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# Differentiation of archaeological Maize (*Zea mays* L.) from native wild grasses based on starch grain morphology. Cases from the Central Pampas of Argentina

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**Abstract**

This paper presents, for the first time, a detailed study, from an archaeological perspective, of the morphological characteristics of the starch grains within the kernels of selected native wild grasses found in the Central Pampas of Argentina. We compared native wild grasses to maize starch grains, which can be distinguished from each other based on their size, shape and other attributes. The majority of the studied grains did not share morphological characteristics with maize starch grains. Considering this, it can be said that, if irregular and polyhedral grains with transverse or radial fissures dominate the starch assemblage, maize identification may be done on the basis of both morphology and size. Additionally, this research contributes to the characterization of the starch grains of the Panicoideae subfamily, which includes maize. Several classes of simple and compound starch grains are described and defined for native species of Pooideae, Chloridoideae, Arundinoideae, and Panicoideae subfamilies. The results obtained may constitute a baseline for the future determination of maize and wild grass use in archaeological contexts belonging to Middle/Late Holocene hunter-gatherers in the Pampas of Argentina and neighboring areas.

**Highlight:**

- Differentiation of maize starch from South American wild grasses by means of their morphology and size.

**Key words**: Archaeobotany; Central Pampas of Argentina; maize; wild grasses; starch.

## 1. Introduction

In the last few years, maize phytoliths and maize starch grain morphology have been profusely studied, providing important information about ancestral and domesticated New World crops (Babot, 2004, 2011; Dickau et al., 2007; Hart et al., 2003; Hocsman et al., 2010; Holst et al., 2007; Inda and del Puerto, 2008; Iriarte, 2003; Iriarte et al., 2004; Pearsall, 2002; Pearsall et al., 2003, 2004a, 2004b; Piperno and Holst, 1998; Piperno and Pearsall, 1993; Piperno et al., 2009; among others). Maize and other grass kernels produce substantial quantities of starch. Maize starch grains have distinctive morphological characteristics, although some of them can overlap with other native Poaceae, which can lead to misidentification. Nonetheless, previous research has demonstrated that starch grain size and morphology are still useful features for differentiating maize from native wild grasses of North, Central and South America (Piperno et al., 2009). Such differentiation still requires regional surveys to study native wild grasses of local relevance, as was the case for the research done by Holst et al. (2007) in the current Venezuelan territory. This is the only comparative study of maize/wild grasses starch from South America available today.

Although archaeobotanical studies of phytoliths and starch grains have been done since the late 1990´s in the Southern Cone of South America (see Zucol et al., 2008), the differentiation of maize from native wild grasses has not been undertaken yet. This is due to, first, the absence of native wild species of the Panicoideae subfamily, which may be a confounder in areas where microfossil studies have been done (Babot, 2004; Korstanje, 2008; Korstanje and Cuenya, 2008; among others); secondly, to a long delayed interest for the study useful food resources in the Pampean region based on microfossil research; thirdly, to a lack of interest and an underestimation of the use of maize kernels and wild grasses by hunter-gatherer groups in the Late Holocene.

Two studies conducted by Iriarte (2003) and by Inda and del Puerto (2008) in the current territory of Uruguay are the only ones where maize was compared to wild grasses, considering their production of ergastic particles, specifically silicaphytoliths. These authors established that size and three-dimensional morphology of cross-shaped phytoliths allow distinguishing maize from Panicoid wild grasses. Secondly, they argued that the application of a multivariate discriminant function analysis, as described by Pearsall and Piperno (1990), together with size and qualitative attributes of cross-shaped phytolith assemblages, allow distinguishing between maize and wild grasses in the grasslands of southeastern Uruguay.

Due to the preliminary finding of maize starch grains in food residues on ceramics (namely *challas*), which correspond to hunter-gatherer contexts of the Pampas region of Argentina (Fig. 1) (Musaubach and Berón, 2011), the discussion about maize versus wild grasses has been renewed. The Pampas is an environment rich in native Poaceae species, so it would be interesting to establish whether wild seeds were used for food and non-food practices among local hunter-gatherers groups. In fact, microfossil characterization of wild grasses is very useful for the comprehension of food practices of hunter-gatherers groups (e.g. Liu et al., 2010, 2011; Mercader, 2009; Mercader et al., 2008; Yang et al., 2012).

We undertook, for the first time, a detailed study of the morphological characteristics of starch grains of selected native wild grasses found in the Central Pampas of Argentina. The aims of this research are: 1. To characterize the morphological variation of kernel starch grains of native wild species of grasses present in the Central Pampas, which belong to *monte* and *espinal* phytogeographical provinces (Cabrera, 1951); 2. To contribute to the characterization of the starch grains of the subfamily Panicoideae; 3. To establish morphological differences between starch grains of maize and native wild grasses that could help in their identification; and 4. To contribute to the building of a reference collection of the starch grains of wild grasses of archaeological interest, by discussing the feasibility of taxa assignation.

## 2. Material and methods

The material was obtained from herbarium specimens hosted at the National University of La Plata Herbarium - LP Herbarium (Appendix A). Twenty three species of native wild grasses were selected according with the present occurrence of the genera on the archaeological locality of Tapera Moreira (Lihué Calel department, La Pampa province, Argentina) (Berón, 1997, 2004; Berón and Curtoni, 1998, 2002), as an example of herbaceous communities belonging to *monte* and *espinal* phytogeographical provinces (Cabrera 1951).

Only mature kernels were used. They were rehydrated in distilled water for 2 to 3 days. To extract the starch grains, the kernels were cut with a razor blade. For light microscopy examination, the kernels were gently scrapped with a histological needle directly onto microscope slides and mounted in a water/glycerin solution. The starch grains were examined and photographed with polarized and unpolarized light at 400X. Measurements were conducted on 50 grains per species using Micrometrics SE Premium software; population parameters were then estimated.

Nomenclature generally follows the proposal of ICSN (2011). Of particular importance to this study was the classification of grains into simple and compound. In order to improve the discrimination of species, subcategories were defined for compound starch grains as follows: a. Compound/discrete aggregates. Aggregates made up of a discrete and known number of component granules; b. Compound/supernumerary aggregates. Aggregates of a variable and supernumerary number of component granules. The latter may or may not have distinctive tridimensional morphologies. Grains in a. and b. may form larger aggregates within the plant tissue, forming a second level of aggregation. Classes a. and b. may present a coating of amorphous starch, cementing grains into cohesive masses. The latter characteristics fit with the definition of “starch chunks” made by Goering (1967), regarding plant tissue compactly filled with starch and coated with it that breaks down into irregular forms.

