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**Spatial Distribution Pattern of the Populations of *Cephalanthera longibracteata* at Mt. Yeon-ae**

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**Abstract** *Cephalanthera longibracteata* (Blume) is an herbaceous and a member of the genus *Cephalanthera* in the family Orchidaceae. It has been investigated the population density and spatial distribution of this species at Mt. Yeon-ae in Korea during 2015. The spatial pattern of *C. longibracteata* was analyzed according to several patchiness indexes, population uniformity or aggregation under different sizes of plots by dispersion indices, and spatial autocorrelation. Population densities (D) varied from 0.207 to 2.111, with a mean of 1.049. The small plots (from 3 m x 3 m to 12 m x 24 m) of *C. longibracteata* were uniformly distributed in the forest community. However, two large plots (24 m x 24 m and 24 m x 48 m) were aggregately distributed. At spatial autocorrelation by Moran's I, significant aggregations were partially observed within III classes (9 m). The comparison of Moran's I values to a logistic regression indicated that a highly significant percentage of individual dispersion in *C. longibracteata* populations at Mt. Yeon-ae could be explained by isolation by distance.

**Keywords** *Cephalanthera longibracteata*, Mt. Yeon-ae, population density, Moran's I, spatial distribution

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### **Introduction**

The predictability of the physical arrangement of plants, at whatever scale it is viewed, is referred to as a spatial pattern. The spatial structure of a plant community in a forest stand is an important signature of forest dynamics because plant survival and growth depend on local environments within a habitat [1-2]. The spatial heterogeneity of populations and communities plays a central role in many ecological theories, for instance the theories of succession, adaptation, maintenance of species diversity, community stability, competition, predator-prey interactions, parasitism, epidemics and other natural catastrophes, ergoclines, and so on [3]. The spatial distribution pattern of plant populations exhibits scale dependence, e.g. a species may show an aggregated distribution at one spatial scale and may change to a random or uniform distribution at a different scale [4]. In aggregated distribution, the distance between neighboring individuals is minimized. Uniform distributions are found in populations in which the distance between neighboring individuals is maximized. Random distribution, also known as unpredictable spacing, is the least common form of distribution in nature and occurs when the members of a given species are found in homogeneous environments in which the position of each individual is independent of the other individuals. The spatial distribution pattern of plant populations exhibits scale dependence, e.g. a species may show an aggregated distribution at one spatial scale and may change to a random or uniform distribution at a different scale [5].

Ecological data are usually characterized by spatial structures due to spatial autocorrelation. Spatial autocorrelation refers to the pattern in which observations from nearby locations are more likely to have similar magnitude than by chance alone. The magnitude, intensity, as well as extent of spatial autocorrelation can be quantified using spatial statistics [6-7]. Spatial autocorrelation coefficients provide an averaged isotropic estimation of spatial autocorrelation intensity at each distance class. When working with plant spatial structures, however, most species will show some degree of spatial anisotropy due to differential response to environmental conditions. Spatial anisotropy can be detected by calculating and comparing the spatial autocorrelation for pairs of locations grouped not only by distance class but also by direction. Many ecologists have adopted several different major schools of spatial analysis from other disciplines [8]. The first of these comes from geography,



and its methods include the use of statistics (e.g., Moran's *I*) to measure spatial autocorrelation [9].

*Cephalanthera longibracteata* (Blume) is an orchid genus *Cephalanthera* in the family Orchidaceae. *C. longibracteata* is found in the far eastern Russia, Korea, Northern China, and Japan in open forests. Plants are autotrophic and usually grow 30-50 cm tall. Stems are slender to somewhat robust, with several basal sheaths, 6-8-leaved, and slightly scabrous toward apex [10]. Leaves are broadly lanceolate to oblong-lanceolate, 6-14 × 1.5-3 cm, abaxially scabrous on veins, and apex long acuminate. Flowers are erect, weakly spreading, white pedicel and ovary 12-16 mm. Sepals are narrowly ovate to lanceolate, 10-13 × 2-3 mm, and apex acute to acuminate. Several white flowers were occurred in May and June.

The point pattern analysis method was adopted to study the distribution pattern of *C. longibracteata* individuals of different size classes and the correlations between various size classes as well as the impact of topographical attributes on the population distribution.

