

# Environmental impact on phytolith morphometric parameters by example crenate morphotype of *Dactylis glomerata* L. leaves (South of Western Siberia, Russia)

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Morphometric parameters of phytoliths are effectively applied in identifying fossil remains of cultivated grass species. The research of intraspecific trait variation in phytolith size and shape will expand the possibilities of applying morphometric studies. The aim of the study is to assess the degree of intraspecific variability of *D. glomerata* crenate phytoliths in response to coenotic and climatic factors. 6 habitats have been studied in the south of Western Siberia (Kulunda lowland and Altai mountains). A high amplitude of intraspecific and intrapopulation variability of morphometric characteristics of crenate phytoliths *D. glomerata* has been revealed. Most of the parameters correlate with the amount of annual precipitation. According to the totality of all 17 morphometric parameters, phytoliths of forest and herbaceous ecosystems differ from each other. Thus, crenate phytolith size and shape are influenced by climatic and coenotic factors.

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## Keywords

Climate effect, *Dactylis glomerata*, leaf epidermis, morphometry, phytoliths

## Introduction

Biosilification is a process inherent to a huge number of organisms. Plants are able to accumulate silicon in a significant amount and form phytoliths that repeat the shape of the cavities containing them. Due to the high phytolith stability in the environment, they are reliable markers of paleoecological conditions (Rovner 1971; Piperno and Becker 1996; Blinnikov et al. 2001; Lu et al. 2006; Khokhlova et al. 2018; Strömberg et al. 2018; Druzhinina et al. 2023). Phytolith analysis is used in many branches of science including archeology and archaeobotany (Verdin et al. 2001; Albert et al. 2008; Wang et al. 2016; Zhang et al. 2016; Ryabogina et al. 2021). The specificity of phytoliths varies in different plant taxa. The *Poaceae* phytoliths are one of the most specific ones. Morphotypes at the level of subfamilies of grasses differ significantly (Twiss et al. 1969; Twiss 2001, Lu and Liu 2003). This is used for paleoecological reconstructions based on the ratios of C3 and C4 morphotypes of *Poaceae* (Bremond et al. 2005, 2008; Biswas et al. 2021).

A high level of specificity is manifested in the morphometry of phytoliths. The study of phytolith size and shape has received its breakthrough with the identification of cultivated cereals. There has been shown the specificity of phytoliths of cultivated grasses including maize, wheat, rye, oats, barley, rice, millet at the level of genera and species (Zhijun et al. 1998; Ball et al. 1996, 1999, 2017; Portillo et al. 2006; Out and Madella 2016; Wang et al. 2019; Yost et al. 2021; Chen et al. 2023). Criteria have been developed to distinguish phytoliths of cultivated grasses from wild ones and other plants that form similar phytoliths in shape for a number of territories. Compatibility of phytolith data and, in particular, morphometry with the phylogeny of grasses allows us to come to the use of phytoliths in the taxonomy of *Poaceae* (Hoškova et al. 2022).

The widespread use of phytolith morphometry determines the relevance of studying the variability of phytolith traits within one species and the impact of environmental conditions on various parameters. So, for example, the degree of silicification is generally affected by the presence of available silica in soils (Wang et al. 2018; Sun et al. 2019). T.B. Ball and J.D. Brotherson (1992) conducted the experiment on the cultivation of two types of grasses in closed ground with an assessment of the effect of three variables on phytolith morphometry such as light, soil composition and watering. The influence of environmental factors on phytolith size was revealed, however, the authors assessed this influence as insufficiently significant. W.A. Out and M. Madella (2016) assessed the intraspecific variability of phytoliths of the *Panicum miliaceum* and *Setaria italica* species in five populations for each. The authors note not only the stability of many parameters, but also the influence of climatic conditions on some variables in *Panicum miliaceum* phytoliths. R. E. Dunn et al. showed the dependence of phytolith sizes on illumination on five types of cereals (Dunn et al. 2015). It is also worth noting the study of some plants species phytoliths, which shows that there is intraspecific variation in phytolith composition and parameters and it may be due to the influence of environmental factors (Lisztes-Szabó et al. 2014, Liu et al. 2016).

The grounds to believe that environmental factors will influence phytolith size are based on plant anatomy research. Plant anatomy research gives grounds to believe that environmental factors will influence phytolith size. Many anatomical traits of grasses are idioadaptations to environmental factors (Gibson 2009). Plants in their structure have a certain phenotypic plasticity, which increases their adaptability (Sultan 2000). The following anatomical features vary in grasses in response to changing environmental conditions: the proportions of sclerenchyma tissue, the mesophyll cell density, the stroma proportions, the size of vessels, the stomatal density, the nature of the deposition of substances in the integumentary and barrier tissues, and the epidermal thickness (Cruz et al. 1992; Thompson et al. 1992; Garnier and Laurent 1994; Wahl and Ryser 2000; Wahl et al. 2001; Han et al. 2008; Lopes et al. 2009; Abd El-Gawad and El-Amier 2017; Guo et al. 2017 and others). The research of the influence of environmental factors on the epidermal structure is

especially important for the study of grasses phytoliths. Invasive grasses have a high plasticity of the anatomical structure (Han et al. 2008). Changes in the structure of the plant epidermis in response to environmental degradation have been shown in representatives of the Asteraceae, Euphorbiaceae, and Nightshade families (Stevovic et al. 2009; Ekpemerechi et al. 2017; Okanume et al. 2017).

