Turczaninowia 28, 3: 145–153 (2025)
https://www.doi.org/10.14258/turczaninowia.28.3.17
http://turczaninowia.asu.ru

ISSN 1560–7259 (print edition)
TURCZANINOWIA
ISSN 1560–7267 (online edition)

УДК 582.52/.59:581.821

Two new morphometric parameters proposed to classify CRENATE-phytoliths of the *Pooideae* Benth.

M. Yu. Solomonova[1, 5]*, A. D. Lyashchenko[1, 2, 6], G. A. Speranskij[1, 7],
M. S. Blinnikov[3, 4, 8], N. Yu. Speranskaya[1, 9]

[1] Altai State University, Lenina Pr., 61, Barnaul, 656049, Russian Federation

[2] Altai Regional Children's Ecological Center, Barnaul, Parkovaya St., 7, 656045, Russian Federation

[3] St. Cloud State University, 4th Avenue South, 720, St. Cloud, MN 56301-4498, United States

[4] Institute of Archaeology and Ethnography SB RAS, Akademika Lavretieva Pr., 17, Novosibirsk, 630090, Russian Federation

[5] E-mail: m_solomonova@list.ru; ORCID iD: https://orcid.org/0000-0002-4154-6048

[6] E-mail: aleny-lea@mail.ru. ORCID iD: https://orcid.org/0000-0003-1135-902X

[7] E-mail: speranskyg@gmail.com. ORCID iD: https://orcid.org/0009-0002-4546-1161

[8] E-mail: msblinnikov@stcloudstate.edu; ORCID iD: https://orcid.org/0000-0003-1069-6307

[9] E-mail: speranskaj@mail.ru; ORCID iD: https://orcid.org/0000-0003-0036-3760

** Corresponding author*

Keywords: grasses, *Meliceae,* phytoliths, *Poeae*, submorphotypes, *Triticeae*.

Summary. The article proposes two additional morphometric parameters (L1 and L2) for classifying Crenate phytoliths of grass leaves into two submorphotypes: Crenate_lobate (CRE_LOB) and Crenate_sinuate (CRE_SIN). L1 characterizes the size of the lobe (wave), and L2 – the degree of its expression. Both morphometric parameters are calculated based on width and inscribed radius characteristics. The study examined 12 species of grasses belonging to three tribes (*Poeae*, *Meliceae* and *Triticeae*). Six species of grasses predominantly have CRE_LOB phytoliths, while the other six have CRE_SIN. Statistical data analysis revealed differences in the phytoliths of the *Meliceae* tribe based on classical morphometric characteristics (width and inscribed radius). The inscribed radius has similar values in the phytoliths of two closely related subtribes of *Poeae* (*Agrostidinae*, *Aveninae*). The two newly proposed parameters (L1 and L2) do not have obvious taxonomic affinity, but they do highlight the differences between the two submorphotypes. Based on our results, boundaries of these morphometric characteristics are proposed to separate the two submorphotypes explicitly. For L1, the critical values are: L1 > 2 for CRE_LOB and L1 < 1 for CRE_SIN. L2 is the most accurate morphometric characteristic for distinguishing submorphotypes, and an exact value for such classification is proposed: CRE_SIN < 1.35 < CRE_LOB.

Два новых морфометрических параметра для классификации CRENATE-фитолитов *Pooideae* Benth.

М. Ю. Соломонова[1], А. Д. Лященко[1, 2], Г. А. Сперанский[1], М. С. Блинников[3, 4], Н. Ю. Сперанская[1]

[1] Алтайский государственный университет, пр. Ленина, д. 61, г. Барнаул, 656049, Россия

[2] Алтайский краевой детский экологический центр, ул. Парковая, д. 7, г. Барнаул, 656045, Россия.

[3] Государственный университет Сент-Клауд, 4-й Южный просп., д. 720, г. Сент-Клауд, Миннесота, MN 56301-4498, США

[4] Институт археологии и этнографии СО РАН, пр. Академика Лаврентьева, д. 17, г. Новосибирск, 630090, Россия

Поступило в редакцию 28.04.2025
Принято к публикации 18.09.2025

Submitted 28.04.2025
Accepted 18.09.2025

Ключевые слова: злаки, фитолиты, подморфотипы, *Poeae*, *Meliceae* и *Triticeae*.