The purpose of this paper was to describe a statistical analysis for detecting a species association, which is valid even when the assumption of within-species spatial randomness is violated. The present study used the point pattern analysis method to investigate the variation in the spatial distribution pattern of *C. longibracteata* at different spatial scales and spatial autocorrelation at different plots in a 24 x 48 m<sup>2</sup> spatial scales at the Mountain Yeon-ae in Korea.

## Materials and Methods

### Surveyed regions

This study was carried out on the populations of *C. longibracteata*, located at Mt. Yeon-ae (35°26'943"N/129°20'278"E) in Busan-ci (Korea). The elevation of community of *C. longibracteata* ranges from 125 to 140 m. The site is characterized by a temperate climate with a little hot and long summer. In this region the mean annual temperature is 15.1 °C with the maximum temperature being 29.2 °C in August and the minimum -0.8 °C in January. Mean annual precipitation is about 1518.8 mm with most rain falling period between June and August.

### Sampling procedure

Many quadrats at Mt. Yeon-ae were randomly chosen for each combination of site x habitat, so that, overall, 90 quadrats were sampled for the complete experiment.

Spatial ecologists use artificial sampling units (so-called quadrats) to determine abundance or density of species. The number of events per unit area are counted and divided by area of each square to get a measure of the intensity of each quadrat. I randomly located quadrates in each plot which I established populations. The quadrat sizes were 2 m x 2 m, 2 m x 4 m, 4 m x 4 m, 4 m x 8 m, 8 m x 8 m, 8 m x 16 m, 16 m x 16 m, and 16 m x 32 m. I mapped all plants to estimate *C. longibracteata* density per plot.

### Index calculation and data analysis

Given the above definition of spatial autocorrelation, it is expected that the x-y coordinates of points (e.g. individual plants) having a spatial structure are more likely to be spatially close than expected by chance alone. Following this simple idea, the nearest neighbor method measures the mean nearest distance for all points  $d_i$ , where  $i = 1$  for the first neighbor [11, 12]. The spatial pattern of *C. longibracteata* was analyzed according to the Nearest Neighbor Rule [13, 14] with Microsoft Excel 2014.

Average viewing distance ( $r_A$ ) was calculated as follows:

$$r_A = \sum_{i=1}^N r_i / N \quad (i = 1, 2, 3 \dots N)$$

Where  $r_i$  is the distance from the individual to its nearest neighbor.  $N$  is the total number of individuals within the quadrat.

The expectation value of mean distance of individuals within a quadrat ( $r_B$ ) was calculated as follows:

$$r_B = 1/2\sqrt{D}$$

Where  $D$  is population density and  $D$  is the number of individuals per plot size.

$$R = r_A / r_B$$

When  $R > 1$ , it is a uniform distribution,  $R = 1$ , it is a random distribution,  $R < 1$ , it is an aggregated distribution. The significance index of the deviation of  $R$  that departs from the number of "1" is calculated from the following formula [14].

$$C_R = \frac{r_A - r_B}{\delta_{rB}}$$

$$\delta_{rB} = 0.2613/\sqrt{ND}$$

When  $C_R > 1.96$ , the level of the significance index of the deviation of  $R$  is 5%, and when  $C_R > 2.58$ , the level is 1%.

One test for spatial pattern and associated index of dispersion that can be used on random-point-to-nearest-organism distances was suggested by Eberhardt [15] and analyzed further by Hines and Hines [16]:



$$I_E = (s/m)^2 + 1$$

Where  $I_E$  = Eberhardt's index of dispersion for point-to-organism distances,  $s$  = observed standard deviation of distances,  $m$  = mean of point-to-organism distances  
Many spatial dispersal parameters were calculated the degree of population aggregation under different sizes of plots by dispersion indices: index of clumping or the index of dispersion ( $C$ ), aggregation index ( $CI$ ), mean crowding ( $M^*$ ), patchiness index (PAI), negative binomial distribution index  $K$ ,  $Ca$  indicators ( $Ca$  is the name of one index) [8] and Morisita index ( $IM$ ) were calculated with Microsoft Excel 2014. The formulae are as follows:

$$\text{Index of dispersion: } C = S^2/m$$

$$\text{Aggregation index } CI = \frac{S^2}{m} - 1$$

$$\text{Mean crowding } M^* = m + \frac{S^2}{m} - 1 = m + CI = m + C - 1 - 1$$

$$\text{Patchiness index } PAI = \frac{m}{\frac{S^2}{m} - 1} = \frac{M^*}{m}$$

$$\text{Aggregation intensity } PI = k = m^2/(S^2 - m) = \frac{m}{CI} = \frac{m}{C-1}$$

$$Ca \text{ indicators } Ca = 1/k$$

$$IM = \frac{n \sum m (m-1)}{nm(nm-1)}$$

Where  $S^2$  is variance and  $m$  is mean density of *C. longibracteata*.

When  $C, M^*, PAI > 1$ , it means aggregately distributed, when  $C, M^*, PAI < 1$ , it means uniformly distributed, when  $CI, PA, Ca > 0$ , it means aggregately distributed, and when  $CI, PA, Ca < 0$  it means uniformly distributed.

The mean aggregation number to find the reason for the aggregation of *C. longibracteata* was calculated [17].

$$\delta = mr/2k$$

Where  $r$  is the value of chi-square when  $2k$  is the degree of freedom and  $k$  is the aggregation intensity.

Green index (GI) is a modification of the index of cluster size that is independent of  $n$  [18].

### Spatial structure

When a plant population or community is sampled, the samples have a spatial relationship with each other. To a certain extent, samples that are close to each other are more likely to be similar. Numerical simulations of previous analyses were performed to investigate the significant differences at various distance scales, i.e., 1.0 m, 1.5 m, 2.0 m, and so on. However, no significant population structure was found within the 3.0 m distance classes by means of Moran's  $I$ , and a significant population structure was revealed beyond 3.0 m. Thus, the distance classes are 0-3.0 m (class I), 3.0-6.0 m (class II), 6.0-9.0 m (class III), 9.0-12.0 m (class IV), 12.0-15.0 m (class V), 15.0-18.0 m (class VI), 18.0-21.0 m (class VII), 21.0-24.0 m (class VIII), 24.0-27.0 m (class IX), and 27.0-30.0 m (class X). The codes of classes are the same as in the distance classes and are listed Table 1.

**Table 1:** Spatial patterns of *Cephalanthera longibracteata* individuals at different sampling quadrat sizes in Mt. Yeon-ae

Quadrat size (m x m)	Density	R	CR	$I_E$	Distribution pattern
3 x 3	2.111	3.611	21.775	1.331	Uniform
3 x 6	2.167	5.167	49.783	1.316	Uniform
6 x 6	1.333	2.865	24.729	1.418	Uniform
6 x 12	1.001	1.819	13.297	1.835	Uniform
12 x 12	0.792	1.378	7.724	1.975	Uniform
12 x 24	0.493	1.058	1.331	2.089	Uniform
24 x 24	0.288	0.877	-3.039	2.370	Aggregation
24 x 48	0.207	0.699	-8.894	2.501	Aggregation

The spatial structure was quantified by Moran's  $I$ , a coefficient of spatial autocorrelation (SA) [9]. As applied in this study, Moran's  $I$  quantifies the similarity of pairs of spatially adjacent individuals relative to the population sample as a whole. The value of  $I$  ranges between +1 (completely positive autocorrelation, i.e., paired individuals have identical values) and -1 (completely negative autocorrelation). Each plant was assigned a value depending on the presence or absence of a specific individual. If the  $i$ th plant was a homozygote for the individual of interest, the assigned pi value was 1, while if the individual was absent, the value 0 was assigned [19].



Pairs of sampled individuals were classified according to the Euclidian distance,  $d_{ij}$ , so that class  $k$  included  $d_{ij}$  satisfying  $k - 1 < d_{ij} < k + 1$ , where  $k$  ranges from 1 to 10. The interval for each distance class was 3.0 m. Moran's  $I$  statistic for class  $k$  was calculated as follows:

$$I(k) = \frac{n \sum i \sum j (i \neq j) W_{ij} Z_i Z_j}{S \sum Z_i^2}$$

where  $Z_i$  is  $p_i - \bar{p}$  ( $\bar{p}$  is the average of  $p_i$ );  $W_{ij}$  is 1 if the distance between the  $i$ th and  $j$ th plants is classified into class  $k$ ; otherwise,  $W_{ij}$  is 0;  $n$  is the number of all samples and  $S$  is the sum of  $W_{ij}$   $\{\sum i \sum j (i \neq j) W_{ij}\}$  in class  $k$ . Under the randomization hypothesis,  $I(k)$  has the expected value  $u_1 = -1/(n - 1)$  for all  $k$ . Its variance,  $u_2$ , has been given, for example, in Sokal and Oden [9]. Thus, if an individual is randomly distributed for class  $k$ , the normalized  $I(k)$  for the standard normal deviation (SND) for the plant genotype,  $g(k) = \{I(k) - u_1\}/u_2^{1/2}$ , asymptotically has a standard normal distribution [20]. Hence, SND  $g(k)$  values exceeding 1.96, 2.58, and 3.27 are significant at the probability levels of 0.05, 0.01, and 0.001, respectively.

## Results and Discussion

The population densities ( $D$ ) per quadrat size vary among scales (Table 1). Small quadrat sizes such as 3 m x 3 m and 3 m x 6 m have a relatively high  $D$  value, whereas larger or wider quadrat sizes such as 24 m x 24 m and 24 m x 48 m have, comparatively, very low  $D$  values. Population densities ( $D$ ) varied from 0.207 to 2.111, with a mean of 1.049 (Table 1). The values ( $R$ ) of spatial distance (the rate of observed distance-to-expected distance) among the nearest individuals were higher than 1 and the significant index of CR was  $> 2.58$ . If by this parameter, the small plots (3 m x 3 m, 3 m x 6 m, 6 m x 6 m, 6 m x 12 m, 12 m x 12 m, and 12 m x 24 m) of *C. longibracteata* at Mt. Yeon-ae were uniformly distributed in the forest community (Table 1). However, *C. longibracteata* were aggregately distributed in two large plots (24 m x 24 m and 24 m x 48 m). The expected value of  $I_E$  in a random population is 1.27. Values below this suggest a regular pattern, and larger values indicate clumping.  $I_E$  values for all quadrates are larger than 1.27 (Table 1). Under the hypothesis, population of *C. longibracteata* is clumping.

The values dispersion index ( $C$ ) at Mt. Yeon-ae were lower at six plots (3 m x 3 m, 3 m x 6 m, 6 m x 6 m, 6 m x 12 m, 12 m x 12 m, and 12 m x 24 m) than 1 except two large plots (24 m x 24 m and 24 m x 48 m) (Table 2). Thus these aggregation indices ( $CI$ ) were negative at Mt. Yeon-ae, which indicate a uniform distribution. Two large plots (24 m x 24 m and 24 m x 48 m) were positive. The mean crowding ( $M^*$ ) and patchiness index ( $PAI$ ) showed positive values for all plots. The three indices  $C$ ,  $M^*$ ,  $PAI$  were  $< 1$  and their values of  $PI$  and  $Ca$  except two plots were also shown smaller than zero, thus it means uniform distributed. In *C. longibracteata*, the two indices,  $C$ ,  $PAI$  were  $> 1$  and their values of  $PI$  and  $Ca$  except four small plots were also shown greater than zero, thus it means aggregately distributed. Thus, most individuals of *C. longibracteata* at Mt. Yeon-ae were clustered and the distribution pattern of the *C. longibracteata* was quadrat-sampling dependent. The values of  $\delta$  were varied from 0.561 for 12 m x 12 m to 3.101 for 24 m x 48 m (Fig. 1).

Morisita index ( $IM$ ) is related to the patchiness index ( $PAI$ ) and showed an overly steep slope at the plot 24 m x 24 m in Mt. Yeon-ae. When the area was smaller than 24 m x 24 m, the degree of aggregation increased significantly with increasing quadrat sizes, while the patchiness indices did not change from the plot 3 m x 3 m to 12 m x 24 m. Green index varied between -0.381 to 0.678 (Fig. 2).

