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

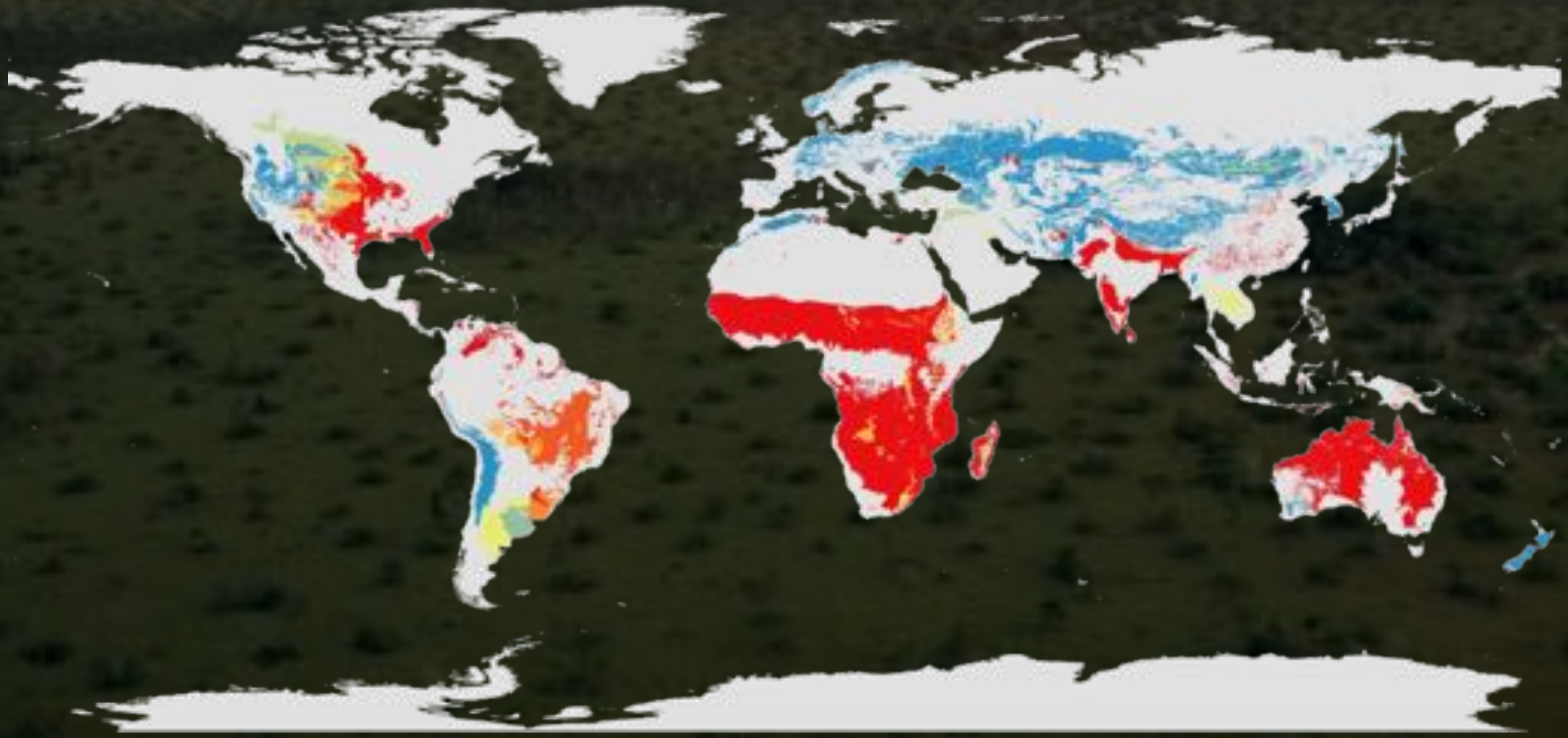
Caroline A.E. Strömberg
Department of Biology & Burke Museum
University of Washington
Seattle, WA

iDigBio Integrated Digitized Biocollections

Burke Museum University of Washington

Grasslands are ecologically vital

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



Color = grass-dominated habitats

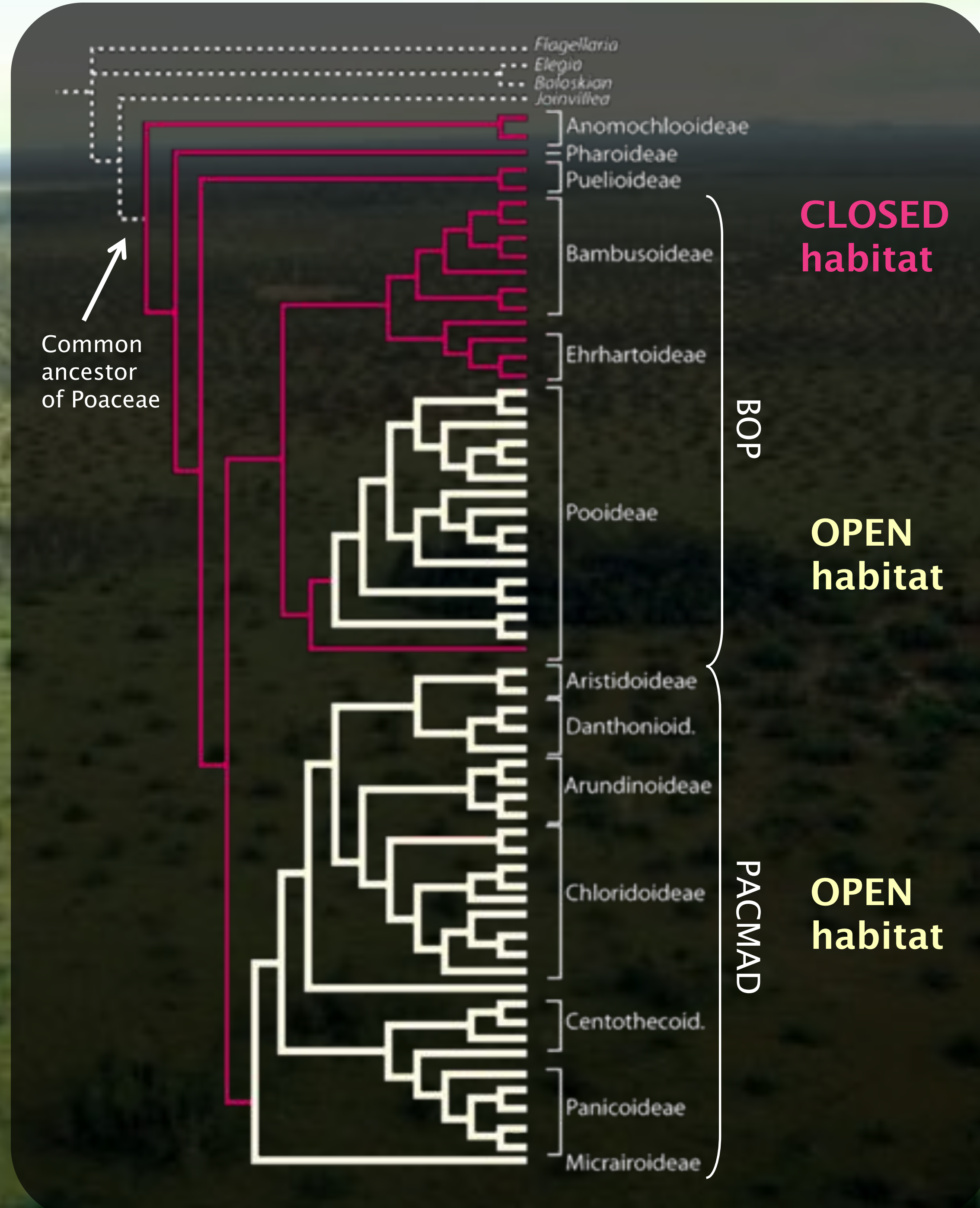
Lehmann et al. (in prep.)