Аннотация. В статье предложены два дополнительных морфометрических параметра (L1 и L2) для классификации Crenate-фитолитов листьев злаков на два подморфотипа: Crenate_lobate (CRE_LOB) и Crenate_sinuate (CRE_SIN). L1 характеризует размер лопасти (волны), L2 – степень её выраженности. Оба морфометрических параметра рассчитаны на характеристиках ширины и вписанного радиуса. В ходе исследования изучено 12 видов злаков, относящихся к трём трибам (*Poeae*, *Meliceae* и *Triticeae*). У половины видов преобладают фитолиты CRE_LOB, у остальных – CRE_SIN.

Статистический анализ данных выявил отличия у фитолитов трибы *Meliceae* по классическим морфометрическим характеристикам (ширина и вписанный радиус). Вписанный радиус имеет близкие значения у фитолитов двух близких потриб *Poeae* (*Agrostidinae*, *Aveninae*). Два новых предложенных параметра (L1 и L2) не имеют очевидных таксономических свойств, но описывают различия между подморфотипами с высокой точностью. По результатам исследования предложены границы этих морфометрических характеристик, разделяющие два подморфотипа. Для L1 крайними значениями являются L1 > 2 для CRE_LOB и L1 < 1 для CRE_SIN. L2 является наиболее точным морфометрическим параметром для выделения подморфотипов, для него предложено точное значение для классификации двух форм: CRE_SIN < 1,35 < CRE_LOB.

Introduction

Crenate (CRE) is an important morphotype of *Pooideae* Benth. phytoliths for taxonomic, paleoecological, and archaeobotanical studies in temperate regions (Blinnikov et al., 2001; Gao et al., 2018a; Romanis et al., 2021). It forms in the short cells of leaf epidermis (Newman, 2019) but often has a rather elongated shape. This does not correspond to the classical understanding of short cells (Esau, 1965) but is accepted in the international phytolith nomenclature (ICPN 2.0, Newman, 2019). In various parts of Eurasia, CRE is more abundantly found under forests and meadows (Blinnikov et al., 1994, 2021; Golyeva, 2001, 2007; Gao et al., 2018a, b). However, there are other studies disputing this fact. In the work of Xu et al., in Northwestern China, it was noted that CRE is used as an indicator of cool and probably dry habitat conditions (Xu et al., 2024). In a recent study of the Qilian Mountains (Li et al., 2024), it was suggested that the relative abundance of CRE increases with altitude up to the subalpine zone, and then slightly decreases in alpine communities (Liu et al., 2024). Differences in the interpretation of the ecological signal of this morphotype may be due to the fact that its individual forms are associated with different, sometimes ecologically opposite, plant communities.

In an early paper on the subject, Kiseleva identified two submorphotypes: “wavy plates” (e. g., in *Koeleria* Pers. and *Agropyron* Gaertn, Crenate_sinuate in ICPN 2.0) and ”double-contour” forms with deeper lobes (*Calamagrostis* Adans. and *Poa* L., Crenate_lobate in ICPN 2.0) (Kiseleva, 1989). Her study did not gain wide recognition, possibly due to the lack of precise criteria for dividing into wavy and lobed morphotypes, and also because it was published only in Russian.

Examples of recent studies on Crenate in Southwestern Siberia (the Altay region) show a relatively high proportion of CRE_SIN in the surface soils of some plant communities from steppes and mountain petrophytic communities in southern Western Siberia (Silantiyeva et al., 2018; Solomonova et al., 2019a, b; Solomonova et al., 2025). CRE_LOB has a different ecological signal indicating more moist communities, e. g., meadows and forests) (Solomonova et al., 2025). There are also data on the taxonomic affiliation of these two forms (Solomonova et al., 2022) to certain groups (tribes or subtribes) of *Pooideae*, which confirms the division made by N. K. Kiseleva (1989).

