05			0.003	



(a) (b)

Fig. 2 (a-b). a) Normal Probability Plot (N= 20) showing the deviation from expected and Observed data; b) Histogram between LP_A and LED showing the mean and SD value of the population.

(r=0.633), R square = 0.401, which implied that only 40.1% of the LED was explained by the LPA (Table 2). The ANOVA analysis showed that the F-value was 12.051 and p-value (significance value) is 0.003 ($p<0.05$), which indicated that regression equation was statistically significant in

linear relation (Table 3). Table 3 is also, provided the qualification of the relationship between LPA and LED. With every increase of one unit in LPA, the LED (on the average) increased by 0.696 (95% CI 0.265 to 0.840) units, $p<0.05$. As indicated in Fig. 2(a), most splashes were close to diagonal, which indicated that standardized residuals were obeyed the normal distribution and Fig. 2(b) histograms indicated that the distribution of the residual satisfies the normality assumption.

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