Morphometric phytolith analysis has achieved great success in phytolith species identification from the generative structures of cultivated grasses (Ball et al. 1996, 2017; Portillo et al. 2006), but even in the study of leaf phytoliths, differences between individual species can be traced (Out and Madella 2016; Wang et al. 2019). Among leaf phytoliths, morphometric studies are most often carried out on morphotypes that are characteristic of cultivated grasses, such as rondels (Yost et al. 2021), bulliforms (Wang et al. 2019) and bilobates (Out and Madella 2016). Similar research on wild grasses is much rarer (e.c. Lisztes-Szabó et al. 2014).

This paper focuses on the phytolith morphometry in the leaves of *D. glomerata* (cock's-foot, orchard grass, “cat grass”). The main morphotype of this species is crenate. The name is given according to ICPN 2.0 (Neumann et al. 2019) This morphotype does not occur in cultivated species and has little studied morphometrically (Lisztes-Szabó et al. 2014, Dunn et al. 2015). *Dactylis glomerata* grows in various ecological conditions and is widely distributed throughout the globe. There is evidence of a high plasticity of the size of the vegetative organs of this species, depending on the growing conditions (Ostgard and Eagles 1971; Garnier and Roy 1998; Harmens et al. 2000; Belesky 2005). The study of *D. glomerata* phytolith parameters in various habitats will allow us to assess not only the limits of variation of its main morphotype, but also the influence of environmental factors on it. Our research includes three areas:

1. evaluation of intrapopulation variability of the crenate morphotype;
2. evaluation of intraspecific variability of this morphotype in *D. glomerata*;
3. evaluation of some environmental factors affecting phytolith morphometries.

## Materials and methods

### Characteristics of the study area

The study included samples of *D. glomerata* from 6 sites (Fig. 1) in the south of Western Siberia (Altai Region and the Republic of Altai, Russia). All sites differ from each other geobotanically, climatically, and the degree of anthropogenic load (Table 1). Sites 1-3, 6 were selected on the territory of the Altai Mountains, sites 4, 5 were taken on the territory of the Kulunda lowland near the border with the Priobsky plateau. The main estimates of climatic parameters were obtained from the hydrometeorological observational data given in the Climate Handbook (Pil'nikova 1993) and data from the Roshydromet state observational network (<http://meteo.ru/>). Five plants of *D. glomerata* were collected from each site. The height of generative shoots and leaf blades from the nodes (including the leaf sheath) was measured. Since the material was collected at the end of the growing season, some of the leaves were in a dry state, so we chose the length of the largest leaf as the main indicator (Suppl. material 1: Table 13).

| № | Vegetation          | Altitude above sea level, m | Precipitation, mm/year | January temperature, °C | July temperature, °C | Coordinates          | Notes             |
|---|---------------------|-----------------------------|------------------------|-------------------------|----------------------|----------------------|-------------------|
| 1 | Hygrophilous meadow | 1471                        | 620                    | -18.4                   | 12.2                 | 51.050833°83.634167° | Horse trails      |
| 2 | Dry meadow          | 803                         | 580                    | -16.5                   | 14.1                 | 51.2931°83.341717°   | Agricultural land |
| 3 | Larch forest        | 541                         | 620                    | -17.0                   | 15.6                 | 51.284333°83.        | Cart-track        |



|   |                       |     |     |       |      |                          |                                          |
|---|-----------------------|-----|-----|-------|------|--------------------------|------------------------------------------|
|   |                       |     |     |       |      | 337667°                  |                                          |
| 4 | Fallow land           | 170 | 450 | -19.2 | 19.3 | 53.466527°81.<br>811660° | Outskirts of<br>the settlement           |
| 5 | Post-forest<br>meadow | 140 | 350 | -19.2 | 19.3 | 53.500951°81.<br>497080° | Roadside                                 |
| 6 | Fir forest            | 616 | 820 | -9.2  | 16.5 | 51.781406°<br>87.604958° | Protected<br>area, power<br>line - 200 m |

**Table 1.** Characteristics of study sites

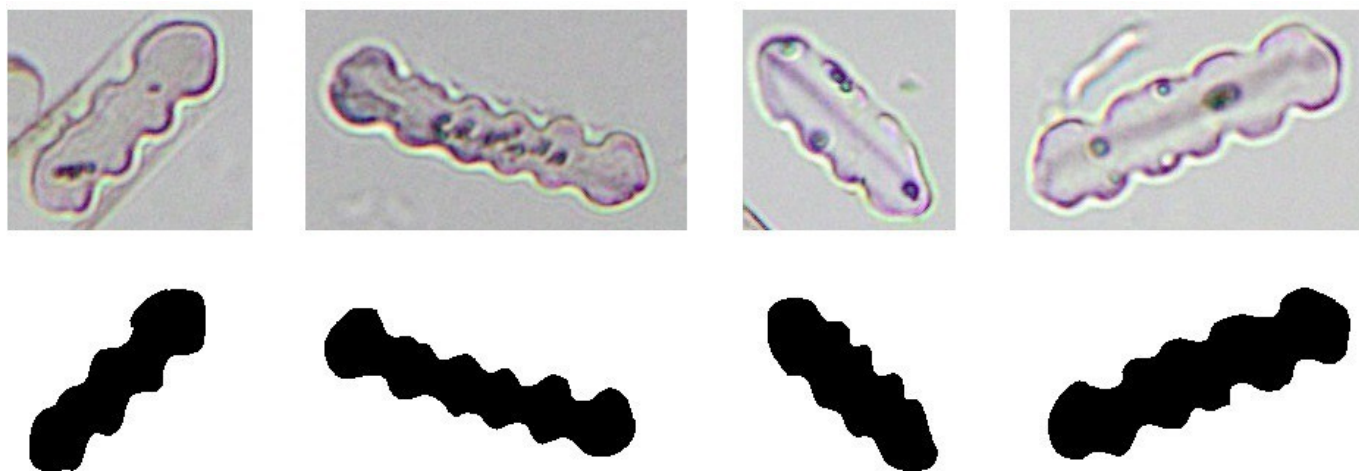


**Figure 1.** Sample collection site map. 1-6 - number of sample sites.

### Protocol of laboratory research

The study of phytoliths was carried out for basal and stem leaves together. Leaf sheaths were also included in a single sample with leaf blades. The study of the material was carried out according to the following protocol:

1. Two stem, two basal leaves of the largest size and 2 dried leaves were selected from each sample. The plant material was washed with distilled water with the addition of a surfactant.
2. Phytoliths were extracted from plant tissue samples using the modified dry oxidation technique of Golyeva (2008). Plant material was carefully rinsed with distilled water, cut into small fragments of about 5 × 5 mm, and ashed in a muffle furnace at 400 °C for 20 h.
3. The resulting ash was treated with 20% hydrochloric acid to remove solutes and washed with distilled water through a nuclear membrane with a pore size of 2 µm.
4. The obtained samples were dried in a water bath at 90°C for 20-30 minutes. A collection of *D. glomerata* phytolith specimens/samples is kept in Biodiversity research laboratory of Altai State University (Barnaul, Russia).
5. The phytoliths were studied and photographed by the Olympus BX-51 light microscope, the Olympus XC-50 camera and the CellSensStandart software.
6. The dominant morphotype of the *D. glomerata* phytolith, crenate, which is formed in short cells of the epidermis, was chosen for research analysis.
7. For morphometric studies, photographs of the morphotype were taken in the projection from above (Fig. 2).
8. The ImageJ software recommended by the International Committee on Phytolith Morphometry, as well as its PhytolithsBatch plugin were used for morphometric measurements of crenates. Standard parameters for the phytolith size and shape were studied (Ball et al. 2016) such as area, convex area, perimeter, convex perimeter, length, fiber length, width, equivalent diameter, inscribed radius, form factor, roundness, convexity, solidity, compactness, aspect ratio, elongation, curl.



**Figure 2.** Crenate phytoliths of *D. glomerata* and area of their measurement in binary format (computer program Image J).

### Evaluation of minimum adequate sample sizes and statistical data analysis

1. Five plant specimens were selected from each site for the phytolith research. 50 phytoliths were investigated from each sample. After determining the minimum sample size, we increased the number of measured phytoliths for 1, 2, 3 and 6 *D. glomerata* habitats up to 100 to analyze intrapopulation variability. The reliability of data on intraspecific variability covers the paths of plants from one site.

2. The minimum sample size was determined according to the equation recommended by the International Committee on Phytolith Morphometry (Ball et al. 2016), assuring a 90 % confidence level that the sample means are within 5 % of the actual population means on the level of plants and sites (populations):

$$N_{\min} = (Z_{\alpha/2})^2 \times S^2 / (ME)^2,$$

where:  $N_{\min}$  the minimum adequate sample size;  $(Z_{\alpha/2})^2 = 1.64$ , which is the square of the two-tailed Z value at  $\alpha = 0.10$ ;  $S^2$  = the variance, and  $(ME)^2$  = the square of the desired margin of error, in this case 0.05 the sample mean.

3. Past 4.03 software was used for statistical analysis of results. Descriptive statistics were analyzed for the marginal indicators: mean, marginal and maximum value, standard deviation.

4. ANOVA was applied to identify differences in data samples. There were carried out two analyses: the first analysis was performed between individual collection points with a sample of 250 phytoliths from each site and an analysis of differences was conducted between samples for sites 1,2,3,6 with a sample of 100 phytoliths from each sample. Tukey's post-hoc tests was used to compare pairwise characteristics of *D. glomerata* phytoliths from different collection sites and different samples.

5. Correlation analysis was carried out on the average values for each sample. The correlation with the height of the generative shoot, the length of the largest leaf, the height of growth above sea level, the annual amount of precipitation and the average temperatures of the coldest and warmest months of the year was checked. We present correlation values (Pearson's coefficient,  $r$ ) and the twotailed probabilities that the columns are uncorrelated ( $\rho$ ). We consider  $\rho$  values less than 0.05 to indicate a significant level of correlation.

6. To analyze the entire set of morphometric features of *D. glomerata* phytoliths, we used a discriminant analysis for each habitat and for groups of forest and herbaceous phytocenoses.

## Result

### Minimum sample size

The test to determine the minimum adequate sample size of sinuates was carried out at two levels: intraspecific and for individual plants. Table 2 contains the maximum of the obtained values. A comprehensive analysis is given in the Supplementary material 1: tables 1, 2. At the intraspecific level, only 7 out of 17 indicators will be reliable when sample size up to 50 specimens. The minimum adequate sample size should be between 100 and 150, respectively for two indicators, i.e. Convex Area and Roundness. Within the presented research, an adequate sample size at the level of one site is achieved by the multiplicity of sample repetitions (5 samples per site). At the level of individual plants, a sample size of 50 measurements covers from 6 to 11 parameters, depending on the population. To analyze intrapopulation variability, we selected populations 1, 2, 3, and 6, for which the sample was increased to 100 phytoliths per plant, which covers the required volume for most parameters.