Morphometric studies of CRE_LOB and CRE_SIN in orchard grass (*Dactylis glomerata* L.) and junegrass (*Koeleria cristata* (L.) Pers.) indicate that the most important parameters for distinguishing these two submorphotypes are elongation, aspect ratio, convexity, and solidity. However, these and other studies have shown that within a single species there is significant variation in parameters depending on ecological conditions (Solomonova et al., 2023). Moreover, both submorphotypes include both small and large forms, making the application of classical morphometric parameters challenging (Solomonova et al., 2025).

This paper aims to introduce and test two additional morphometric parameters that would allow even better quantification of shape difference between CRE_SIN and CRE_LOB.

Materials and methods

We studied 12 species of *Pooideae* Benth. subfamily of grasses common in our region, whose phytolith assemblages have one or the other form (more than 90 % of all CRE) commonly found (Table 1, Fig. 1). Genus *Melica* L. has some confusing subtypes (no more than 20 %), but we included them as well because they generally have smaller sized phytoliths and to increase overall taxonomic diversity of species assessed. We followed the extraction protocol published earlier (Solomonova et al., 2023).

Table 1. Taxonomic affinity, basic submorphotype of CRENATE and the number of phytoliths studies per species

№	Species	n of phytoliths	Basic CRE-submorphotype	Tribe / subtribe(*)
1	*Dactylis glomerata* L.	305	CRE_LOB	*Poeae* R. Br. / *Dactylidinae* Stapf
2	*Agrostis gigantea* Roth	210	CRE_LOB	*Poeae* / *Agrostidinae* Fr.
3	*Calamagrostis epigeios* (L.) Roth	373	CRE_LOB	*Poeae* / *Agrostidinae*
4	*Melica nutans* L.	199	CRE_LOB	*Meliceae* Link. ex Endl.
5	*Melica altissima* L.	107	CRE_LOB	*Meliceae*
6	*Glyceria triflora* L.	111	CRE_LOB	*Meliceae*
7	*Agropyron pectinatum* (M. Bieb.) P. Beauv.	204	CRE_SIN	*Triticeae* Dumort.
8	*Agropyron cristatum* (L.) Gaertn.	277	CRE_SIN	*Triticeae*
9	*Elymus excelsus* Turcz. ex Griseb.	104	CRE_SIN	*Triticeae*
10	*Leymus racemosus* (Lam.) Tzvelev	105	CRE_SIN	*Triticeae*
11	*Koeleria cristata* (L.) Pers.	301	CRE_SIN	*Poeae* / *Aveninae* J. Presl
12	*Koeleria delavignei* Czern. ex Domin	224	CRE_SIN	*Poeae* / *Aveninae*

Note: * – tribes and subtribes affiliations follow Soreng et al. (2017, 2022).