**Table 2:** Changes in gathering strength of *Cephalanthera longibracteata* at different sampling quadrat sizes

Quadrat size (m x m)	No. Quadrat	Aggregation indices						
		<i>C</i>	<i>CI</i>	<i>M*</i>	<i>PAI</i>	<i>PI</i>	<i>Ca</i>	<i>IM</i>
3 x 3	11	0.313	-0.687	0.259	0.274	-1.377	-0.726	0.290
3 x 6	8	0.356	-0.644	0.481	0.427	-1.746	-0.573	0.437
6 x 6	6	0.454	-0.546	0.514	0.485	-1.940	-0.515	0.494
6 x 12	5	0.647	-0.353	0.421	0.544	-2.193	-0.456	0.459
12 x 12	3	0.709	-0.291	0.435	0.599	-2.494	-0.401	0.606
12 x 24	3	0.780	-0.220	0.497	0.693	-3.262	-0.307	0.703
24 x 24	2	1.071	0.071	0.854	1.091	10.948	0.091	1.099
24 x 48	1	1.161	0.161	0.935	1.209	4.792	0.209	1.215

**Table 3:** Spatial autocorrelation coefficients (Moran's  $I$ ) among plots of *Cephalanthera longibracteata* for ten distance classes

I	II	III	IV	V	VI	VII	VIII	IX	X
0.643***	0.605***	0.214*	0.107	0.022	-0.055	-0.119	-0.384**	-0.409**	-0.593***

\*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .



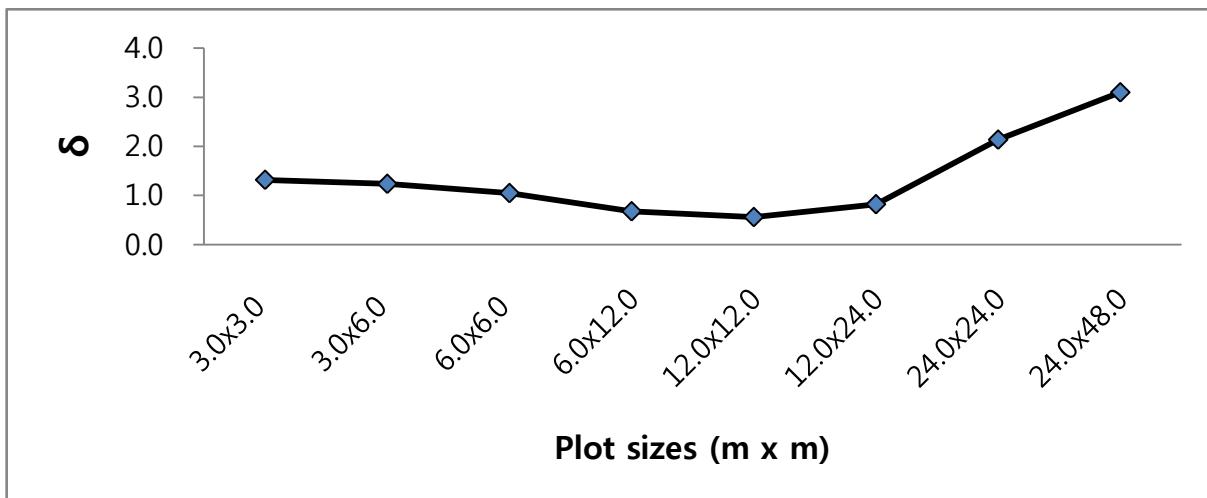


Figure 1: The mean aggregation number to find the reason for the aggregation of *Cephalanthera longibracteata*.