In order to distinguish between maize starch and native wild grasses based on morphological attributes and grain size we chose species that showed simple starch grains and discrete aggregates made up of faceted component granules. For qualitative and quantitative description of starch grains we followed Holts et al. (2007), who identified different shape and surface features for maize and its wild relatives. Additionally, we considered other qualitative and quantitative variables related to morphology and optical properties of grains (Babot, 2007; Babot et al., 2007). Systematic classification of the taxa was done according to Rúgolo de Agrassar et al. (2005).

Maize starch grains are simple, typically irregular, “with no definable shape, because they vary in form when they are rotated” (Holst et al., 2007: 17611) with deep compression facets. The two-dimensional shape of the faces is polygonal, with four to six sides of different length; a rough, grooved surface in grains found in horny endosperm, and a smooth surface in the ones in floury endosperm. The hilum is typically described as spherical, V-shaped or linear, where cracks or radial/stellate fissures originate; the lamella is indistinct (Holst et al., 2007; Korstanje and Babot, 2007; Medina and Salas, 2008; Pagán Jimenez, 2007; Piperno, 2009; Piperno and Holst, 1998; Winton and Winton, 1932). It has a central Maltese cross, symmetric, with four visible arms which intersect at right angles. The starch grains may have pressure facets due to the compact filling of the cells (Fig. 2, Table A).

## 3. Results

The results obtained from the qualitative and quantitative analysis of starch grains are summarized on Tables B, C and D. The taxa were grouped according to the simple/compound criteria, as described below. Six species showed simple starch grains: *Sorghastrum pellitum, Bromus auleticus*, *B. bonariensis, B. brevis, B. catharticus,* and *Panicum urvilleanum* (Fig. 3).

Compound starch grains of class a (discrete aggregates) were observed in three species: *Elionorus muticus, Aristida adscencionis* and *Sporobolus rigens.* Every component granule in the aggregates is irregular in shape due to the fact that they develop pressure facets and rounded portions. Only *S. rigens* forms larger aggregates, filling the plant tissue in a second level of aggregation. These aggregates present a coat of amorphous starch, cementing grains.

*Aristida mendocina, Bothriochloa alta, B. laguroides, Nasella clarasii, Piptochaetium napostaense*, and *Sorghastrum pellitum,* all show compound starch grains of class b (supernumerary aggregates).

Aggregates of classes a and b are fragile and disarticulate easily, releasing the component granules.

Unfortunately,—*Amelichloa brachychaeta, Aristida subulata, Cortaderia selloana, Cynodon dactylon, Cynodon hirsutus, Imperata brasiliensis, Jarava ichu, Paspalum dilatatum* subsp *dilatatum*, and *Paspalum vaginatum* cannot be assigned to any category because of the lack of distinct starch in the kernels sampled.

**4. Discussion**

Previous examinations of starch grain size and morphology in the northern USA, southern Central America, and South America, where teosinte does not occur, indicated that maize starch grains can be distinguished from those of native wild grasses. The distinctive morphology allows identification of maize in starch grain assemblages recovered from archaeological stone tools, pottery, and sediments. In most wild non-*Zea* grasses studied by Holst et al. (2007) and others (Tateoka, 1962), grain size ranges from a mean length of 3 to 11 µm and a maximum length of 2 to 11 µm. A few grass species have starch grains as large as those of maize, but in each case their morphological characteristics appear to distinguish them from maize (Holst et al., 2007).

Environment may affect starch grain size, but morphology and grain size are genetically controlled (Lindeboom et al., 2004). In this manner, the environmental effects are not as important as the ones related with species, cultivated varieties and maturity degree (Shannon & Garwood, 1984). Therefore, in spite of some size plasticity and variability of starch grains of grasses and maize, the other morphological features of the largest starches still allow for discriminating between them. This is consistent with what has been shown by previous research and by the results obtained in this work.

According to Holst et al. (2007) irregular grains are exceptionally found in wild grasses, and compression facets are slight. Oval, round and bell-shaped grains predominate, frequently showing a continuous double border, and lacking the transverse fissures “that cut across the greater part of the breadth of the grain” (Holst et al. 2007: 17611).

Regarding the grain shape of the species with simple grains studied here, only irregular or polyhedral starch grains of *Sorghastrum pellitum* may be confounded with maize due to the presence of pressure facets and transverse fissures, and also because it can reach 20 µm in maximum length. In maize, the maximum length of starch grains commonly ranges from 8 to 25 µm (Holts et al., 2007) but grains that surpass these limits have been reported, reaching 2-35 µm in length, with a mean of 11.1 to 15.8 µm (Holst et al., 2007; Korstanje and Babot, 2007; Medina and Salas, 2008; Pagán Jimenez, 2007; Winton and Winton, 1932). Grains with a maximum length above 20 µm are important in order to identify maize unambiguously when *Sorghastrum* is expected. Fortunately, the simple grains of this species only exceptionally have defined pressure facets and transverse fissures. Besides, only a minor proportion of them are produced by the plant, and the starch assemblages of *S. pellitum* show mainly compound grains in the form of supernumerary aggregates made up of small granules that fill the plant tissue. As we mentioned previously, they present a coat of amorphous starch, cementing grains. This latter class of starch is absent in maize. In sum, the possibility of an overlap between the simple starch maize grains and Sorghastrum is negligible, and can be controlled by a comprehensive study of all the forms of starch.

In the three species in which compound starch grains were observed as discrete aggregates class a, (*Elionorus muticus*, *Aristida adscencionis* and *Sporobolus rigens*), their component grains are plano-convex or irregular in shape due to the development of pressure facets and rounded portions. Nevertheless, component granules show a few (1 to 4) slight pressure facets and do not have fissures or rough surfaces, unlike maize. Additionally, the maximum length of the granules is under 10 µm. Therefore, even in the case that the aggregates disjoin, the component granules of these species clearly imply a "compound origin", and thus they may be differentiated from maize starch. Interestingly, the natural color of *Elionorus muticus* starch grains is red-brown.

Previous work on starch grains of wild grass endosperm described the presence of compound grains, of flat simple grains in plain view of circular or oval shape, and of simple spherical grains with or without angles (Tateoka, 1962). Our results follow these general trends, but show some differences, too. As was mentioned, flat and rounded simple starch grains, spherical, ellipsoid, oval, and reniform in shape were observed in some species of the Central Pampas (*Bromus auleticus*, *B. bonariensis*, *B. brevis*, *B. catharticus*, and *Panicum urvilleanum*). Irregular and polyhedral starch grains were registered as simple particles (*Sorghastrum pellitum*). Additionally, two classes were described within the compound starch grain category, including discrete (*Elionorus muticus*, *Aristida adscencionis* and *Sporobolus rigens*) and supernumerary aggregates (*Aristida mendocina*, *Piptochaetium napostaense*, *Nasella clarasii*, *Sorghastrum pellitum*, *Bothriochloa alta* and *B. laguroides*) (Table B).