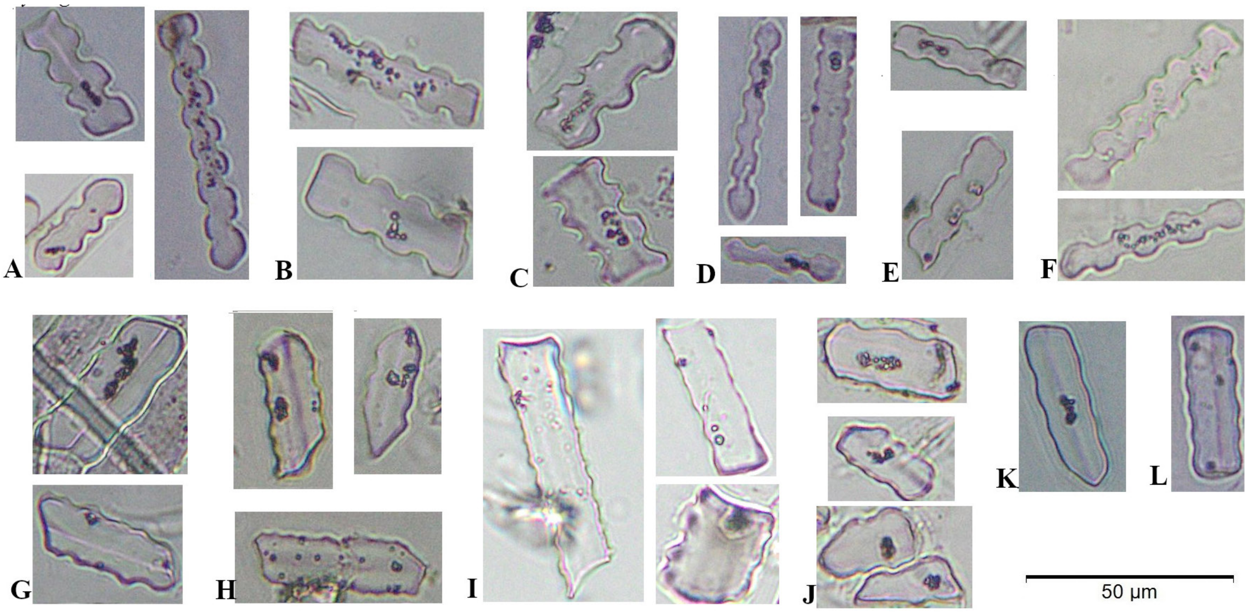

Fig. 1. Examples of typical CRENATE-phytoliths in the studied grass species: A – *Dactylis glomerata* L.; B – *Agrostis gigantea* Roth; C – *Calamagrostis epigeios* (L.) Roth; D – *Melica nutans* L.; E – *Melica altissima* L., F – *Glyceria triflora* L., G – *Agropyron pectinatum* (M. Bieb.) P. Beauv., H – *Agropyron cristatum* (L.) Gaertn.; I – *Elymus excelsus* Turcz. ex Griseb.; J – *Leymus racemosus* (Lam.) Tzvelev; K – *Koeleria cristata* (L.) Pers.; L – *Koeleria delavignei* Czern. ex Domin.; A–F – CRE_LOB; G–I – CRE_SIN.

Phytoliths were studied under an Olympus BX-51 optical microscope, an Olympus XC-50 camera, and cellSens Standart software. The magnification used was ×20. Microphotographs of the phytoliths were taken from the upper base side of the phytolith (OPS). For morphometric measurements of Crenate, the ImageJ program with the “PhytolithsBatch” plugin, recommended by the International Committee for Phytolith Morphometry, was used. Standard, widely accepted morphometric parameters of phytolith size and shape were measured (Ball et al., 2016). One hundred phytoliths were measured per sample. For each species, one to three leaves from different plants were sampled and examined.

In this study, we propose two new morphometric parameters (L1 and L2) characterizing the differences between lobed (CRE_LOB) and wavy (CRE_SIN) phytoliths. These characteristics were calculated using the maximum width of the phytolith and the inscribed radius (radius of the inscribed circle).

The size of the largest lobe of the phytolith (L1) is calculated as:

$L1 = (\text{Width} - 2 \times \text{Inscribed Radius}) / 2$

The degree of lobe expression is calculated as:

$L2 = \text{Width} / (2 \times \text{Inscribed Radius})$

Statistical processing of the results included the following types of analysis: Shapiro-Wilk test to assess the normality of each sample distribution and basic descriptive statistics (the mean and the standard deviation). For the studied parameters L1 and L2, data visualization graphs (violin plot and box plot: median, quartiles, outliers) were constructed, and a one-way analysis of variance (ANOVA) was performed to assess significance of the mean difference for the parameters between groups of grasses with dominance of CRE_SIN vs. CRE_LOB. At the species level, a post-hoc Tukey HSD test was performed for pairwise comparisons.

Results

The Shapiro-Wilk test confirmed that the analyzed samples conform to a normal distribution ($p < 0.05$). The width and inscribed radius in both identified groups (dominated by CRE_LOB or CRE_SIN) vary (Table 2). Among the species group with a predominance of CRE_LOB, species with smaller phytolith widths are more common. However, some species with CRE_SIN phytoliths have a lower average width compared to the phytoliths of *A. gigantea* Roth. The inscribed radius of CRE_LOB phytoliths is smaller than that of CRE_SIN, but the most reliable differences are observed in the representatives of the *Meliceae* Link. ex Endl. tribe. In contrast, the values of this parameter in representatives of *Agrostidinae* Fr. (*A. gigantea* and *C. epigeios* (L.) Roth) are comparable to the phytoliths of representatives of *Aveninae* J. Presl.