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



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

Direct evidence for past grasslands

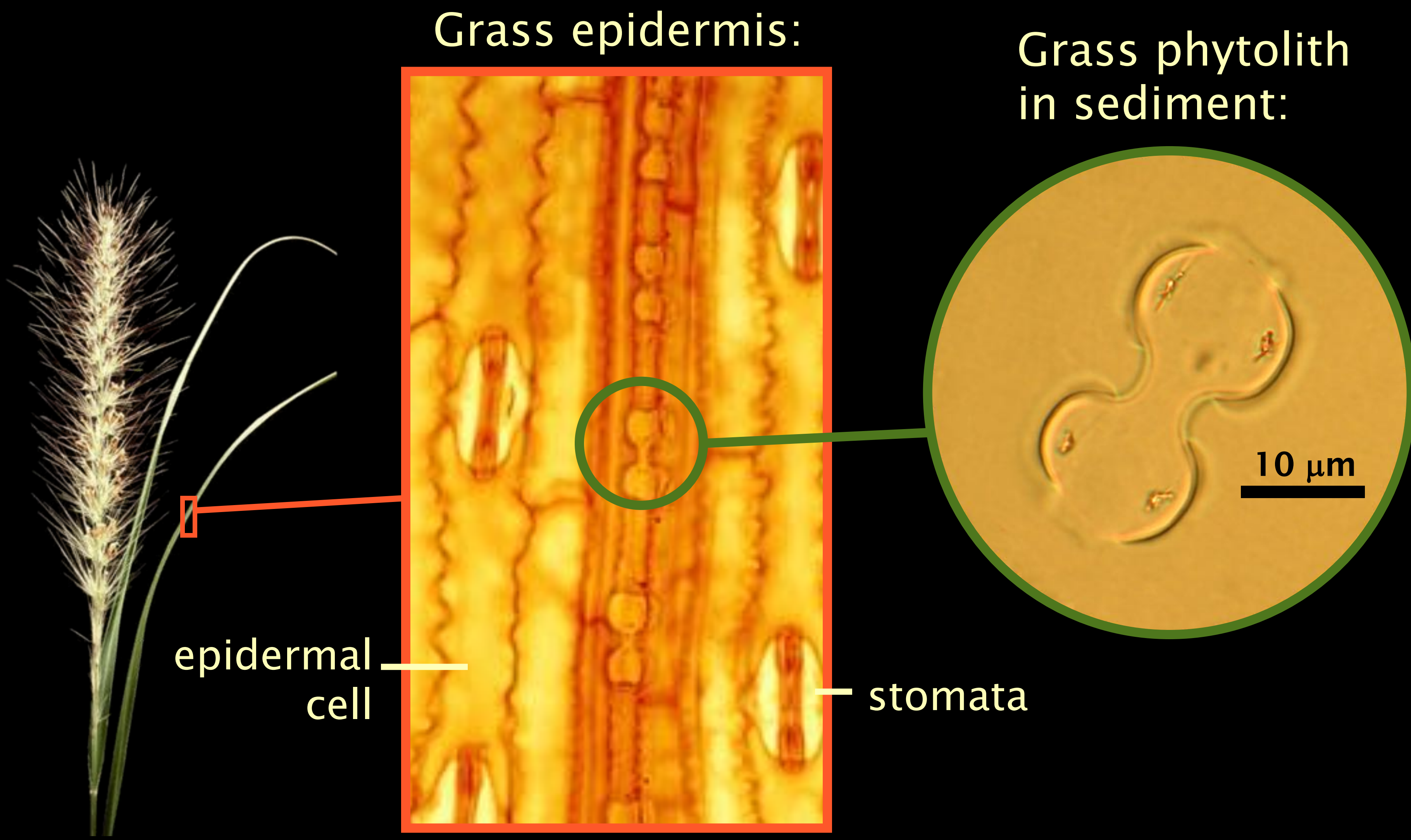
- Grass mesofossils and pollen are rare until the late Miocene—and often hard to interpret taxonomically