Starch grains of the Panicoid morphotype have been characterized for different purposes such as for traditional systematic studies (Tateoka, 1962) or for archaeobotanical analysis (Liu et al., 2011). This type of starch comprises isolated grains, characterized by having facetted shapes with either angular or rounded edges; the numbers of facets vary from four to six; the hilum is centric, and often appears as a deep depression; pronounced star fissures often radiate toward the periphery; and the arms of extinction crosses are mostly straight. In several cases, lamellae are visible in a part of the granule. Grains within a cell are not very variable in size, although the grains within the cells near the seed coat are smaller than those in the cells of the inner parts. The Panicoid type has been found in a large number of Paniceae, in many species of Andropogoneae, Eragrosteae, Chlorideae, Pappophoreae, Arundinelleae, and Arthropogoneae tribes, and several other tribes (Bambuseae, Unioleae, Phaenospermeae, Brachyelytreae, Garnotieae, Isachneae and Boivinelleae). Starch grains of this type are usually 4-10 µm in diameter, but in some genera such as *Phaenosperma* Benth, *Brachyelytrum* P. Beauv., *Cenchrus* L., *Sorghum* Moench, etc., they are larger, reaching 30-40 µm in diameter (Tateoka, 1962). Other morphotypes, such as elliptic or reniform grains, were not previously assigned to Panicoid grasses.

According to our research, only scarce simple grains (n=5) present in *Sorghastrum pellitum* correspond to the Panicoid starch grain morphotype defined by Tateoka (1962) and Liu et al. (2011), but their size does not exceed 20 µm maximum length (Fig. 4). Additionally, this species has mainly compound starch grains belonging to the class of supernumerary aggregates with small component granules. Even when *Panicum urvilleanum* presents single starch grains, they have a spherical three-dimensional morphology, which does not correspond to a classic Panicoid starch grain (Tateoka 1962). Other native wild grasses that belong to the subfamily Panicoideae (Table B) did not fit in the Panicoid morphotype because of the presence of compound grains/discrete aggregates (*Elionorus muticus*) or of compound grains/supernumerary aggregates (*Bothriochloa alta* and *B. laguroides).*

Species with compound grains of class b are not discussed here. They will be studied in comparison with other useful South American taxa in further contributions.

**5. Conclusion**

In summary, our results indicate that the size and morphological attributes of starch grains can be very useful for separating native wild grasses of the Central Pampas from maize in the archaeological record, similarly to how maize is distinguished from teosinte. Potential size overlapping and its variation due to environmental and maturity degree are irrelevant when considering other morphological features of the largest starches which still allow for discriminating between grasses and maize.

In the case of the Pampas environments, when irregular and polyhedral starch grains with transverse or radial fissures, dominate the starch assemblage, and when these grains include at least some particles larger than those of *Sorghastrum pellitum* (20 µm maximum length), the identification of maize may be based on of both, morphology and size. The absence of compound grains/supernumerary aggregates in Pampean microfossil assemblages constitutes an additional criterion for identifying them as maize instead of *Shorghastrum*. Except for this species, the rest of the wild grasses studied do not overlap with maize when multiple morphological attributes are considered together with size.

Several classes of simple and compound starch grains of native wild grasses were analyzed here. This is of particular implication for the knowledge of Panicoid grasses, which are currently known mainly by the so-called Panicoid morphotype. Besides, this will allow identifying in further studies the past uses of wild grasses of Poaceae sub-families other than Panicoid.

Beyond the controversy on maize versus native wild grasses and the characterization of the Panicoid starch grain types, the data analyzed here showed a partial overlapping with Amaranthaceae-Chenopodiaceae and Cucurbitaceae families (Korstanje and Babot, 2007) which comprise several useful species which could potentially be found in Andean and Pampean archaeological assemblages (Babot, 2011; Musaubach et al., 2011). The discussion of that evidence will be a matter of future contributions.

In the pursuit for solving archaeological questions regarding the past uses of plants, the need for a correct characterization of the starch grain and phytolith assemblages of native wild grasses is evident. This implies the necessity of a multiproxy analysis of the evidence provided by starch and phytoliths (Boyd et al., 2006; Coil et al., 2003;

Korstanje and Babot, 2007) and, if possible, by macro-remains and isotopic analysis. As Korstanje and Babot (2007) said, comprehensive knowledge of the whole microfossil record is a very important for solving archaeological questions. Additionally, it is necessary to study Poaceae biodiversity in a regional scale in order to avoid mistakes in the uncritical application of some morphotypes defined for other regions.

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| **Starch grain morphology** | Winton &Winton 1932 | Holst et al 2007 | Korstanje & Babot 2007 | Medina & Salas 2008 | Pagán Jimenez 2007[1] |
|---|---|---|---|---|---|
| Shape | polygonal, round, triangular, rectangular | irregular, round, bell | polyhedral, spherical, irregular-elongated | polyhedral, irregular | ellipsoid, polyhedral (sic “truncated”), spherical, among other |
| Hilum Position | central | centric | centric | concentric | centric, eccentric, indistinct |
| Hilum Form | ------ | cavity | v-shaped, dot or line | dot or line | spherical |
| Pressure facets | ------ | slight, defined | present | ------ | ----- |
| Fissures | rosette of rifts or single rift | transverse | radiating | radial | radial, perpendicular, T-shape |
| Lamella | ------ | ------ | indistinct | ------ | indistinct, exceptionally distinct |
| Length range in µm | varies up to 30 | 6 to 26 | 2 to 35 | 1,72 to 29,15 | 7 to 20 |

Table A. Starch grain characteristics in maize. [1] Archaeological maize from Northern Chile.