| Parameters | Populations | Plant (sample) |
|------------|-------------|----------------|
|            |             |                |

|                     |     | Population number |     |     |     |    |     |
|---------------------|-----|-------------------|-----|-----|-----|----|-----|
|                     |     | 1                 | 2   | 3   | 4   | 5  | 6   |
| Area                | 90  | 60                | 100 | 80  | 110 | 80 | 100 |
| Convex Area         | 100 | 70                | 120 | 120 | 110 | 80 | 110 |
| Perimeter           | 60  | 50                | 60  | 80  | 50  | 30 | 60  |
| Convex Perimeter    | 60  | 50                | 60  | 80  | 40  | 40 | 60  |
| Length (Feret)      | 60  | 60                | 80  | 100 | 50  | 50 | 80  |
| Fiber Length        | 70  | 60                | 80  | 100 | 50  | 40 | 80  |
| Width               | 30  | 30                | 20  | 20  | 40  | 30 | 30  |
| Equivalent Diameter | 20  | 20                | 30  | 20  | 30  | 20 | 30  |
| Inscribed Radius    | 50  | 90                | 40  | 70  | 60  | 60 | 50  |
| Form Factor         | 70  | 80                | 50  | 100 | 50  | 40 | 80  |
| Roundness           | 150 | 180               | 90  | 130 | 80  | 70 | 210 |
| Convexity           | 10  | 10                | 10  | 10  | 10  | 10 | 10  |
| Solidity            | 10  | 10                | 10  | 10  | 10  | 10 | 10  |
| Compactness         | 30  | 40                | 20  | 40  | 20  | 20 | 50  |
| Aspect Ratio        | 90  | 90                | 80  | 130 | 90  | 80 | 90  |
| Elongation          | 90  | 90                | 70  | 130 | 80  | 70 | 90  |
| Curl                | 10  | 10                | 10  | 10  | 10  | 10 | 10  |

**Table 2.** The minimum required sample size for the different sampling levels, based on calculations for each sample (plant) and population separately

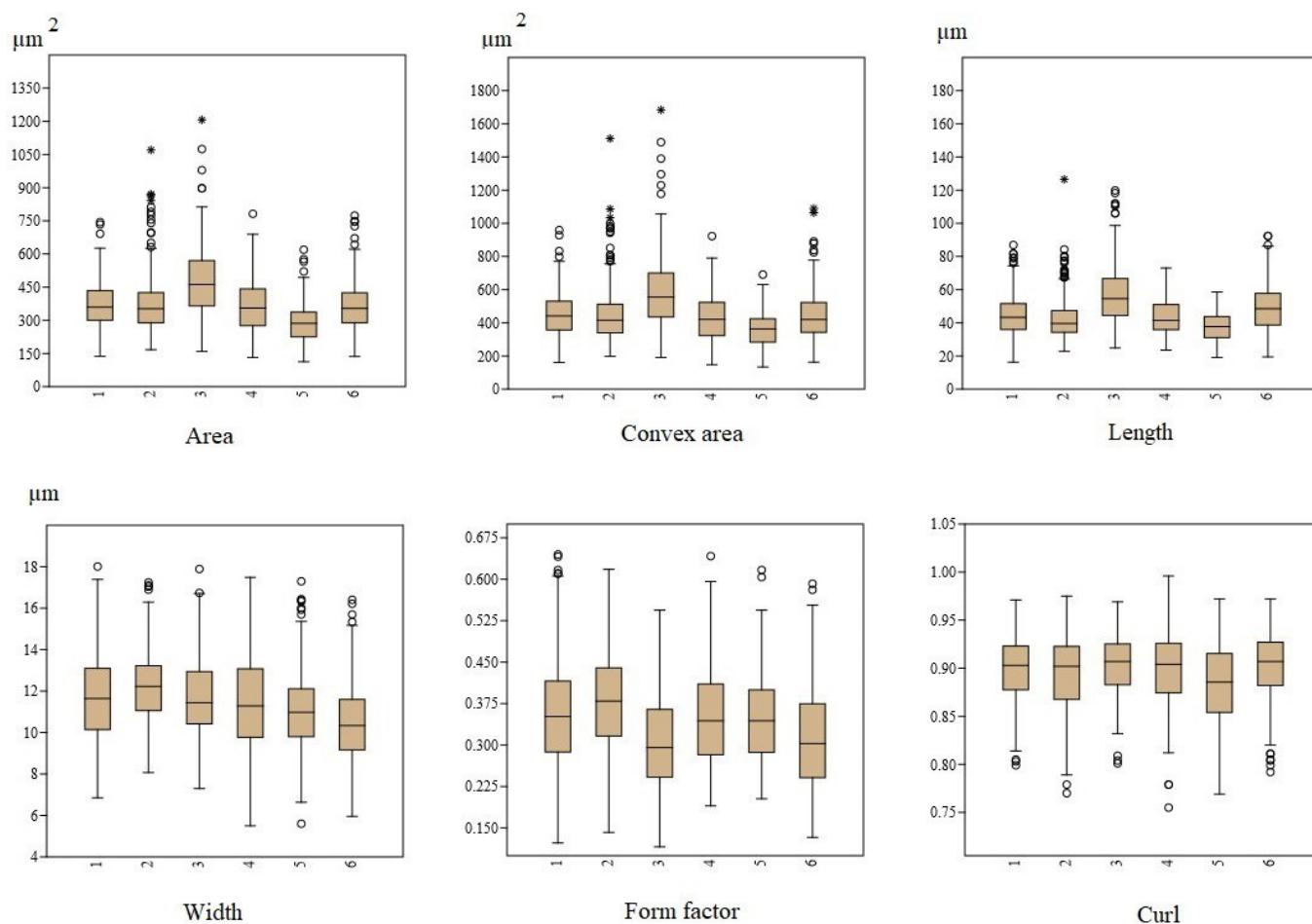
### Morphometric analysis at the intraspecific level

Descriptive statistics include measurements of 1500 phytoliths of the crenate morphotype to assess intraspecific variability. The results of statistical processing are presented in Table 3, Figure 3 and the Supplementary material 1: Table 1, 2.