Pollen

Leaves

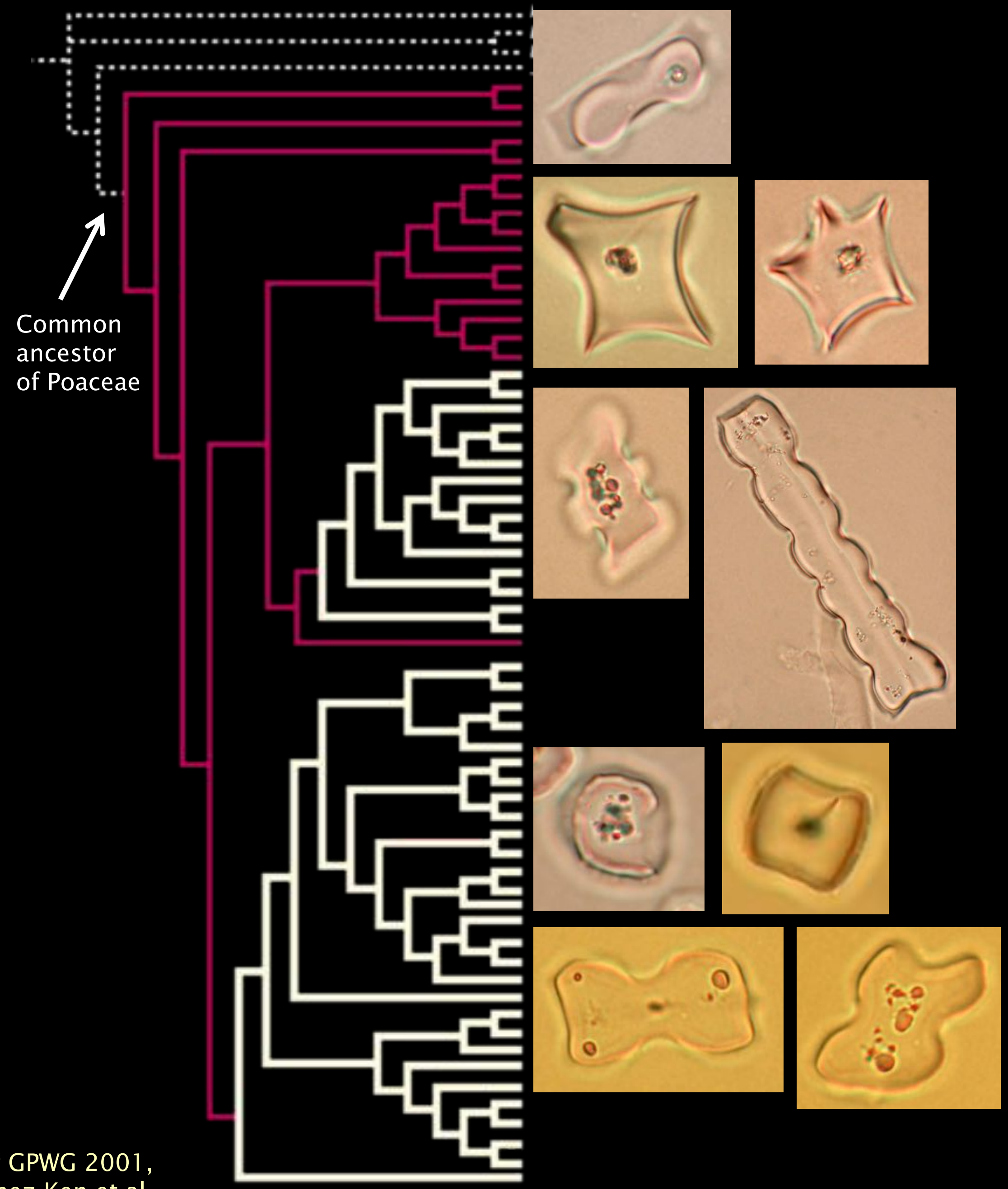
Fruit

Phytoliths *(plant silica)*



Phytoliths

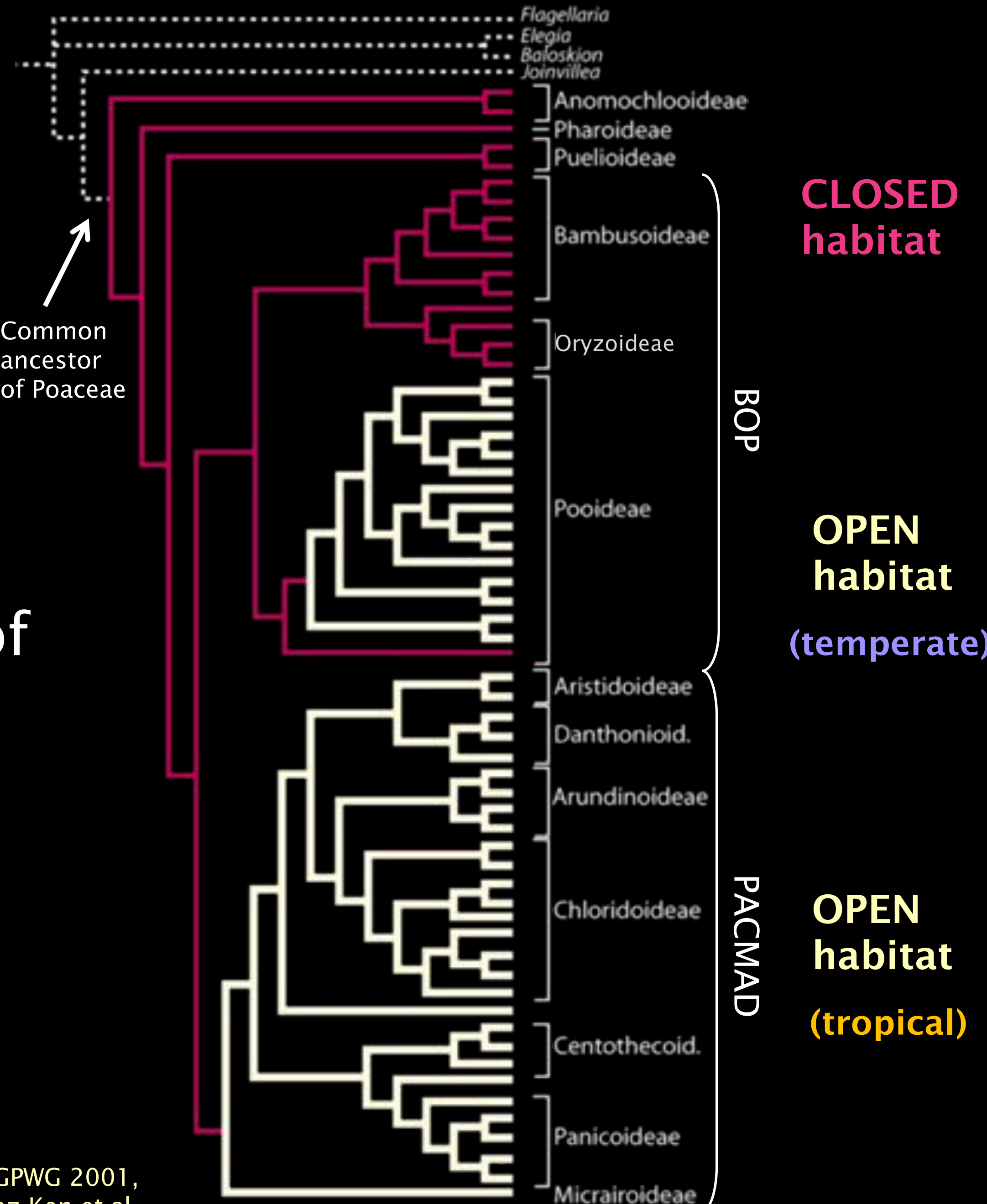
- Taxonomically useful within the grass family (Poaceae)



(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

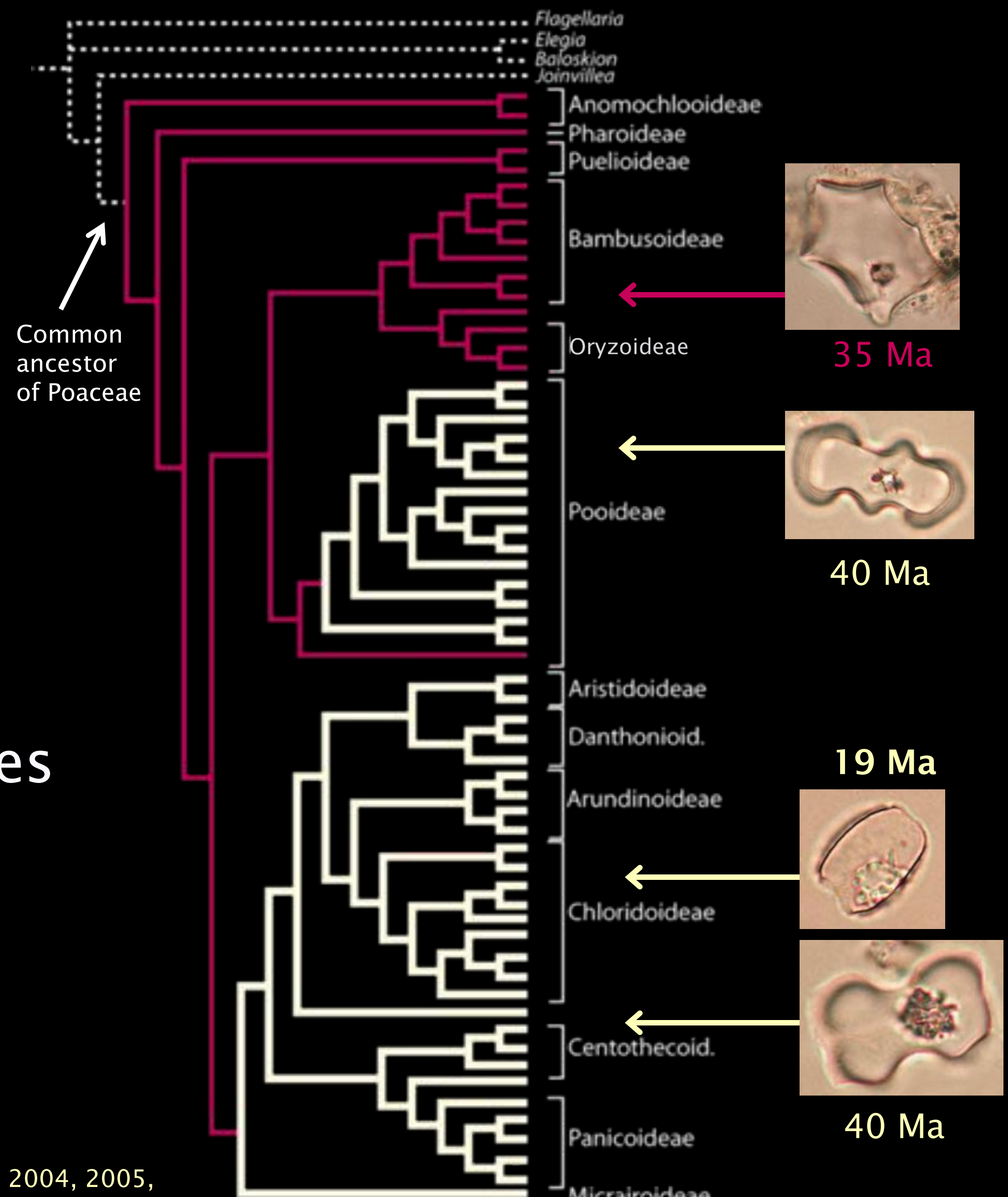


(after GPWG 2001, Sanchez-Ken et al. 2007)

Radiation of open-habitat grasses

- Fossil phytolith morphotypes (Americas, Eurasia):

→ Open-habitat grasses diversified by 40 Ma



(Strömberg 2004, 2005, Strömberg et al. 2007, 2013, Miller et al. 2012)