| | | Starch grain class |
|---|---|---|
| **Subfamily PANICOIDEAE** | | |
| **Tribe** | | |
| Andropogoneae | *Sorghastrum pellitum* | Compound grains/supernumerary primary aggregates with distinct shape -class b |
| | *Imperata brasiliensis* | Indeterminate |
| | *Elionorus muticus* | Compound grains /discrete aggregates -class a |
| | *Bothriochloa alta* | Compound grains/supernumerary primary aggregates with distinct shape -class b |
| | *Bothriochloa laguroides* | Compound grains/supernumerary primary aggregates with distinct shape -class b |
| Paniceae | *Paspalum dilatatum* | Indeterminate |
| | *Paspalum vaginatum* | Indeterminate |
| Panicum | *Panicum urvilleanum* | Simple grains |
| **Subfamily POOIDEAE** | | |
| **Tribe** | | |
| Piptochaetium | *Piptochaetium napostaense* | Compound grains/supernumerary primary aggregates with distinct shape -class b |
| Poeae | *Bromus auleticus* | Simple grains |
| | *Bromus bonariensis* | Simple grains |
| | *Bromus brevis* | Simple grains |
| | *Bromus catharticus* var. *rupestris* | Simple grains |
| | *Nasella clarazzi* | Compound grains/supernumerary primary aggregates with distinct shape -class b |
| | *Jarava ichu* | Indeterminate |
| | *Amelichloa brachychaeta* | Indeterminate |
| **Subfamily CHLORIDOIDEAE** | | |
| **Tribe** | | |
| Aristideae | *Aristida adscensionis* | Compound grains/discrete aggregates -class a |
| | *Aristida mendocina* | Compound grains/supernumerary primary aggregates with distinct shape -class b |
| | *Aristida subulata* | Indeterminate |
| Cynodonteae | *Cynodon dactylon* | Indeterminate |
| | *Cynodon hirsutus* | Indeterminate |
| Sporoboleae | *Sporobolus rigens* | Compound grains/discrete aggregates -class a |
| **Subfamy ARUNDINOIDEAE** | | |
| **Tribe** | | |
| Arundineae | *Cortaderia selloana* | Indeterminate |

Table B. List of wild grasses studied for starch grain characterization.

| | *Panicum urvilleanum* n= 50 | | *Bromus auleticus* n= 50 | | *Bromus bonariensis* n= 50 | | *Bromus brevis* n= 50 | | *Bromus catharticus* n= 50 | | *Sorghastrum pellitum* n=5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Length | Width | Length | Width | Length | Width | Length | Width | Length | Width | Length | Width |
| X | 4.33 | 4.00 | 6.79 | 4.99 | 5.75 | 3.66 | 4.40 | 2.69 | 4.88 | 3.00 | 14.6 | 12.6 |
| Mode | 3.16 | 3.70 | 6.74 | 2.63 | 7.80 | 3.26 | 3.80 | 3.2 | 6.00 | 4.00 | 3.71 | 12 |
| SD | 1.21 | 1.20 | 2.91 | 1.80 | 2.11 | 1.54 | 1.90 | 1.00 | 1.96 | 1.34 | 3.71 | 2.88 |
| Range | 1.19-7.03 | 1.1-6.68 | 0.94-13.8 | 0.94-8.16 | 0.57-10.8 | 0.35-6.86 | 1.2- 9.55 | 1.2-5.71 | 1-10 | 1-6 | 10-20 | 9-17 |

Table C. Starch grain size in wild native grasses with simple grains (in microns).

| **Starch grain morphology** | *Panicum urvilleanum* | *Bromus auleticus* | *Bromus bonariensis* | *Bromus brevis* | *Bromus catharticus* | *Sorghastrum pellitum* |
|---|---|---|---|---|---|---|
| Shape | spherical | spherical ellipsoid, irregular, ovoid-flattened | spherical, ellipsoid ovoid-flattened | spherical ellipsoid ovoid-flattened | spherical ellipsoid irregular, ovoid-flattened | irregular spherical |
| Hilum Position | centric | centric | centric | centric | centric | centric |
| Hilum Form | deep depression | elongated | elongated | elongated | elongated | deep depression |
| Pressure facets | slight | slight | slight | slight | slight | defined |
| Fissures | radial | transverse | absent | absent | absent | transverse |
| Lamella | not visible | visible | visible | visible | visible | not visible |

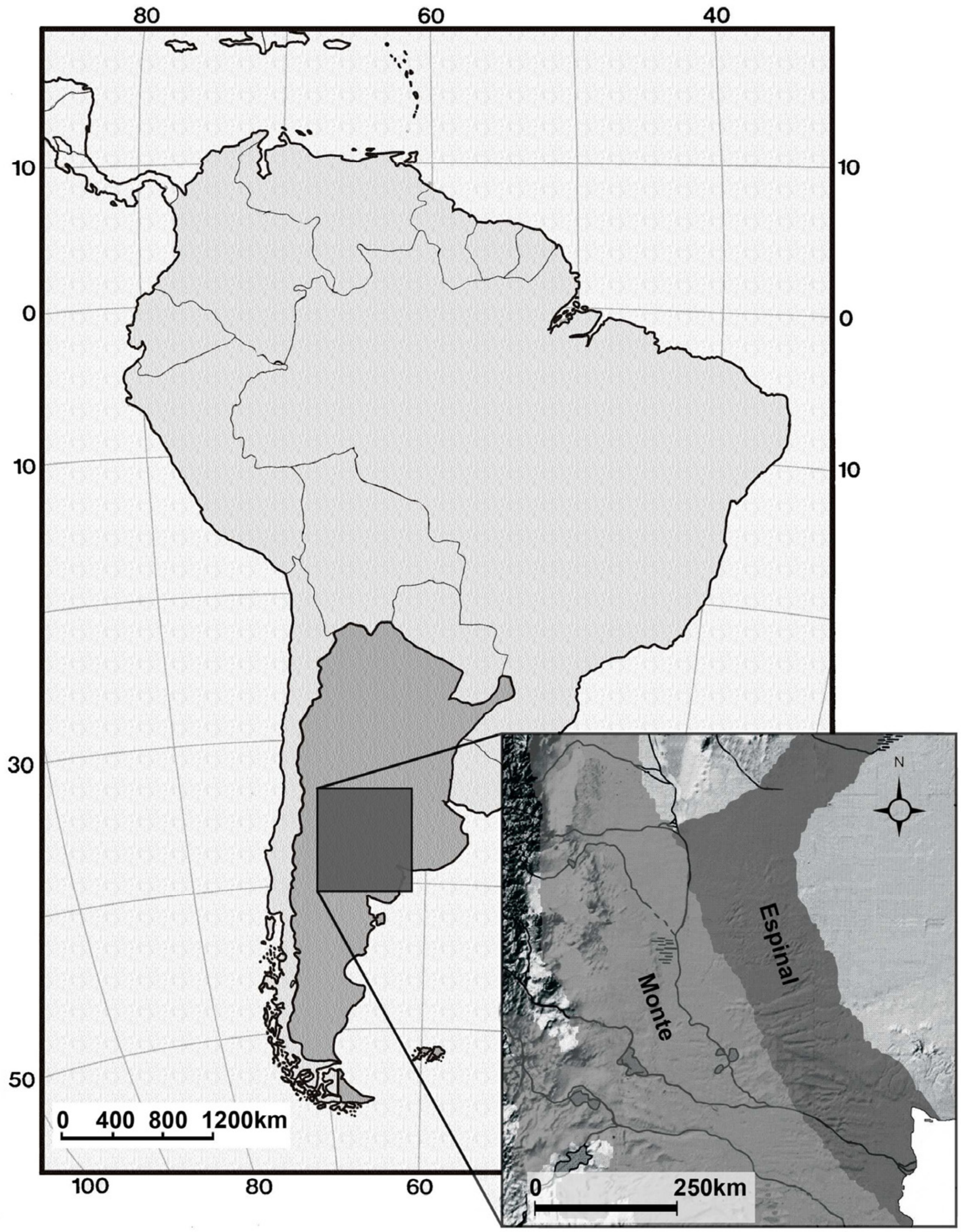
Table D. Starch grain characteristics in wild grasses species with simple grain.