Table 2. Mean and standard deviation of some morphometric parameters in studied grasses

Dominant phytolith morphotype												
	CRE_LOB						CRE_SIN					
Species	*D. glomerata*	*A. gigantea*	*C. epigeios*	*M. nutans*	*M. altissima*	*G. triflora*	*A. pectinatum*	*A. cristatum*	*E. exelsus*	*L. racemosus*	*K. cristata*	*K. delavignei*
Width												
Mean	11.7	13.0	12.3	8.0	9.3	9.3	15.8	13.0	15.5	11.3	11.8	11.5
SD.	2.14	2.39	2.51	1.61	1.22	1.56	3.03	2.73	2.36	2.54	2.26	1.82
Inscribed radius												
Mean	3.5	4.4	4.0	2.5	3.2	2.5	6.5	5.2	6.2	4.7	4.9	4.7
SD	0.94	0.89	0.95	0.78	0.51	0.62	1.30	1.23	0.99	1.11	1.06	0.77
L1												
Mean	2.3	2.1	2.2	1.5	1.5	2.3	1.3	1.3	1.5	0.97	0.99	1.1
SD	0.87	0.77	0.79	0.48	0.49	0.70	0.56	0.58	0.64	0.56	0.35	0.42
L2												
Mean	1.7	1.5	1.6	1.7	1.5	1.9	1.2	1.3	1.3	1.2	1.2	1.3
SD	0.41	0.20	0.27	0.34	0.20	0.45	0.09	0.14	0.10	0.14	0.09	0.11

L1 is a characteristic reflecting the size of the lobe (Table 2, Fig. 2). In the representatives of the first group, this parameter is expectedly larger, but there is a significant overlap of data between the small phytoliths of the first group in *Melica* and the large phytoliths of the second group from the *Triticeae* Dumort. tribe. When analyzing the data between the two conditional groups (Fig. 2), it is evident that representatives with a predominance of CRE_SIN phytoliths have, on average, smaller lobes.