Grassland evolution in North America

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

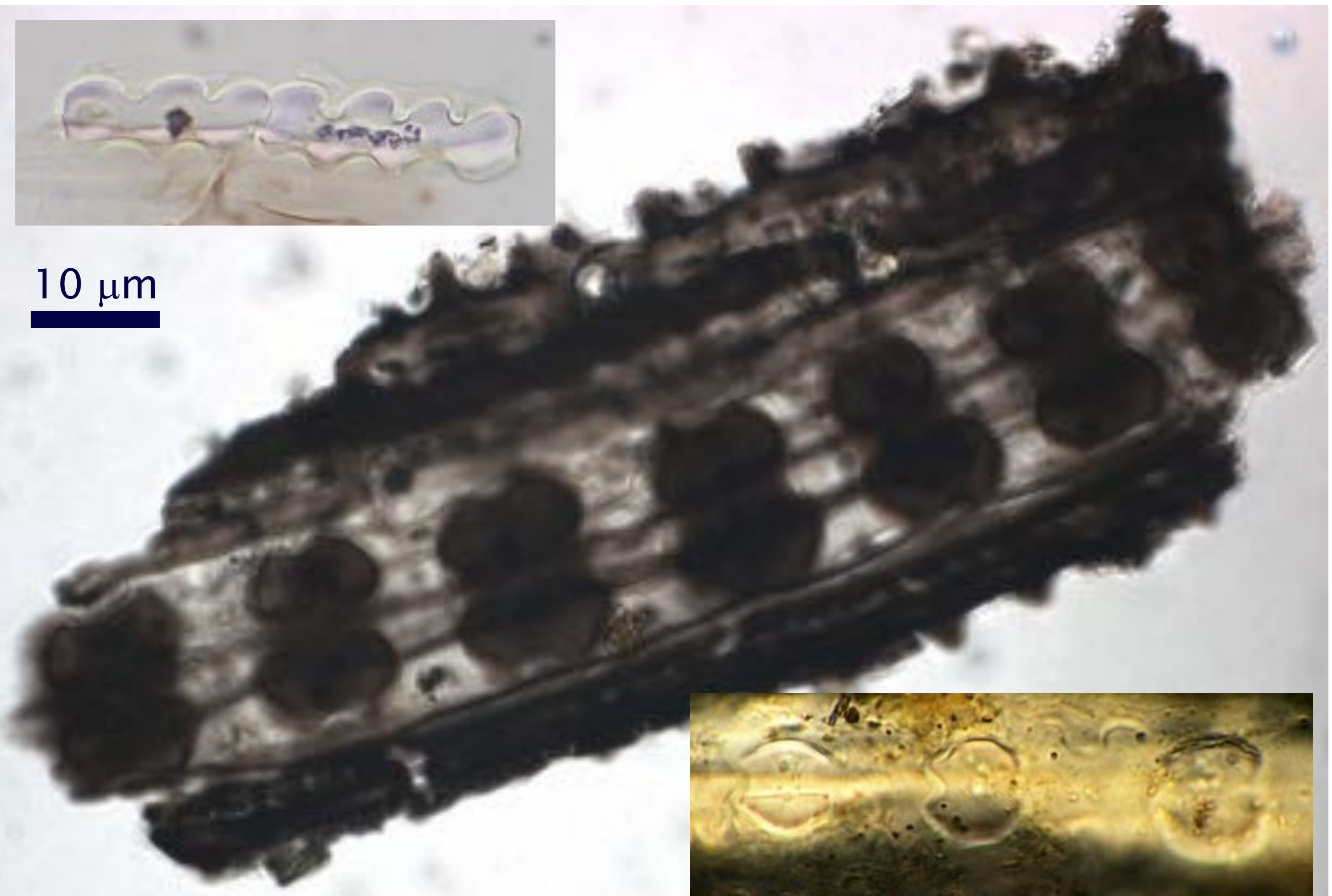
- Tropical, dry-adapted (C_4) chloridoids spread during the latest Miocene

(Strömberg 2004, 2005, 2011, Strömberg et al. 2007, 2013, Edwards et al. 2010)

Early grass diversification

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

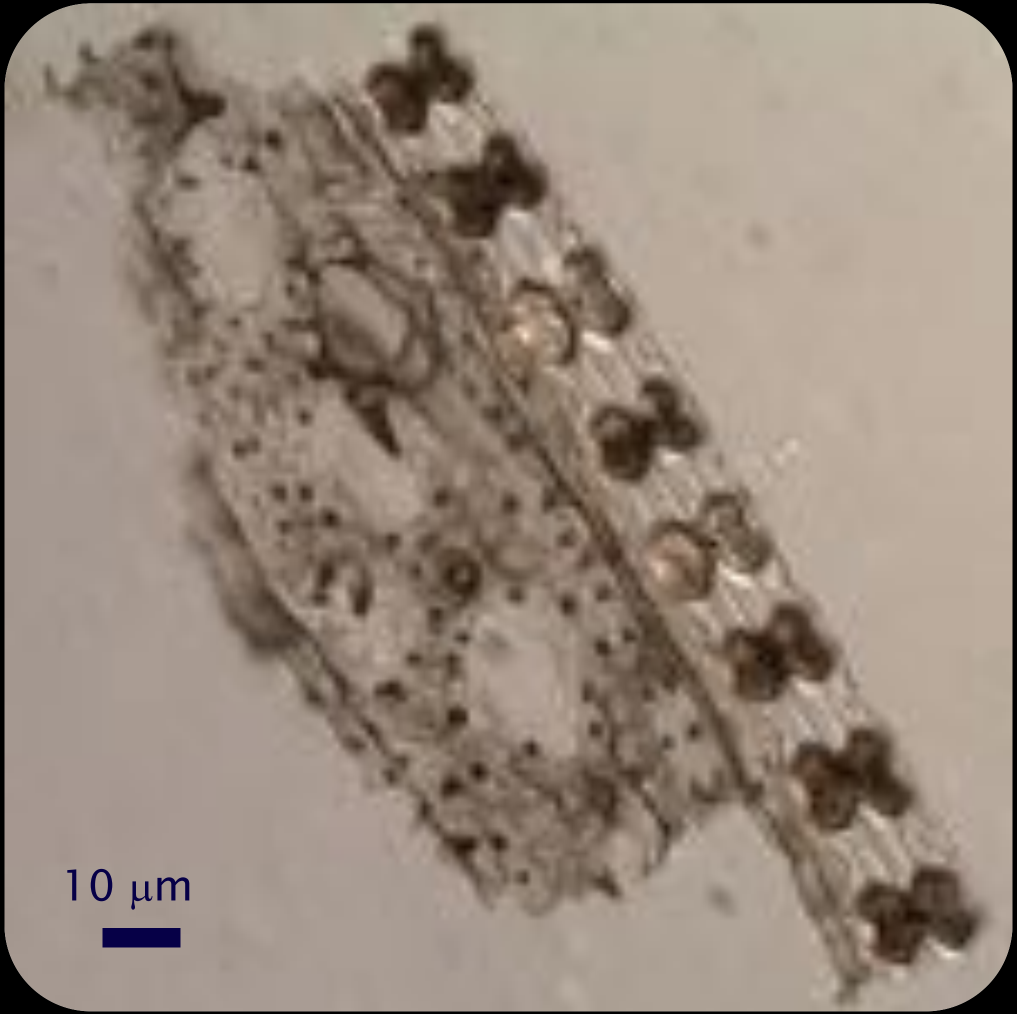
Prasad et al. (2005, 2011)



Early grass diversification

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

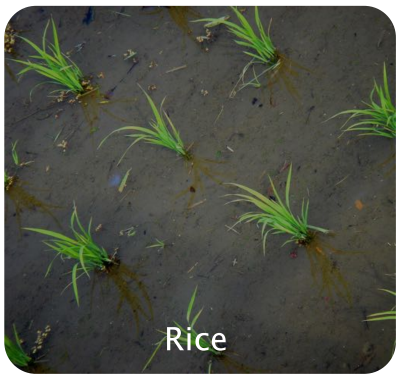
- Dating of phylogeny using the Late K phytoliths