ANOVA (Suppl. material 1: Table 5) showed significant differences in all parameters. Table 3 presents the average values and standard deviations of the parameters of *D. glomerata* phytoliths. We observe the similarity of *D. glomerata* phytoliths in sites 1, 2, 4 and 6 in most size parameters, except for width and inscribed radius. According to the last two parameters, phytoliths of plants from the second and sixth sites differ, which is confirmed by Tukey's test (Suppl. material 1: Table 6). Samples from site 3 have larger phytoliths than samples from other sites, most of the size parameters except for width and inscribed radius show significant differences according to the Tukey's test. Samples from site 5 are smaller, the significance of differences is also confirmed by Tukey's test. Almost all morphometric size parameters of the crenate morphotype in *D. glomerata* have close minimum values except for width and inscribed radius and high variability of maximum values.

Populations of *D. glomerata* 1, 4, and 5, as well as 3 and 6, are similar in a number of phytolith form parameters such as form factor, roundness, compactness. Plants pairs of sites 1, 4 and 3, 6 are also similar in phytolith parameters for aspect ratio and elongation. Phytoliths in site 5 differs from the rest in terms of convexity, solidity, aspect ratio and curl, as well as from sites 2 and 3 in other shape indicators. In terms of the entire set of parameters, the closest morphometric characteristics are found in samples of *D. glomerata* from site 1 (mountain hygrophilous meadow) and 4 (fallow land in steppe conditions), and the characteristics of phytoliths from site 5 differ from sites 2 and 3 in a larger number of parameters (Table 4).





**Figure 3.** The range of values of some morphometric characteristics of crenate phytoliths *D. glomerata* from different sites: 1-6 - numbers of material collection sites.

|                     | 1     |        | 2     |        | 3     |        | 4     |        | 5     |        | 6     |        |
|---------------------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|
|                     | Mn    | S. dv. | Mn    | S. dv. | Mn    | S. dv. | Mn    | S. dv. | Mn    | S. dv. | Mn    | S. dv. |
| Area                | 370.7 | 105.1  | 380.6 | 136.1  | 479.0 | 155.9  | 364.1 | 113.5  | 288.6 | 85.00  | 363.1 | 112.0  |
| Convex Area         | 450.3 | 137.0  | 455.4 | 175.2  | 586.0 | 219.8  | 434.6 | 139.7  | 356.5 | 102.0  | 444.0 | 147.7  |
| Perimeter           | 117.6 | 29.00  | 114.5 | 32.67  | 146.2 | 43.21  | 116.0 | 26.99  | 103.0 | 20.17  | 124.1 | 32.55  |
| Convex Perimeter    | 100.8 | 24.00  | 97.44 | 26.45  | 125.7 | 36.14  | 99.7  | 22.56  | 86.32 | 16.31  | 107.9 | 28.32  |
| Length (Feret)      | 44.71 | 12.18  | 42.44 | 12.98  | 57.30 | 18.00  | 44.06 | 11.23  | 37.59 | 8.214  | 49.09 | 14.44  |
| Fiber Length        | 49.77 | 13.49  | 47.48 | 14.68  | 63.58 | 20.41  | 49.10 | 12.55  | 42.55 | 9.196  | 54.37 | 15.69  |
| Width               | 11.70 | 1.973  | 12.23 | 1.792  | 11.65 | 1.815  | 11.42 | 2.340  | 11.03 | 1.928  | 10.51 | 1.846  |
| Equivalent Diameter | 21.51 | 3.100  | 21.71 | 3.635  | 24.38 | 3.938  | 21.27 | 3.371  | 18.97 | 2.799  | 21.25 | 3.268  |
| Inscribed Radius    | 3.440 | 0.908  | 3.671 | 0.791  | 3.477 | 0.904  | 3.387 | 0.856  | 3.054 | 0.832  | 3.088 | 0.792  |
| Form Factor         | 0.355 | 0.096  | 0.381 | 0.088  | 0.304 | 0.099  | 0.351 | 0.085  | 0.349 | 0.079  | 0.316 | 0.093  |
| Roundness           | 0.259 | 0.093  | 0.288 | 0.086  | 0.207 | 0.073  | 0.255 | 0.083  | 0.272 | 0.078  | 0.217 | 0.091  |
| Convexity           | 0.859 | 0.034  | 0.855 | 0.035  | 0.867 | 0.035  | 0.862 | 0.039  | 0.840 | 0.040  | 0.870 | 0.035  |

| y            |       |       |       |       |       |       |       |       |       |       |       |       |
|--------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Solidity     | 0.830 | 0.053 | 0.842 | 0.040 | 0.830 | 0.055 | 0.842 | 0.047 | 0.812 | 0.054 | 0.824 | 0.047 |
| Compactness  | 0.501 | 0.089 | 0.531 | 0.080 | 0.447 | 0.080 | 0.498 | 0.081 | 0.517 | 0.073 | 0.456 | 0.092 |
| Aspect Ratio | 3.958 | 1.362 | 3.528 | 1.097 | 5.028 | 1.740 | 4.017 | 1.290 | 3.500 | 0.967 | 4.852 | 1.737 |
| Elongation   | 4.396 | 1.484 | 3.937 | 0.895 | 5.571 | 1.931 | 4.465 | 1.395 | 3.956 | 1.055 | 5.363 | 1.869 |
| Curl         | 0.899 | 0.034 | 1.207 | 0.038 | 0.903 | 0.031 | 0.898 | 0.038 | 0.884 | 0.041 | 0.901 | 0.035 |