FIGURE 1: Map of study area, with Argentinean phytogeographical provinces of Central Pampas detailed.

FIGURE 2: Range of variation in archaeological maize starch. A, D and G: Simple starch grains, five to six two-dimensional polygonal sides. B: Assemblage of simple starch grains. C: Same starch grains with polarized light. E: Irregular starch grain with deep compression facets. F: Same starch grain with polarized light. H: Assemblage of simple starch grains. I: Same starch grains with polarized light. (Scale bars: 10 µm).

FIGURE 3: Pampean native wild grasses from reference collection. A-C: Simple starch grains. A: *Panicum urvilleanum,* B and C: *Bromus auleticus*. D-E: Compound starch grains as discrete aggregates of class a. D: *Sporobolus rigens*. E: *Elionorus muticus*. F-G: Compound starch grains, supernumerary aggregates of class b. F: *Aristida mendocina*. G: *Nasella clarasii*. (Scale bars: 10 µm).

FIGURE 4: Classic Panicoid starch grain morphotype of *Sorghastrum pellitum*. A: Example of centric hilum as a deep depression. B: Same starch grain with polarized light. C: Example of facetted shapes with angular edges. D: Same starch grain with polarized light. E: Example of simple starch grains with transverse fissures. F: Same starch grain with polarized light. (Scale bars: 10 µm).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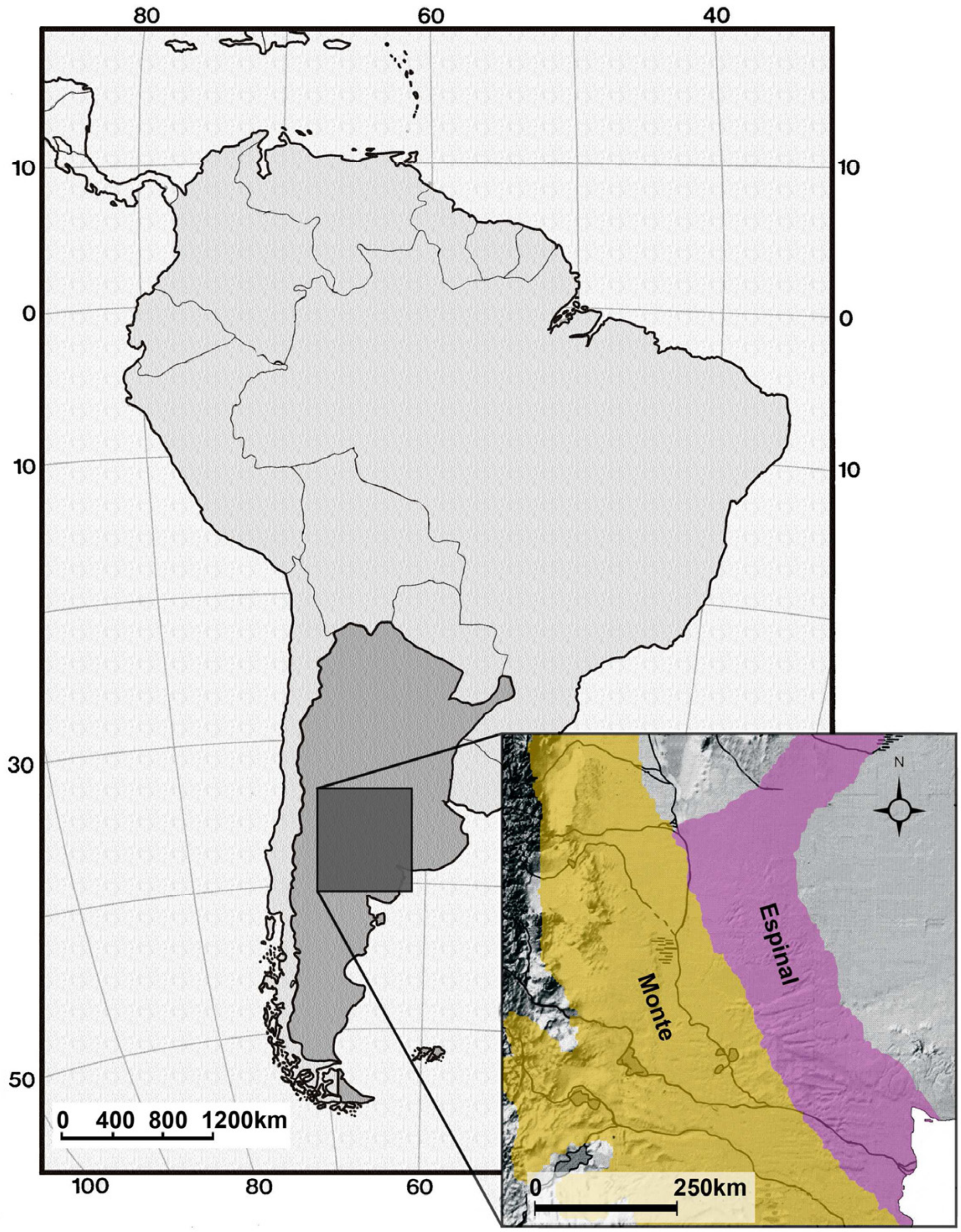