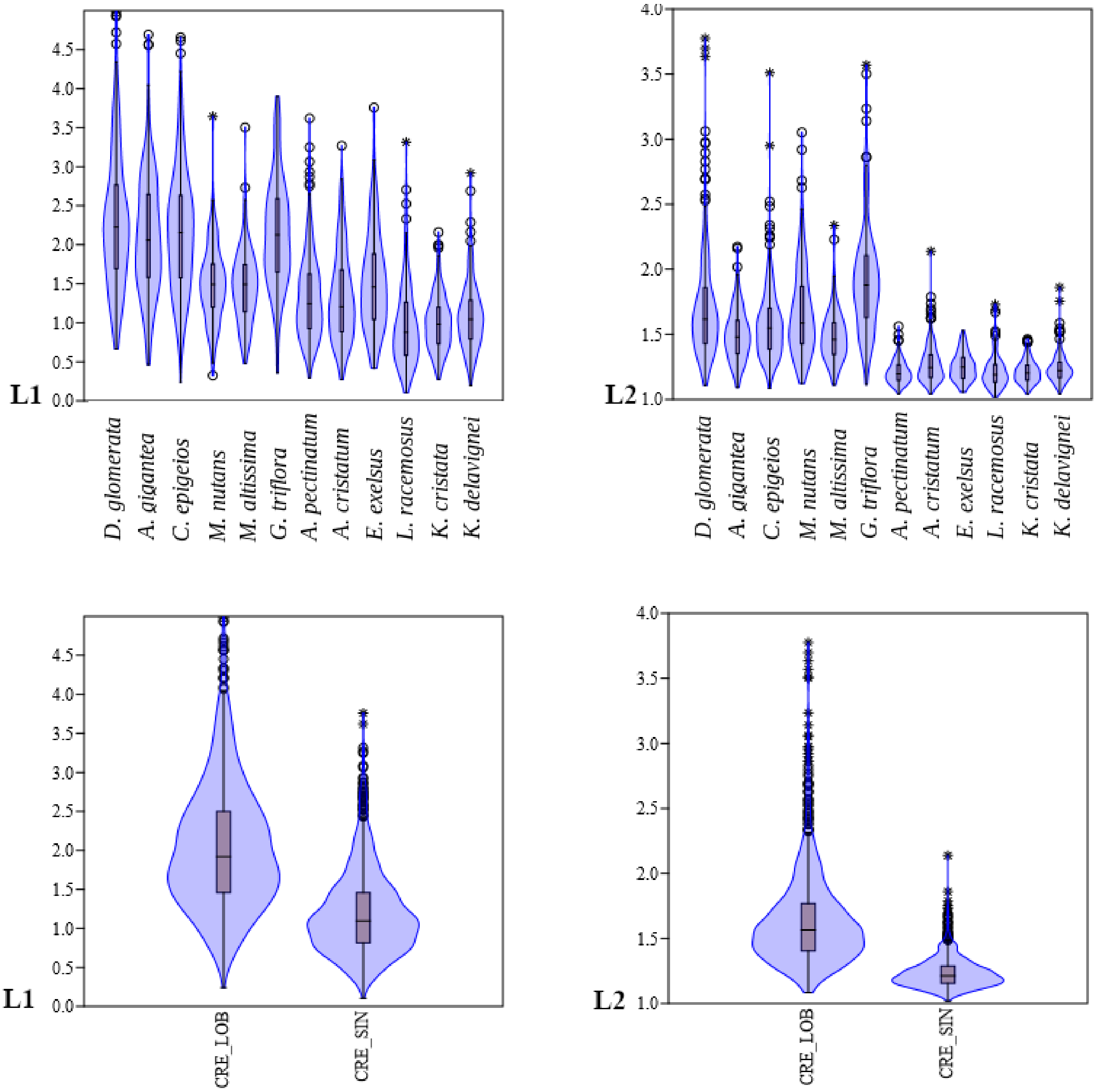

Fig. 2. Variability of L1 and L2 parameters between different species (upper graphs) and grass groups (lower graphs).

L2 characterizes the degree of lobe expression relative to the size of the phytolith (width). This parameter shows the greatest differentiation of phytoliths into two submorphotypes (Table 2, Fig. 2). All species with a predominance of CRE_SIN have less pronounced lobes and, despite their different taxonomic positions in different tribes, form a single group. The overlap of data for L2 between the groups (Fig. 2) is significantly smaller than for L1.

Analysis of variance indicates differences between species for both studied parameters (for L1: F = 135, $p < 0.001$; for L2: F = 179, $p < 0.001$). When comparing individual samples by species using the Tukey test (Table 3), no clear patterns are observed between the phytoliths of the two grass groups for the L1 parameter; however, differences are observed between the phytoliths within one group. For the L2 phytolith characteristic, differences between the conditional species groups are pronounced (Table 3). Within the group dominated by CRE_LOB, there are cases of differences between even closely related species (e. g., *M. altissima* L. and *G. triflora* L.). Within the group dominated by CRE_SIN, such differences are not observed. All species in this group are characterized by Crenate phytoliths with similar L2 values.

Table 3. Post-hoc Tukey HSD test to assess significance of difference between means of the two morphometric parameters L1 and L2

	L1										
	CRE_LOB					CRE_SIN					
	A. gigantea	*C. epigeios*	*M. nutans*	*M. altissima*	*G. triflora*	*A. pectinatum*	*A. cristatum*	*E. exelsus*	*L. racemosus*	*K. cristata*	*K. delavignei*
D. glomerata	–	–	+	+	–	+	+	+	+	+	+
A. gigantea		–	+	+	–	+	+	+	+	+	+
C. epigeios			+	+	–	+	+	+	+	+	+
M. nutans				–	+	–	–	–	+	+	+
M. altissima					+	–	–	–	+	+	+
G. triflora						+	+	+	+	+	+
A. pectinatum							–	–	+	+	+
A. cristatum								–	+	+	+
E. exelsus									+	+	+
L. racemosus										–	–
K. cristata											–
	L2										
	CRE_LOB					CRE_SIN					
	A. gigantea	*C. epigeios*	*M. nutans*	*M. altissima*	*G. triflora*	*A. pectinatum*	*A. cristatum*	*E. exelsus*	*L. racemosus*	*K. cristata*	*K. delavignei*
D. glomerata	+	+	–	+	+	+	+	+	+	+	+
A. gigantea		+	+	–	+	+	+	+	+	+	+
C. epigeios			–	–	+	+	+	+	+	+	+
M. nutans				–	+	+	+	+	+	+	+
M. altissima					+	+	+	+	+	+	+
G. triflora						+	+	+	+	+	+
A. pectinatum							–	–	–	–	–
A. cristatum								–	–	–	–
E. exelsus									–	–	–
L. racemosus										–	–
K. cristata											–