**Table 3.** Intraspecific variability *D. glomerata crenata*. Means (Mn) and standard deviation (S.dv.)

Notes: Three-digit numbers are rounded to tenths, two-digit numbers are rounded to hundredths, and values less than 10 are rounded to thousandths. All size measurements are in  $\mu\text{m}$  and  $\mu\text{m}^2$ . N 250 sinuetes in 5 plants measured for each mean.

|   | 2 | 3  | 4  | 5  | 6  |
|---|---|----|----|----|----|
| 1 | 8 | 12 | 1  | 14 | 11 |
| 2 |   | 12 | 7  | 17 | 12 |
| 3 |   |    | 12 | 17 | 9  |
| 4 |   |    |    | 13 | 12 |
| 5 |   |    |    |    | 11 |

**Table 4.** The number of features showing a significant level of differences in morphometric characteristics based on the Tukey's Test between pairs of data sites of the research

To identify the possibility of distinguishing *D. glomerata* phytoliths from different environmental conditions, a discriminant analysis was carried out based on the totality of all morphometric indicators (Suppl. material 1: Tables 9, 10). As a result, most of the phytoliths were classified incorrectly. The classification accuracy was 39.14%. We applied the second variant of discriminant analysis at the group level: forest and herbaceous communities (Suppl. material 1: Tables 11, 12) In this case, phytolith groups are classified correctly by 71.78%. The most significant variables for classification are area and convex area.

### **Intrapopulation variability of crenate *D. glomerata* phytolith assemblages**

To identify the variability of phytoliths between individual plant specimens, we studied populations 1, 2, 3, and 6. The sampling for each sample size was 100 phytoliths; in total, we analyzed the indicators of 2000 phytoliths (Suppl. materials: Tables 3, 4).

ANOVA results (Suppl. material: Table 7) show that among the parameters of *D. glomerata* phytoliths from site 1, the perimeter value is stable. Convex area and curl differ only in one pair of samples, which is confirmed by the Tukey's test (Suppl. material 1: Table 8). For all other size indicators, there is a difference only in one sample from the rest with the exception of width, and some shape indicators such as form factor, roundness, elongation. All other indicators are more variable. The phytoliths of the second site differ between the samples. The Tukey's test showed differences in phytoliths from one (inscribed radius) to eight pairs of samples (form factor). In addition to inscribed radius, width (3 pairs of samples), roundness, compactness, aspect ratio (2 pairs of samples) also show low variability. In population 3, all parameters have a high level of variability except for one. ANOVA showed that the inscribed radius of phytoliths does not differ between samples. In site 6, phytoliths from different samples do not differ from each other in the area parameter. Differences between one pair based on the Tukey's Test are found for convex area and equivalent diameter. One sample differs from the others in 7 parameters perimeter, convex perimeter, length, etc. The convexity and aspect ratio indicators are the most variable in phytoliths of this population.

## Influence of plant size and climatic environmental factors on the characteristics of phytoliths

**Characterization of the size of plant samples and morphometry of phytoliths.** At each site of the study, 5 specimens of plants were selected. For each plant specimen, we had the height of the generative shoot and the largest length of the basal leaf. We found the largest specimens of *D. glomerata* in mountain meadows (sites 1 and 2) and fir forests (site 6). Some specimens from the roadside meadow community (site 5) are comparable with plants from the above mentioned sites. The plants from site 4 and two specimens of site 5 have the lowest generative shoots. The leaves of specimens of site 4 are comparable with plants at other sites of the study. Correlation analysis (Table 5) showed a weak dependence of the average values of phytolith parameters in short cells of the leaf epidermis on the size of plant generative organs. Nevertheless, a significant positive correlation was found for one morphometric indicator, i.e. solidity. The largest sheet length is positively correlated with four parameters such as width, equivalent diameter, inscribed radius and solidity.