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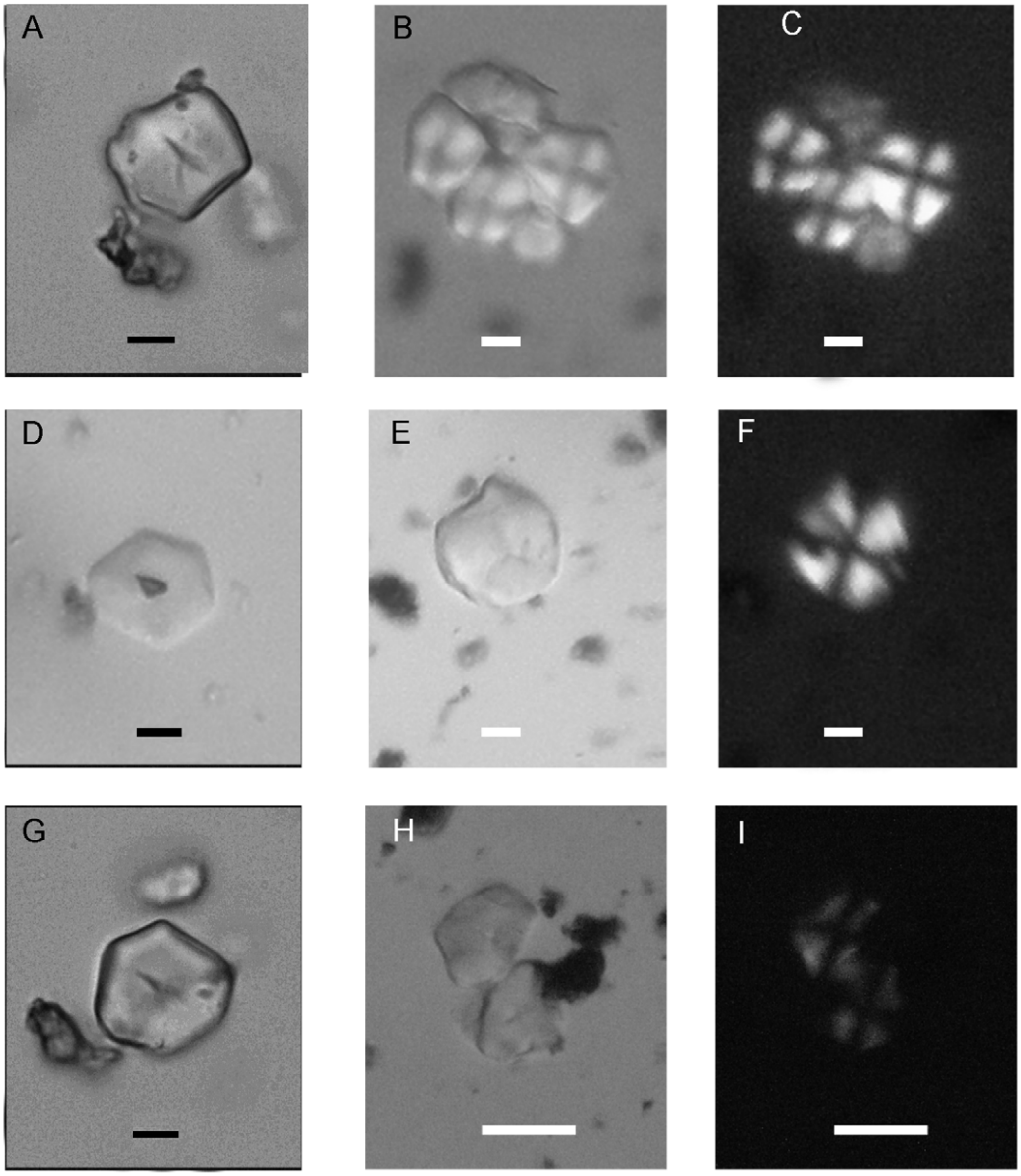
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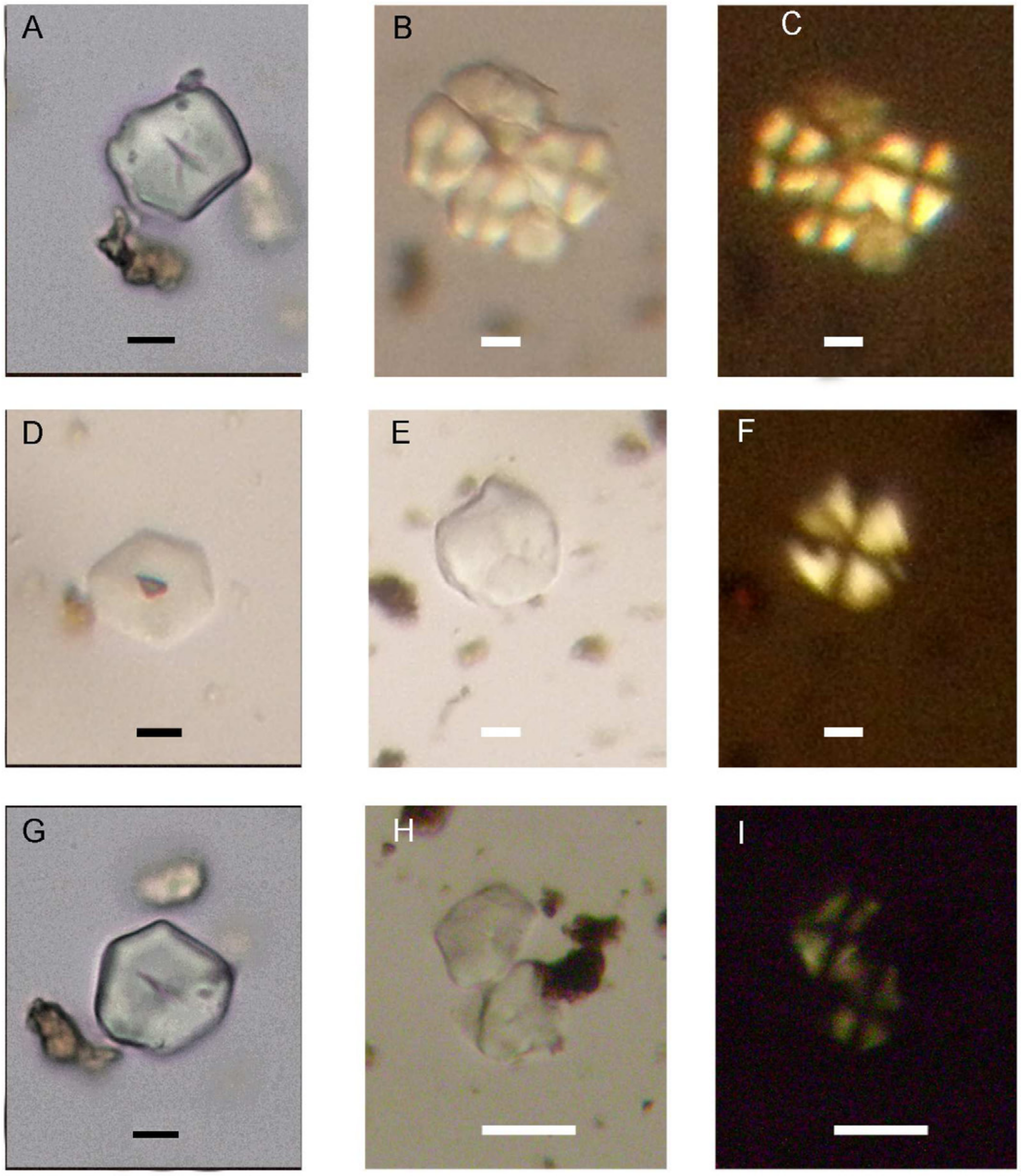