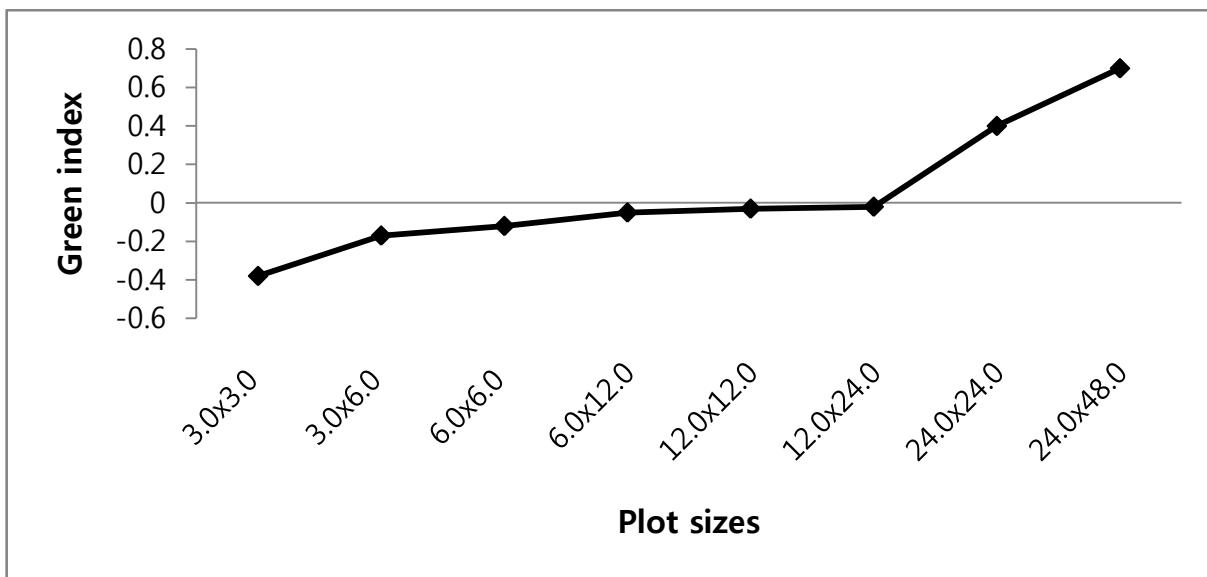


Figure 2: The curves of patchiness in two areas of *Cephalanthera longibracteata* using values of Green index.

The spatial autocoefficient, Moran's  $I$  is presented in Table 3. Separate counts for each type of joined individuals and for each distance class of separation were tested for significant deviation from random expectations by calculating the SND. Moran's  $I$  of *C. longibracteata* significantly differed from the expected value in 6 of 10 cases (60%). Three of these values (30.0%) were significant negative, indicating a partial dissimilarity among pairs of individuals at the III distance class scales (9 m). Three of the significant values (30.0%) were significant positive, indicating similarity among individuals in the first three distance classes (I~III), i.e., pairs of individuals can separate by more than 9.0 m. Namely significant aggregations were partially observed within III classes. As a matter of course, the positive SND values (IV and V classes) and negative SND values at classes VI and VII, however they are not shown significance.

The comparison of Moran's  $I$  values to a logistic regression indicated that a highly significant percentage of individual dispersion in *C. longibracteata* populations at Mt. Yeon-ae could be explained by isolation by distance.

The overall significance of individual correlograms was tested using Bonferroni's criteria. The results revealed that patchiness similarity was shared among individuals within up to a scale of a 3.0 m~9.0 m distance. Thus it was looked for the presence of dispersion correlations between neighbors at this scale. Spatial autocorrelation by allozyme analysis for *C. longibracteata* revealed significant positive genetic correlations among plants located



<10 m, with relatedness at <3 m comparable to that expected for half sibs and first cousins [21]. This genetic structure supports the prediction that the majority of seed dispersal occurs over distances of less than 10 m and is responsible for generating substantial overlap in seed shadows within *C. longibracteata* populations [21].

In many real populations, there may not be any obvious individual populations or substructure at all, and the populations are continuous [22]. The results from this study are consistent with the supposition that a plant population is subdivided into local demes, or neighborhoods of related individuals [23, 24]. Spatially distributed genetic populations that compete locally for resources and mate only with sufficiently close neighbors, may give rise to spontaneous pattern formation [25].

### Conclusion

If by nearest neighbor parameter, the small plots (3 m x 3 m, 3 m x 6 m, 6 m x 6 m, 6 m x 12 m, 12 m x 12 m, and 12 m x 24 m) of *C. longibracteata* at Mt. Yeon-ae were uniformly distributed in the forest community. However, *C. longibracteata* were aggregate distributed in two large plots (24 m x 24 m and 24 m x 48 m). Under Eberhardt's index of dispersion for point-to-organism distances, population of *C. longibracteata* is clumping. The comparison of Moran's *I* values to a logistic regression indicated that a highly significant percentage of individual dispersion in *C. longibracteata* populations at Mt. Yeon-ae could be explained by isolation by distance.

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