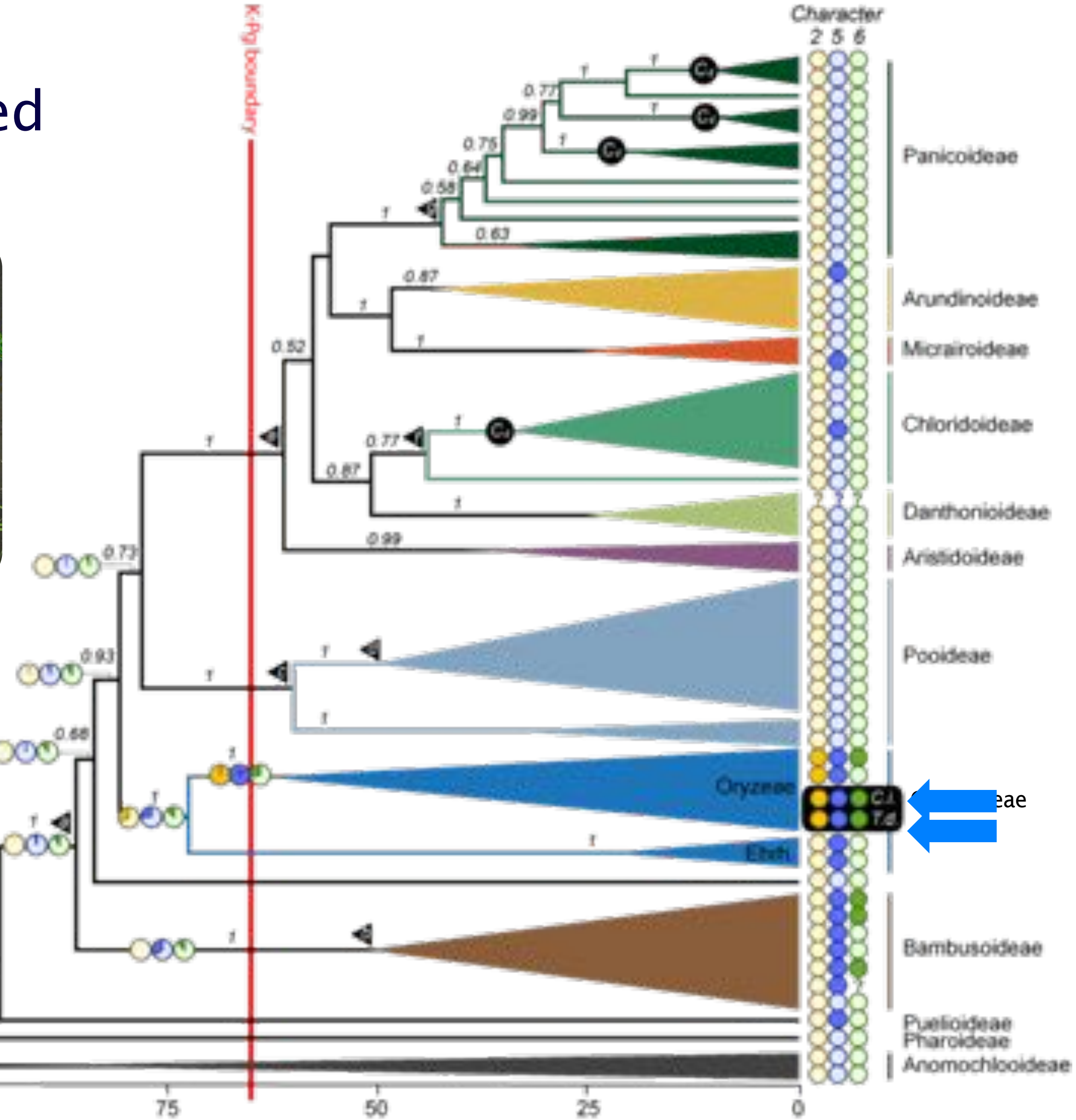
Prasad et al. (2011)

Early grass diversification

- Fossils are nested within Oryzeae



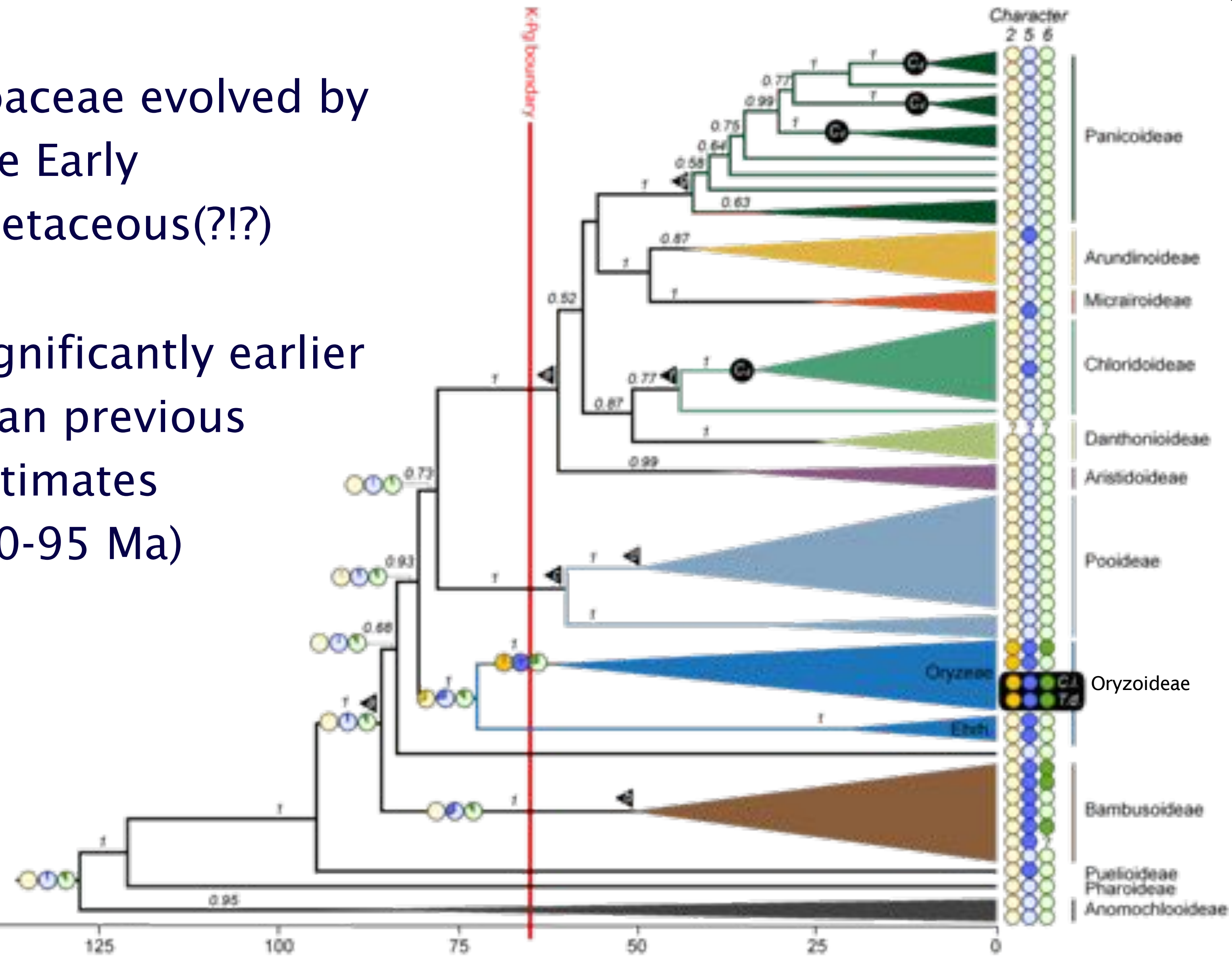
Characters:
2. Vertical bilobates
5. Papilla on long cells
6. Papilla on stomatal subsidiaries