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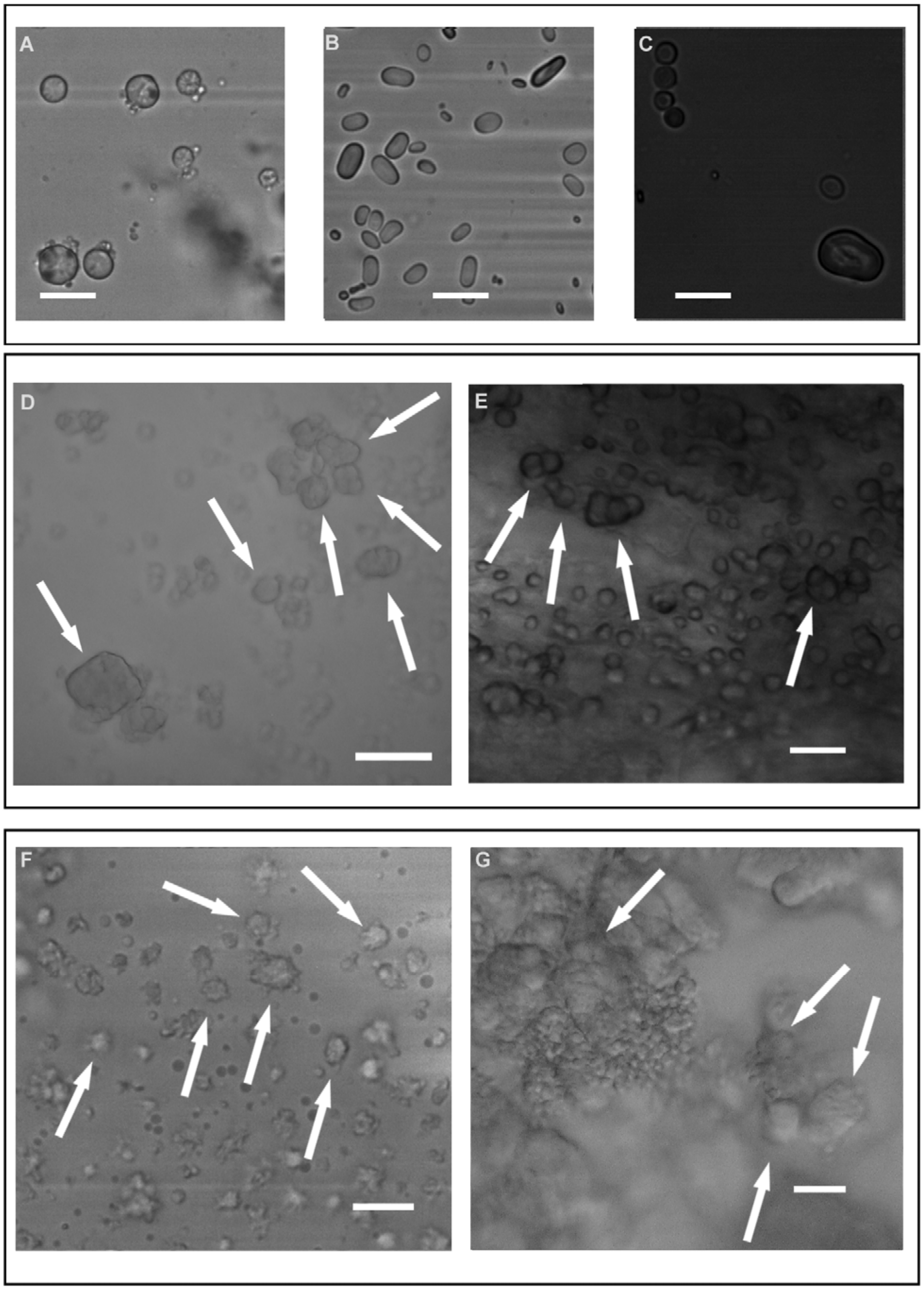
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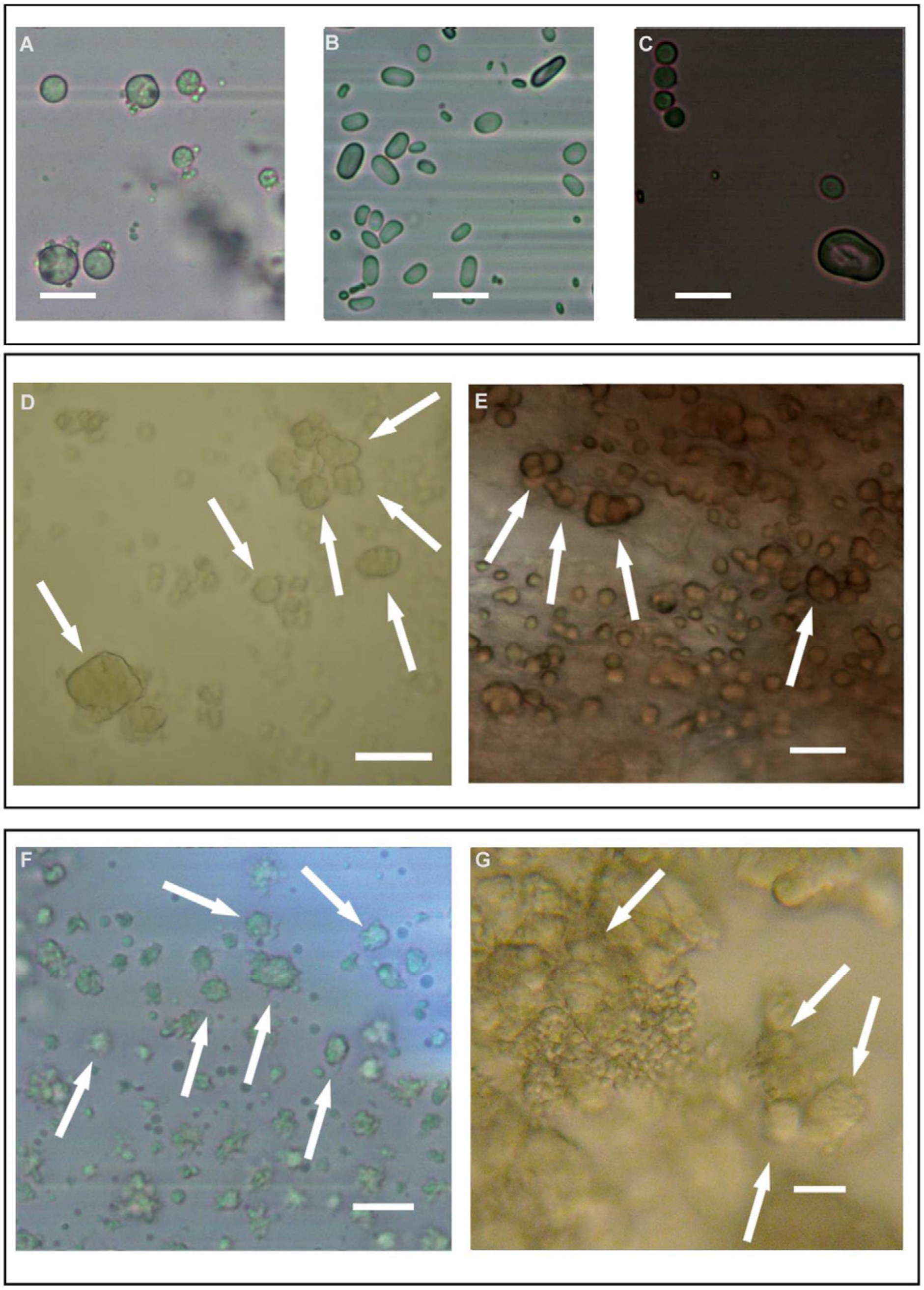