|                     | Height       |        | 2           |        | 3    |        | 4            |        | 5            |        | 6     |        |
|---------------------|--------------|--------|-------------|--------|------|--------|--------------|--------|--------------|--------|-------|--------|
|                     | r            | $\rho$ | r           | $\rho$ | r    | $\rho$ | r            | $\rho$ | r            | $\rho$ | r     | $\rho$ |
| Area                | 0.08         | 0.69   | 0.34        | 0.07   | 0.25 | 0.18   | <b>0.38</b>  | 0.04   | 0.1          | 0.62   | -0.35 | 0.06   |
| Convex Area         | 0.13         | 0.5    | 0.27        | 0.14   | 0.25 | 0.19   | <b>0.38</b>  | 0.04   | 0.11         | 0.58   | -0.33 | 0.07   |
| Perimeter           | 0.11         | 0.54   | 0.17        | 0.38   | 0.17 | 0.37   | <b>0.44</b>  | 0.02   | 0.23         | 0.23   | -0.23 | 0.22   |
| Convex Perimeter    | 0.09         | 0.65   | 0.19        | 0.31   | 0.19 | 0.32   | <b>0.5</b>   | 0.005  | 0.28         | 0.13   | -0.25 | 0.19   |
| Length (Feret)      | 0.08         | 0.66   | 0.17        | 0.37   | 0.18 | 0.34   | <b>0.53</b>  | 0.003  | 0.32         | 0.09   | -0.23 | 0.22   |
| Fiber Length        | 0.09         | 0.65   | 0.15        | 0.42   | 0.16 | 0.39   | <b>0.5</b>   | 0.005  | 0.3          | 0.11   | -0.22 | 0.25   |
| Width               | 0.19         | 0.3    | <b>0.44</b> | 0.015  | 0.26 | 0.16   | -0.15        | 0.43   | <b>-0.37</b> | 0.04   | -0.35 | 0.06   |
| Equivalent Diameter | 0.09         | 0.65   | <b>0.38</b> | 0.04   | 0.28 | 0.14   | <b>0.4</b>   | 0.03   | 0.11         | 0.57   | -0.36 | 0.046  |
| Inscribed Radius    | -0.02        | 0.9    | <b>0.54</b> | 0.002  | 0.3  | 0.1    | 0.03         | 0.87   | 0.22         | 0.23   | -0.4  | 0.03   |
| Form Factor         | -0.06        | 0.75   | 0.15        | 0.4    | 0.1  | 0.61   | -0.3         | 0.11   | 0.3          | 0.11   | -0.11 | 0.55   |
| Roundness           | 0.03         | 0.87   | 0.07        | 0.69   | 0    | 0.99   | <b>-0.47</b> | 0.01   | <b>-0.41</b> | 0.02   | -0.03 | 0.89   |
| Convexity           | -0.19        | 0.3    | 0.15        | 0.41   | 0.16 | 0.39   | <b>0.43</b>  | 0.02   | 0.33         | 0.07   | -0.15 | 0.42   |
| Solidity            | <b>-0.37</b> | 0.045  | <b>0.39</b> | 0.03   | 0.09 | 0.65   | 0.07         | 0.72   | -0.05        | 0.8    | -0.13 | 0.49   |
| Compactness         | 0.009        | 0.96   | 0.07        | 0.71   | 0.01 | 0.96   | <b>-0.5</b>  | 0.005  | <b>-0.44</b> | 0.02   | -0.01 | 0.97   |
| Aspect Ratio        | -0.004       | 0.98   | 0           | 0.99   | 0.06 | 0.75   | <b>0.59</b>  | 0.001  | <b>0.49</b>  | 0.01   | -0.06 | 0.74   |
| Elongation          | 0.003        | 0.99   | -0.02       | 0.93   | 0.05 | 0.8    | <b>0.57</b>  | 0.001  | <b>0.48</b>  | 0.01   | -0.05 | 0.79   |
| Curl                | -0.1         | 0.6    | 0.25        | 0.18   | 0.27 | 0.14   | <b>0.47</b>  | 0.01   | 0.27         | 0.15   | -0.27 | 0.14   |

**Table 5.** Correlation analysis\*

Notes: Height - generative shoot height, leaf length - longest leaf length, precipitation - annual amount of precipitation, altitude - height above sea level, T<sub>01</sub> - january average temperature, T<sub>07</sub> - july average temperature; r - Pearson's coefficient,  $\rho$  - two-tailed probabilities.

**Correlation of morphometric characteristics with some climatic indicators.** We did not find a significant correlation between the height of plant specimens above sea level and the average values of the phytolith morphometric characteristics. To the greatest extent, the size and shape of phytoliths correlate with the amount of annual precipitation. 11 parameters show a positive correlation and 2 parameters show a negative one (Table 5). Three phytolith parameters have a negative correlation coefficient with the average January temperature, while two phytolith parameters have a positive one. Two morphometric indicators have a negative correlation with the value of the average July temperature.

## Discussion

### Minimum sample size

Sample size data show high variability in the studied phytolith morphotype. The most variable parameters are Area, Convex Area and Roundness, they require the largest sample of phytoliths for morphometry. Only 5 parameters of crenates are estimable with a sample size of 50 or less. Other phytolith morphotypes often require a smaller sampling for morphometric analysis. For example, for rondels of inflorescences in barley (*Hordeum*) and wheat (*Triticum*), morphometric data will be reliable for sample size from 5 for shape morphometry to 15-45 for size morphometry (Ball et al. 1999), most rondel parameters in oat (*Avena*) will be correct at 50 sampling (Portillo et al. 2006). W.A. Out and M. Madella (2016) showed that in the morphometry of bilobate phytoliths from *Panicum miliaceum* and *Setaria italica* leaves, a sample size of 50 phytoliths is not enough for many parameters, and in some cases it is advisable to measure up to 165 particles. Our data are consistent with the studies of bilobate phytoliths conducted by W.A. Out and M. Madella (2016) and indicate a high plasticity of the parameters of leaf phytoliths, in contrast to the results obtained on phytoliths of short inflorescence cells. A sample size of 100 and above is recommended for many parameters of long dendritic cells in inflorescences, while aspect ratio, roundness, and some others will be reliable with a sample of less than 50 (Ball et al. 2017).

It is worth noting that crenates in the leaves of *D. glomerata* in different populations have different plasticity. Two sites numbered 4 and 5 turned out to be less demanding on the minimum sample value. They differ from the other four sites by being located in a more arid climate in disturbed habitat. Possibly, climatic or anthropogenic factors influence the variability of phytolith sizes.

### Morphometric analysis

Analyzing the climatic and geobotanical indicators of the growing conditions of the studied populations, we assumed that plants from pairs of sites 2, 3 and 4, 5 would have similar sizes of phytoliths, since these pairs of material collection points are located in similar physical and geographical habitats and are close to each other. Nevertheless, we observe that phytoliths from site 1 (hygrophilous meadow), which differ in hydrothermal regime. The phytoliths of these two populations are of medium size and shape. At the same time, there is a commonality of a number of indicators for phytoliths of two forest populations (3 and 6). Despite the differences in the geobotanical and climatic characteristics of these two sites, the crenate phytoliths of *D. glomerata* have the most irregular and elongated shape. Moreover, plant phytoliths from these two sites have a greater length. The results obtained for *D. glomerata* phytoliths from forest communities are consistent with model experiments on the effect of illumination on phytoliths (Ball and Brotherson 1992; Dunn et al. 2015). Having studied the works on the effect of light on plant anatomy (Knapp and Gilliam 1985; Allard et al. 1991; Marques et al. 1999.), R. Dunn and his research team suggest that plants under high solar radiation have short cells and a shorter shape due to an increase in the number of stomata and trichomes (Dunn et al. 2015). The light factor also affects the leaf area for *Dactylis glomerata*, i.e. the leaf area in young plants increases with a decrease in light intensity (Belesky et al. 2005). This fact can also affect the enlargement of epidermal short cells in grasses of forest phytocenoses. T.B. Ball and J.D. Brotherson showed a