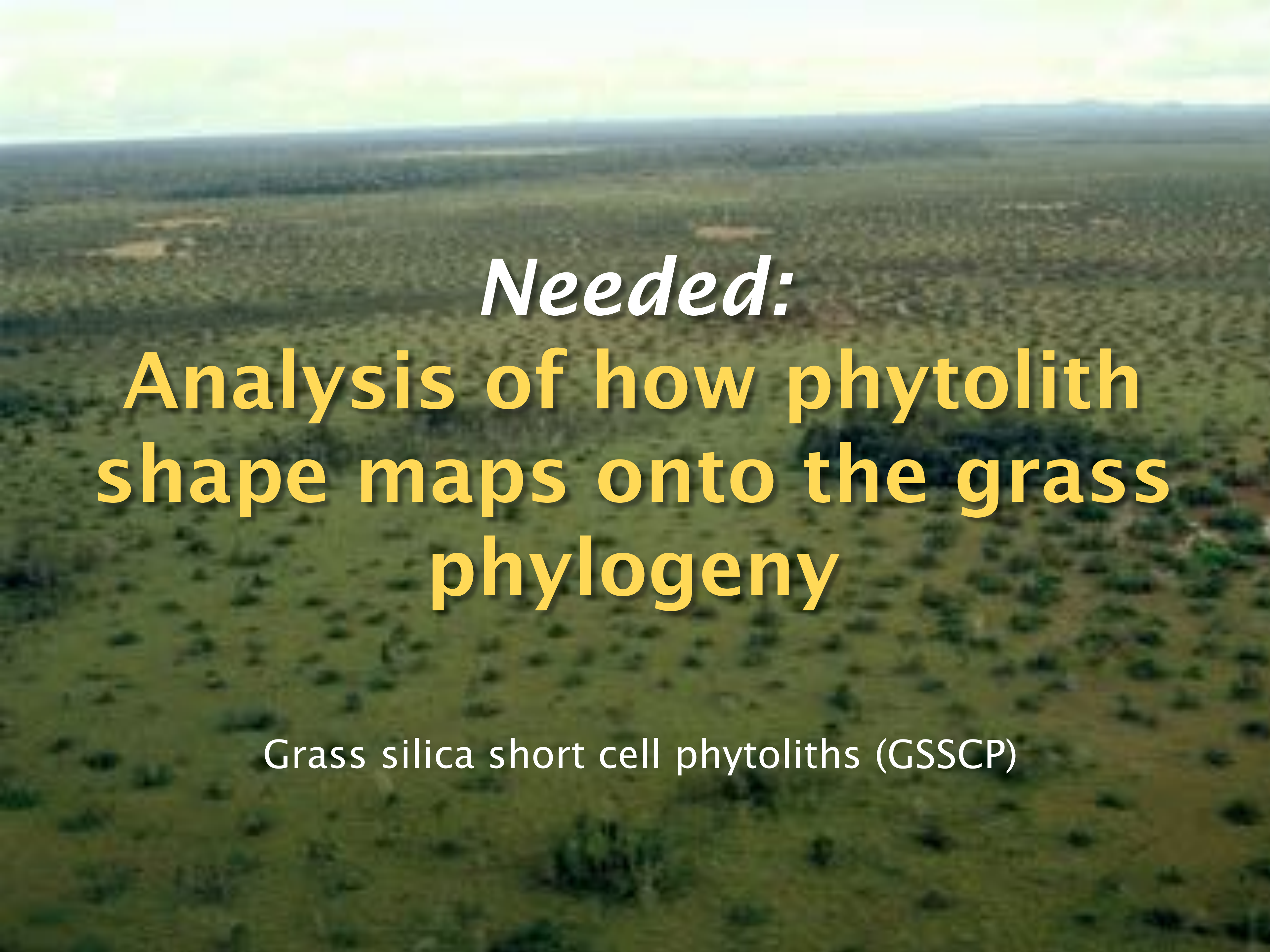


Prasad, Strömberg et al. (2011), *Nature Communications*

Early grass diversification

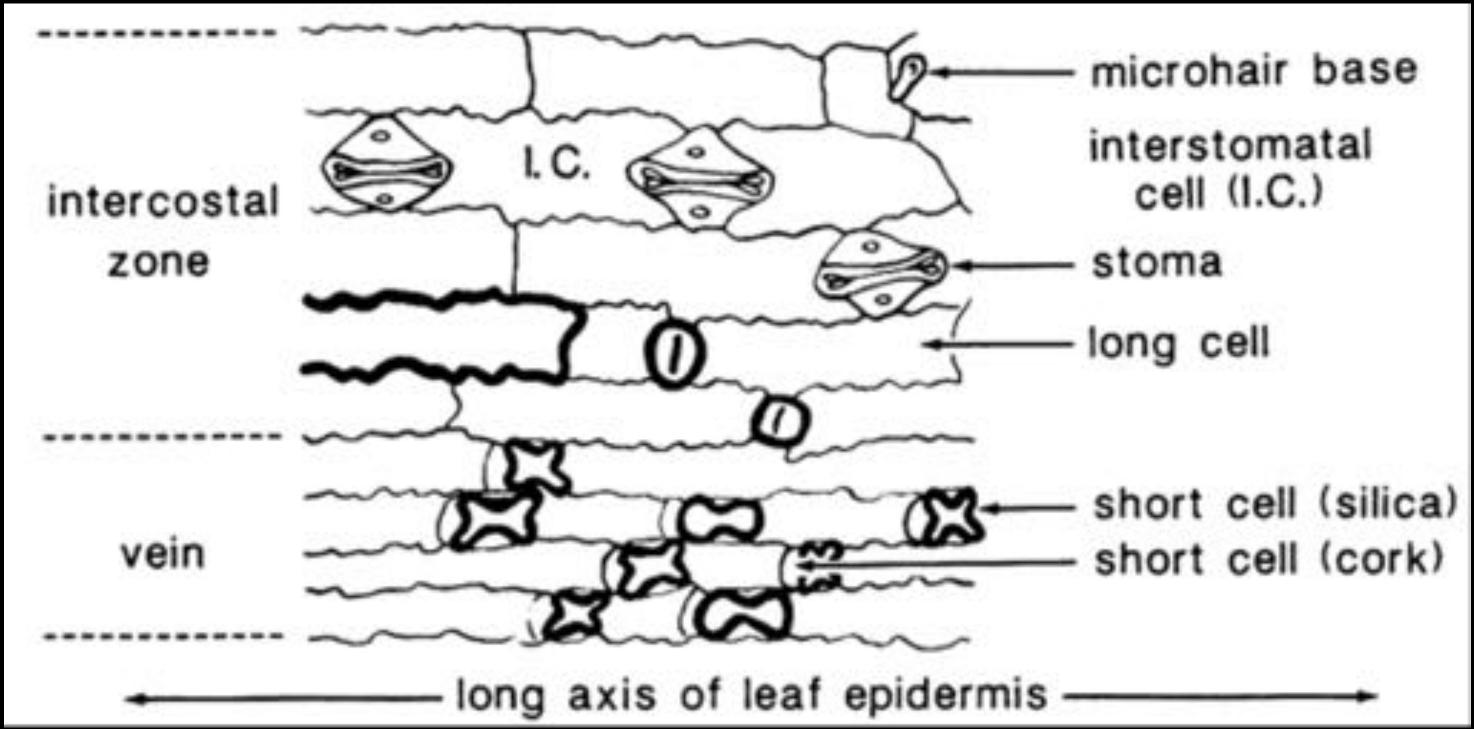
- Poaceae evolved by the Early Cretaceous(?!?)
- Significantly earlier than previous estimates (70-95 Ma)




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

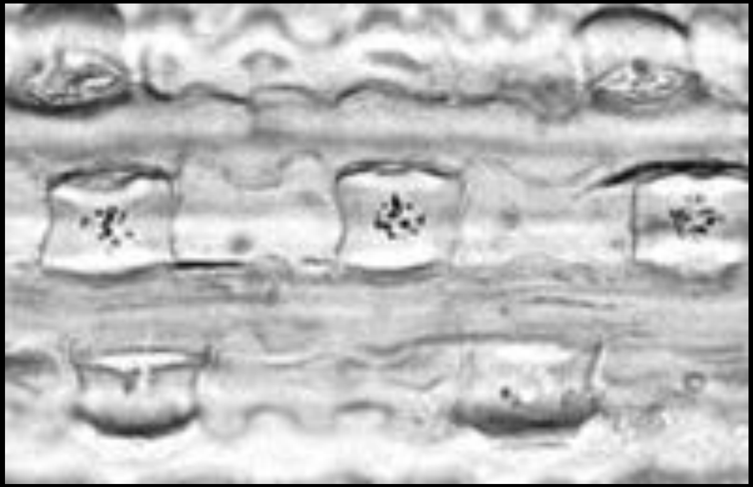


(Metcalfe 1960)

Inadequate current GSSCP shape keys

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

"saddle-shaped"