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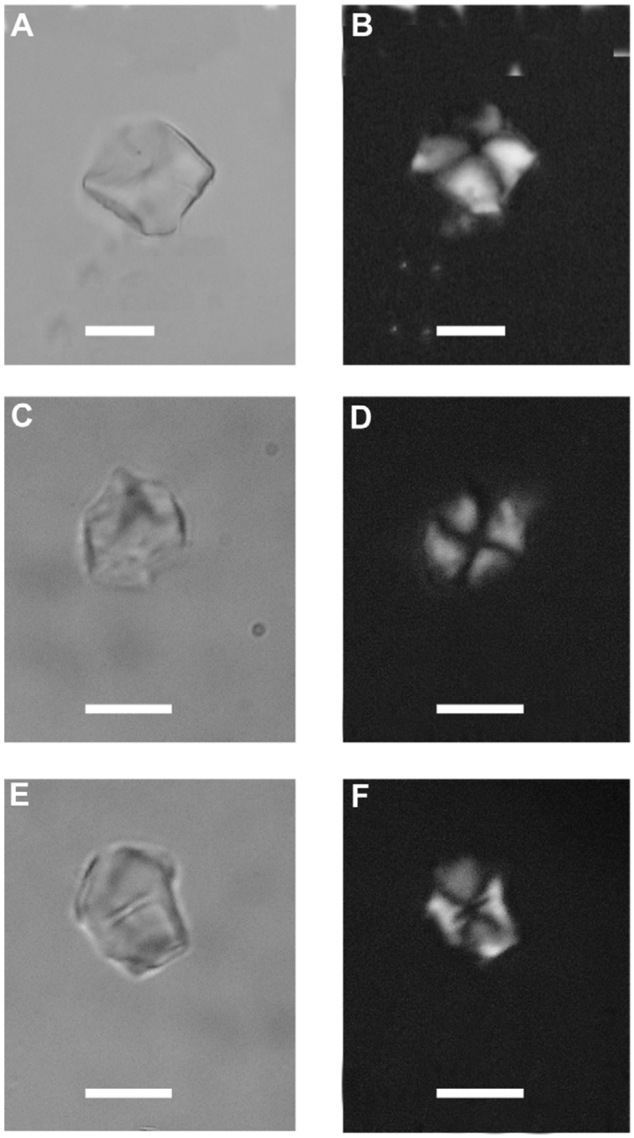
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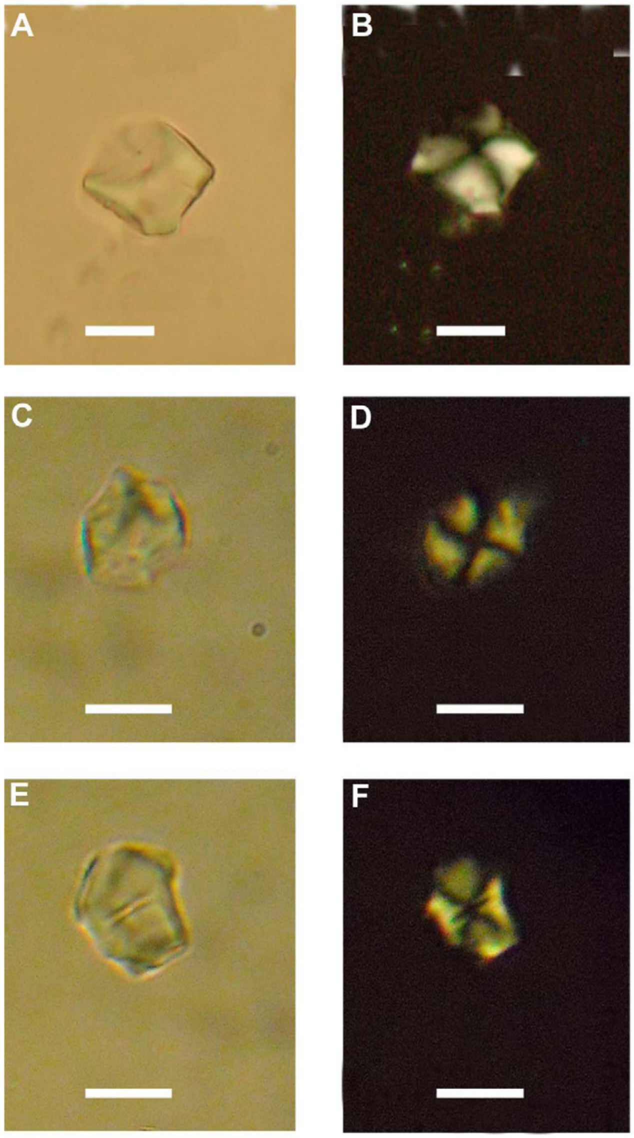
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**Highlight:**

- Morphological characterization of the starch of native wild grasses of Argentina.
- Comparison of native wild grasses to maize starch based on size and shape.
- Characterization of the starch of the Panicoideae subfamily, which includes maize.