Discussion

The obtained results of the study of some morphometric characteristics show that the parameters of width and inscribed radius cannot be significant characteristics for dividing phytoliths into two Crenate groups. Similar results were previously reported for other morphometric characteristics using *D. glomerata* and *K. cristata* (Solomonova et al., 2025). An exception is the inscribed radius of phytoliths of the *Meliceae* tribe, which are distinguished by small sizes (Solomonova et al., 2024). This is likely due to the significant phylogenetic isolation of the tribe from the other two studied tribes (Soreng et al., 2017; Arthan et al., 2024). Besides the features of CRE phytoliths of *Meliceae*, another taxonomic pattern is observed in the values of the inscribed radius. This parameter in the phytoliths of *Agrostidinae* (*A. gigantea* and *C. epigeios*) and *Aveninae* is similar, reflecting the belonging of these grasses not only to the same tribe but also to the same chloroplast group 1 (Soreng et al., 2022).

The obtained morphometric data for the two new characteristics show a significant spread of data from their mean value. This is another proof of the problem of phytolith redundancy, meaning that several morphotypes or submorphotypes can form within one species (e. g. Lisztes-Szabo et al., 2014). Thus, despite the dominance of one submorphotype (CRE_LOB or CRE_SIN) in each specific grass, phytoliths with different degrees of lobe expression can also be found. This property is important for determining specific values for the classification of the two submorphotypes when analyzing the obtained data distribution.

The study results indicate that the L2 parameter is the one most suited for classifying the two submorphotypes. The larger range of data overlap observed for L1 between phytolith groups suggests that it can only be used at its extreme values.

The graphs illustrating the differences between the two phytolith submorphotypes in Figure 2 allow proposing the following values for determining the phytolith submorphotype: L2 > 1.35 = CRE_LOB, L2 < 1.35 = CRE_SIN. L1 > 2 and any value of L2 = CRE_LOB, L1 < 1 and any value of L2 = CRE _ SIN. The proposed boundaries of the two parameters are preliminary and require additional testing on a larger number of plant species and phytoliths from modern soils. In controversial cases, it is necessary to study additional morphometric characteristics of phytoliths (elongation, aspect ratio, degree of concavity and convexity), which were previously studied using two species (Solomonova et al., 2025).

L1 and L2 parameters can potentially be applied not only to CRENATE phytoliths but also to other lobed morphotypes, such as TRAPEZIFORM BILOBATE of feather grass (Solomonova et al., 2019), CROSS, BILOBATE, and POLYLOBATE in *Panicoideae* Link. (Neumann et al., 2019).

Conclusion

The proposed morphometric parameters have high potential for classifying the two submorphotypes of CRENATE (CRENATE_LOBATAE and CRENATE_SINUATE). Since these parameters describe the expression of lobes (waves) and their size, they are more significant for determining the studied submorphotypes than classical morphometric parameters. The recommended values for L1 and L2 can be considered debatable without sufficient testing on a larger number of species and in surface soils of phytocenoses. However, at the same time, they are an important tool for phytolith analysis in temperate regions. The presented study also proves that the visual determination of the phytolith submorphotype is objective.

Acknowledgements

This study has been funded by the Russian Science Fund № 23-74-01026, https://rscf.ru/project/23-74-01026/. The authors express their gratitude to all colleagues at the department of botany of Altay State University: M. M. Silantyeva (DSc), N. V. Elesova (PhD), T. V. Korniyevskaya (PhD), and P. D. Gudkova (PhD) for their help in collecting and identifying plant species in our herbarium.

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