Chloris

Sinobambusa

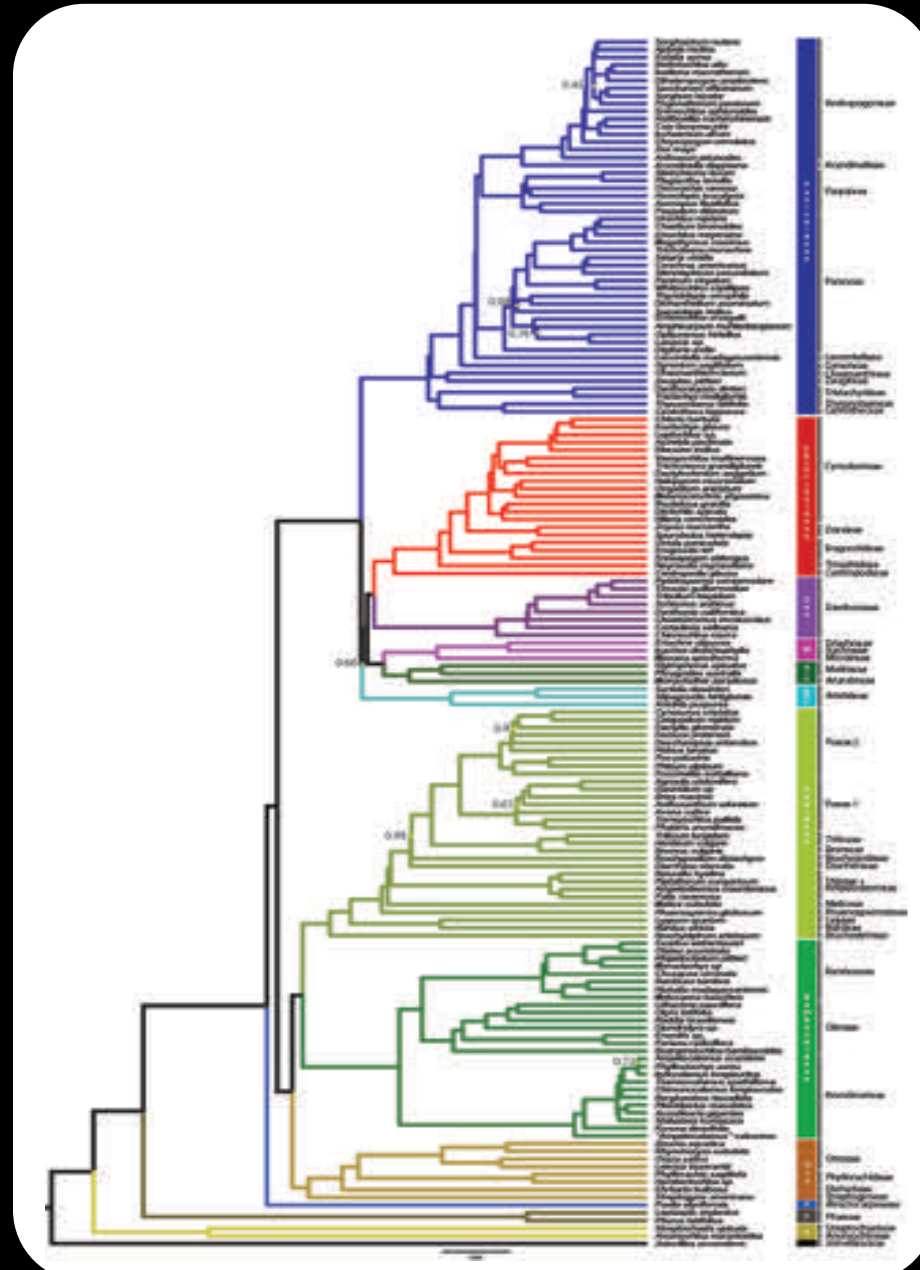
Dry-adapted, C_4 open-habitat grass

Mesophytic, tree-forming C_3 bamboo

(Watson and Dallwitz 1992-)

