Image analysis of modern and fossil plant silica bodies (phytoliths): Unlocking the evolution of grasses and grassland ecosystems

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Grasslands are ecologically vital

- Grassy biomes make up >40% of Earth’s land surface

*Color = grass-dominated habitats*  
Lehmann et al. (in prep.)
When and how did grassland ecosystems come to be?

• When did the grass family first originate and diversify?

• When did open-habitat grasses diversify and become ecologically dominant?
The grass family (Poaceae)

- Open-habitat habit evolved twice within Poaceae

(Common ancestor of Poaceae)

(after GPWG 2001, Sanchez-Ken et al. 2007)
Direct evidence for past grasslands

- Grass mesofossils and pollen are rare until the late Miocene—and often hard to interpret taxonomically.
**Phytoliths** *(plant silica)*

- **Grass epidermis:**
  - epidermal cell
  - stomata

- **Grass phytolith in sediment:**
  - 10 µm
Phytoliths

- Taxonomically useful within the grass family (Poaceae)

(after GPWG 2001, Sanchez-Ken et al. 2007)
Phytoliths

- Taxonomically useful within the grass family (Poaceae):
  - Diversification of ancient grass lineages
  - Ecology of past grass communities

(after GPWG 2001, Sanchez-Ken et al. 2007)
Radiation of open-habitat grasses

- Fossil phytolith morphotypes (Americas, Eurasia):

  → Open-habitat grasses diversified by 40 Ma

Grassland evolution in North America

• Earliest (early Miocene) grasslands were dominated by cool-temperate stipoid pooids

• Tropical, dry-adapted ($C_4$) chloridoids spread during the latest Miocene

Early grass diversification

- Phytoliths (+cuticle) from Late Cretaceous dinosaur coprolites and sediment, central India

Prasad et al. (2005, 2011)
Early grass diversification

- Phytolith characters (distribution, shape) from modern grasses in combined molecular-morphological phylogenetic analysis

- Dating of phylogeny using the Late K phytoliths

Prasad et al. (2011)
Early grass diversification

• Fossils are nested within Oryzeae

Characters:
2. Vertical bilobates
5. Papilla on long cells
6. Papilla on stomatal subsidiaries

Prasad, Strömberg et al. (2011), Nature Communications
Early grass diversification

- Poaceae evolved by the Early Cretaceous (?!?)
- Significantly earlier than previous estimates (70-95 Ma)
Needed:
Analysis of how phytolith shape maps onto the grass phylogeny

Grass silica short cell phytoliths (GSSCP)
Inadequate current GSSCP shape keys

- **Problem 1:** Qualitative or semi-quantitative, 2-D descriptions of GSSCP morphotypes
Inadequate current GSSCP shape keys

- **Problem 1:** Qualitative or semi-quantitative, 2-D descriptions of GSSCP morphotypes

  "saddle-shaped"

*Chloris*  
Dry-adapted, C\textsubscript{4} open-habitat grass

*Sinobambusa*  
Mesophytic, tree-forming C\textsubscript{3} bamboo

(Watson and Dallwitz 1992-)

Inadequate current GSSCP shape keys

- Problem 2: Outdated grass taxonomy
- Recent phylogenies have dramatically changed understanding of Poaceae relationships

(Gallaher et al. in prep.)
Creating a phylogenetic key to grass phytolith shape
Team GRASS:

Postdoc: Tim Gallaher

Grad students: Camilla Crifò, William Brightly

Undergraduates: Anna Schorr, Nik Pershing, Elie Aboulafia, Brittany McManus, Casey O’Keefe, Ashly Senske, Claire Marvet, Brian Connor, Sultan Akbar
Creating a phylogenetic key to grass phytolith shape

**Goals:**
- Measure 3-D shape of GSSCPs using geometric morphometrics
- Map 3-D shape onto current phylogeny
- Correlate with ecological and physiological characters

→ Trace evolution of GSSCP shape and size across Poaceae
→ Establish GSSCP shape/size diagnostic of particular clades/ecologies/physiologies
Unique properties of phytoliths

1. Phytoliths consists mainly of SiO$_2$ (66–91%), organic carbon OC (1–6%), H$_2$O (0–11%), Al (0.01–4.55%), and Fe (0–2.1%)

→ Phytoliths do not auto-fluoresce

→ The outer surface of phytoliths does not readily stain—or stain evenly (e.g., FITC)

(Neethirajan et al. 2009)
2. GSSCP phytoliths are small (~7-40 micrometers)

Resolution of e.g., micro-CT (100-200 micrometers) is not fine enough
3. Most grass species make >>1 type of GSSCPs

*Anomochloa marantoidea*

→ **GSSCPs have to be studied like assemblages**
Unique properties of phytoliths

4. Similar GSSCP shapes can be oriented differently in the tissue in different species

$\rightarrow$ GSSCPs have to be studied in situ
Materials and methods

**Taxa sampled:**
- >200 grass genera from all Poaceae subclades
- Leaf material

(Gallaher et al. in prep.)
Materials and methods

Data collected:
• Orientation and distribution of GSSCP shape
Materials and methods

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• Orientation and distribution of GSSCP shape
• Relative abundances of GSSC types in GSSCP assemblages extracted from leaves

>200 GSSCP /sample
**Materials and methods**

*Data collected:*
- Orientation and distribution of GSSCP shape
- Relative abundances of GSSC types in GSSCP assemblages extracted from leaves
- 3-D shape within each GSSCP type using confocal microscopy of extracted GSSCPs
- >10 specimens /GSSCP morphotype
3-D data workflow: Image acquisition

- Detailed workflow protocol to ensure consistency
3-D data workflow: *Image processing and analysis*

- Use 3-D surface meshes to calculate measures of size such as length, width, height, surface area and volume
- Transform and align meshes using Procrustes superimposition to remove size
3-D data workflow: Image processing and analysis

- Use 3-D surface meshes to calculate measures of size such as length, width, height, surface area and volume

- Transform and align meshes using Procrustes superimposition to remove size
3-D data workflow: **Outcomes**

- Quantified shape for morphometric analysis and phylogenetic mapping
- Animations and 3-D printable objects

*Anomochloa marantoidea*
Preliminary results

• Counts and confocal images of 3-D shape of GSSCPs for the Oryzoideae, Bambusoideae and early-diverging grasses
Problems encountered so far

- Finding stains that stain *all of* and *only* the GSSCPs

Rough mesh before smoothing algorithms have been applied

*Oryza*  
*Pharus*
Problems encountered so far

• Finding stains that stain all of and only the GSSCPs

Solutions:
1. Tinkering with filters and brightness thresholds in the imaging software to get rid of “holes” and smooth surface
3. Development of a new stains in the form of hybridization probes specific to silica
Problems encountered so far

• Processing time for Procrustes analysis for meshes prohibitive

• Finding the ideal way to analyze 3-D data

Solutions:
1. Find optimal number of vertices in meshes

2. Landmark-free algorithms? (e.g., Pomidor et al. 2016)
Sharing data

• Online platform where people can use phylogenetic key, download images, videos, printable models etc. (= Morphobank?)
Conclusions

• GSSC phytoliths contain shape data that are phylogenetically relevant

• Collecting and analyzing these data are complicated by the unique properties of phytoliths

• Stay tuned (and suggestions welcome)!
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Thank you for your attention!
Part II: Problems encountered so far

- Finding stains that stain *only* and *all* of the GSSCPs

GSSCPs in leaf epidermis

Montage of the individual slices from fluorescent confocal microscopy: 20 out of 210 slices shown.

Isolated GSSCP