decrease in the size of *Panicum virgatum* phytoliths under more lit conditions under the experiment (Ball and Brotherson 1992). In the present study, phytoliths are the smallest in the hottest and driest environmental conditions (site 5), despite the fact that they are in partial shade at the edge of the forest. As a result of the correlation analysis, we found that most morphometric characteristics depend on the indicators of the annual precipitation level. The impact of climatic factors on size is shown for short cells and lanceolate phytoliths of *Phragmites communis* (Liu et al. 2016). Thus, it can be assumed that the level of illumination is indirect. It affects the evaporation of moisture and even its lack in plants can cause shredding of short cells of integumentary tissues. Phytoliths of *D. glomerata* from the fallow (site 4), which is under the same climatic conditions as site 5, are larger (but smaller than in forests). Possibly tall fallow vegetation (*Artemisia*, *Urtica*) prevents excessive drying of the soil, and there is also a more favorable soil composition due to fertilization and plowing in the past. Thus, the morphometric parameters of phytoliths are affected by a diverse set of factors.

Despite the identified differences between individual populations resulting in multivariate analysis, we see sufficient homogeneity of the data obtained. The classification accuracy is less than 40% when trying to detect differences between all 6 sites, which is a low value for this method. For example, the classification accuracy of bilobate leaf phytoliths at the species level between *Panicum miliaceum* and *Setaria italica* is 88% as identified by Out and Madella (2016). Thus, despite the high variability of morphometric parameters of phytoliths, our research confirms sufficient intraspecific specificity of size and shape. At the same time, we observe a good accuracy of classification by the discriminant analysis method when dividing into forest and herbaceous communities, which indicates the influence of ecological and cenotic factors on the size and shape of phytoliths. The results obtained are consistent with studies of phytolithic complexes of background soils in the Altai Mountains phytocenoses. The lobed and more elongated form of the crenate morphotype (polylobate trapeziform) is more often found in forest and subalpine mountain communities, while the wavy, shorter and flattened form (wavy plates) is found in petrophytic steppe and shrubby phytocenoses (Solomonova et al. 2019a, 2019b). The first form is produced by *Dactylis glomerata*, the genera *Agriostis*, *Calamagrostis*, *Melica*, and the second by *Agropyron*, *Koeleria*, some species of *Festuca* and *Poa*. The analysis revealed the systematic significance of these two forms of crenates at the level of tribes and subtribes (Solomonova et al. 2023). Morphometric studies of the crenate morphotype at the taxonomic and phytocenotic levels may be promising in solving problems of the evolution and ecology of grasses.

## Conclusions

The analysis of the morphometric parameters of crenate phytoliths (*D. glomerata*) reveals a high level of variation in the phytolith size and shape. In addition to the revealed influence of coenotic and climatic factors on the phytolith parameters, the influence of other environmental indicators, for instance, soil properties, is also to be studied in the future. The variability of crenate phytoliths limits their use to distinguish between individual grass taxa, compared to other short particles. It may be possible to achieve less variable results when examining leaf sheaths and leaf blades separately. The obtained data on the dependence of the phytolith size and shape on the precipitation index and the type of phytocenosis indicate that this phytolith morphotype is promising as an ecological index (proxy index). The development of this research direction requires morphometric studies of crenate phytoliths in cereal species with different requirements for environmental conditions and the study of these phytoliths in surface soils of various phytocenoses.

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## Supplementary material 1

**Table 1. Descriptive statistics and minimum sample size for intraspecific variability of crenate phytoliths *Dactylis glomerata* L. (Size).**

**Table 2. Descriptive statistics and minimum sample size for intraspecific variability of crenate phytoliths *Dactylis glomerata* (Form).**

**Table 3. Descriptive statistics on intrapopulation variability of crenate phytoliths *Dactylis glomerata* L. (Size).**

**Table 4. Descriptive statistics on intrapopulation variability of crenate phytoliths *Dactylis glomerata* L. (Form).**

**Table 5. Analysis of variance of intraspecific differences in crenate phytoliths *Dactylis glomerata*.**

**Table 6. Significance of Tukey's criteria for intraspecific differences in crenate phytoliths of *Dactylis glomerata* .**

**Table 7. ANOVA of intrapopulation differences in crenate phytoliths of *Dactylis glomerata* on the example of sites 1, 2, 3, 6.**

**Table 8. The number of pairs with significant differences according to the Tukey test (%)**

of the total number of matched pairs).

**Table 9. Discriminant analysis of crenate parameters of *D. glomerata* phytoliths from different research sites. Confusion matrix.**

**Table 10. Discriminant analysis of crenate parameters of *D. glomerata* phytoliths from different research sites.**

**Table 11. Discriminant analysis of crenate parameters of *D. glomerata* phytoliths from various groups of plant communities.**

**Table 12. Discriminant analysis of crenate parameters of *D. glomerata* phytoliths from various groups of plant communities.**

**Table 13. Plant sample sizes.**

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Data type: tables

Explanation note: The tables present the results of morphometric studies of *D. glomerata* crenate phytoliths.

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