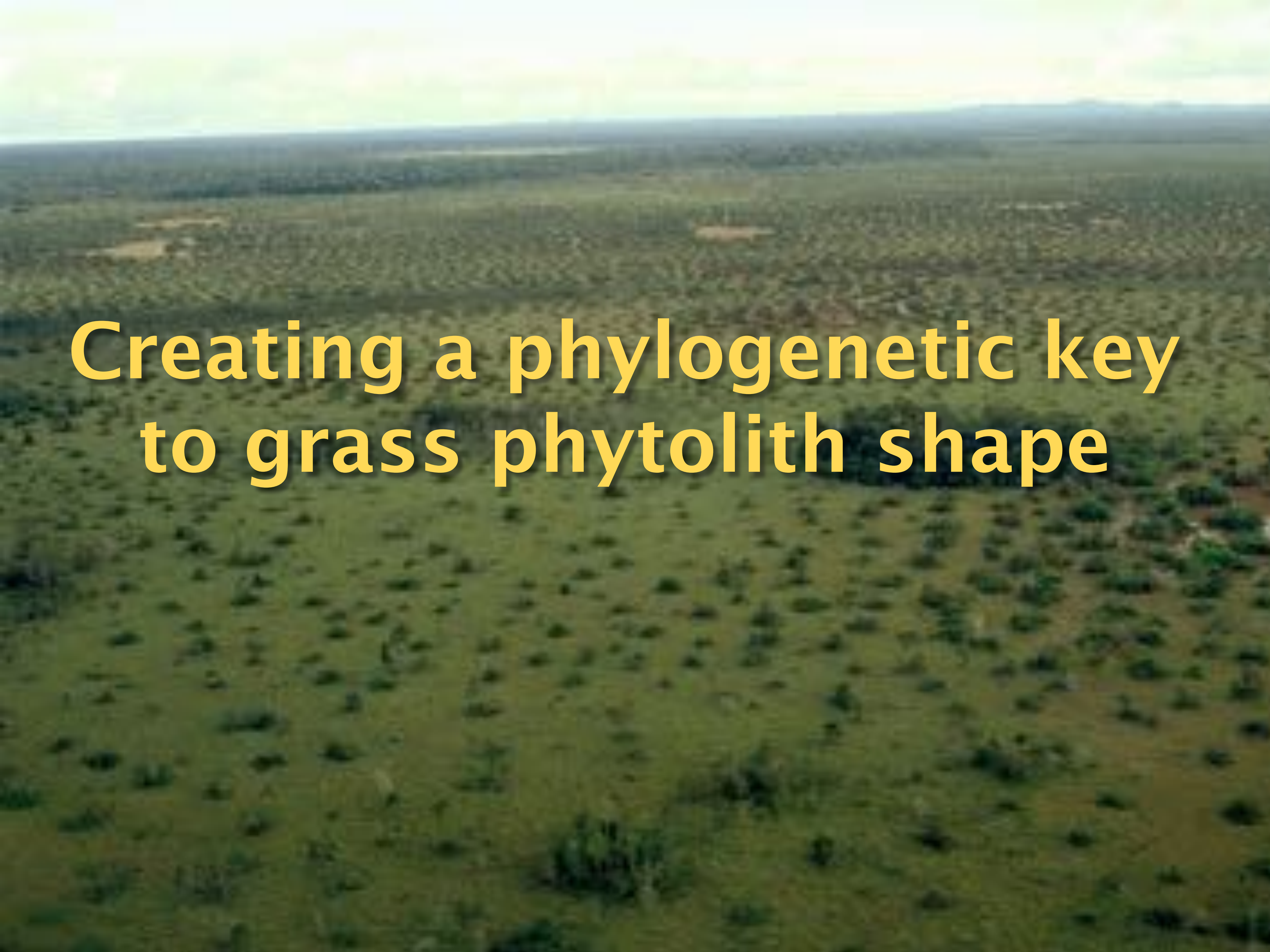
Inadequate current GSSCP shape keys

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

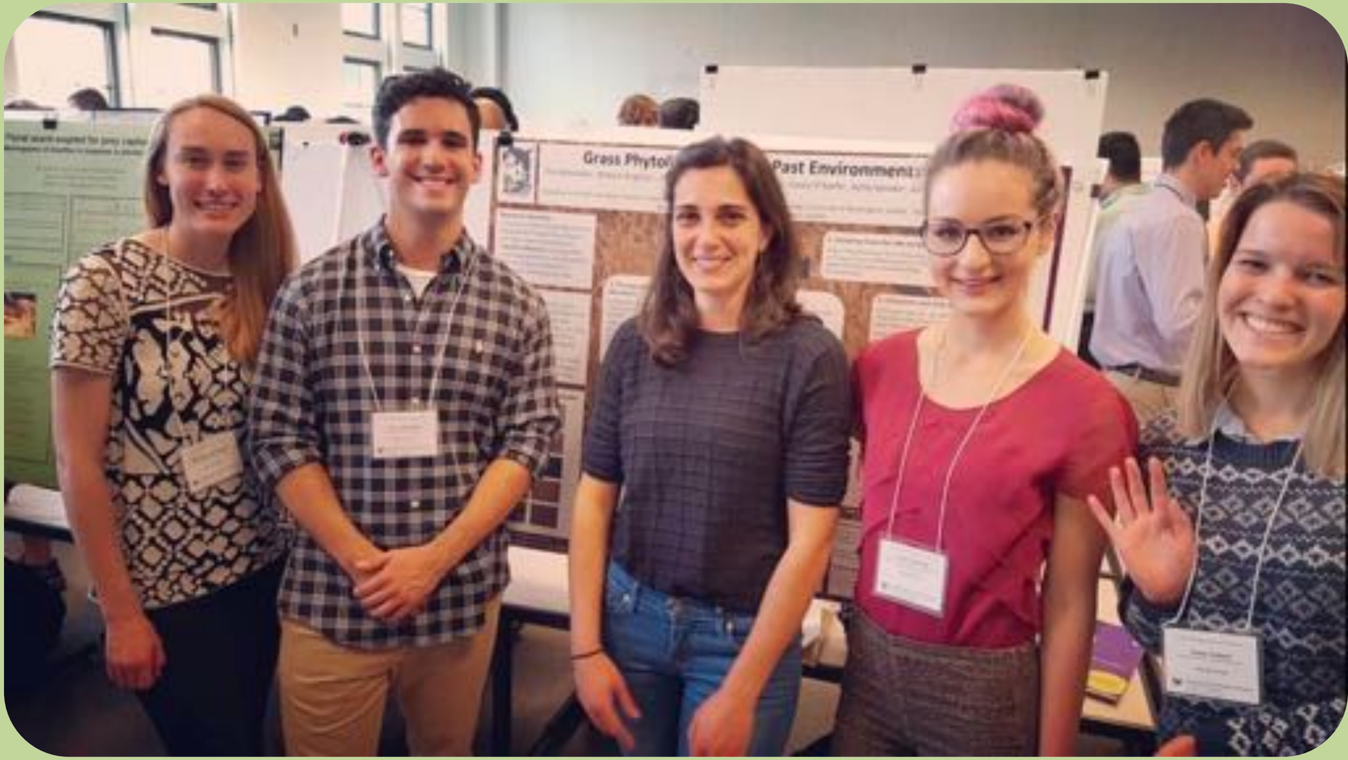


(Gallaher et al. in prep.)

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

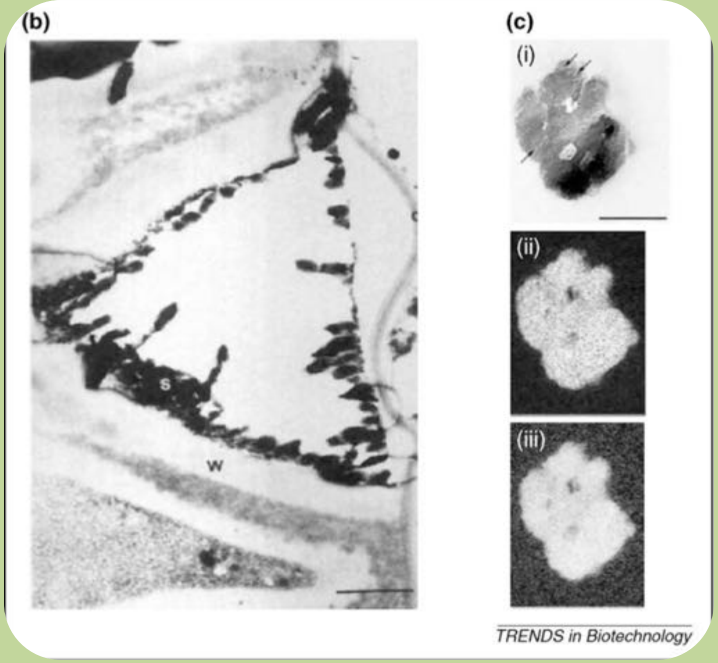
Unique properties of phytoliths

1. Phytoliths consists mainly of SiO_2 (66–91%), organic carbon OC (1–6%), H_2O (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)

Developing phytolith



(Neethirajan et al. 2009)

Unique properties of phytoliths

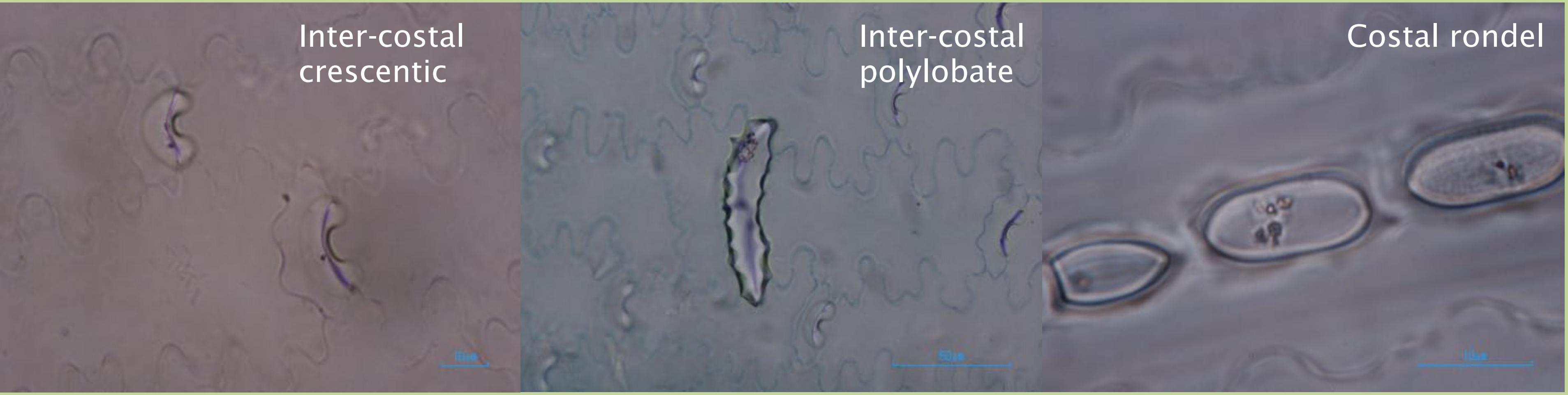
2. GSSCP phytoliths are small (~7-40 micrometers)

→ Resolution of e.g., micro-CT (100-200 micrometers) is not fine enough

Unique properties of phytoliths

3. Most grass species make >>1 type of GSSCPs

Anomochloa marantoidea



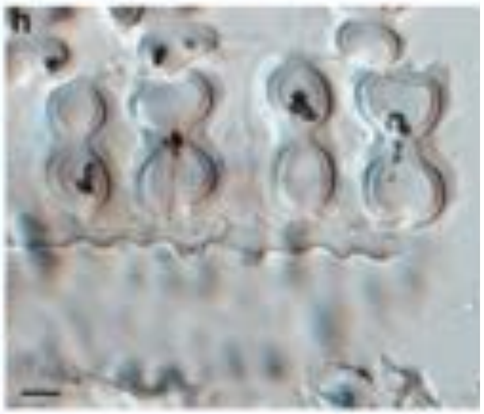
→ ***GSSCPs have to be studied like assemblages***

Unique properties of phytoliths

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

Potamophila

Zizania



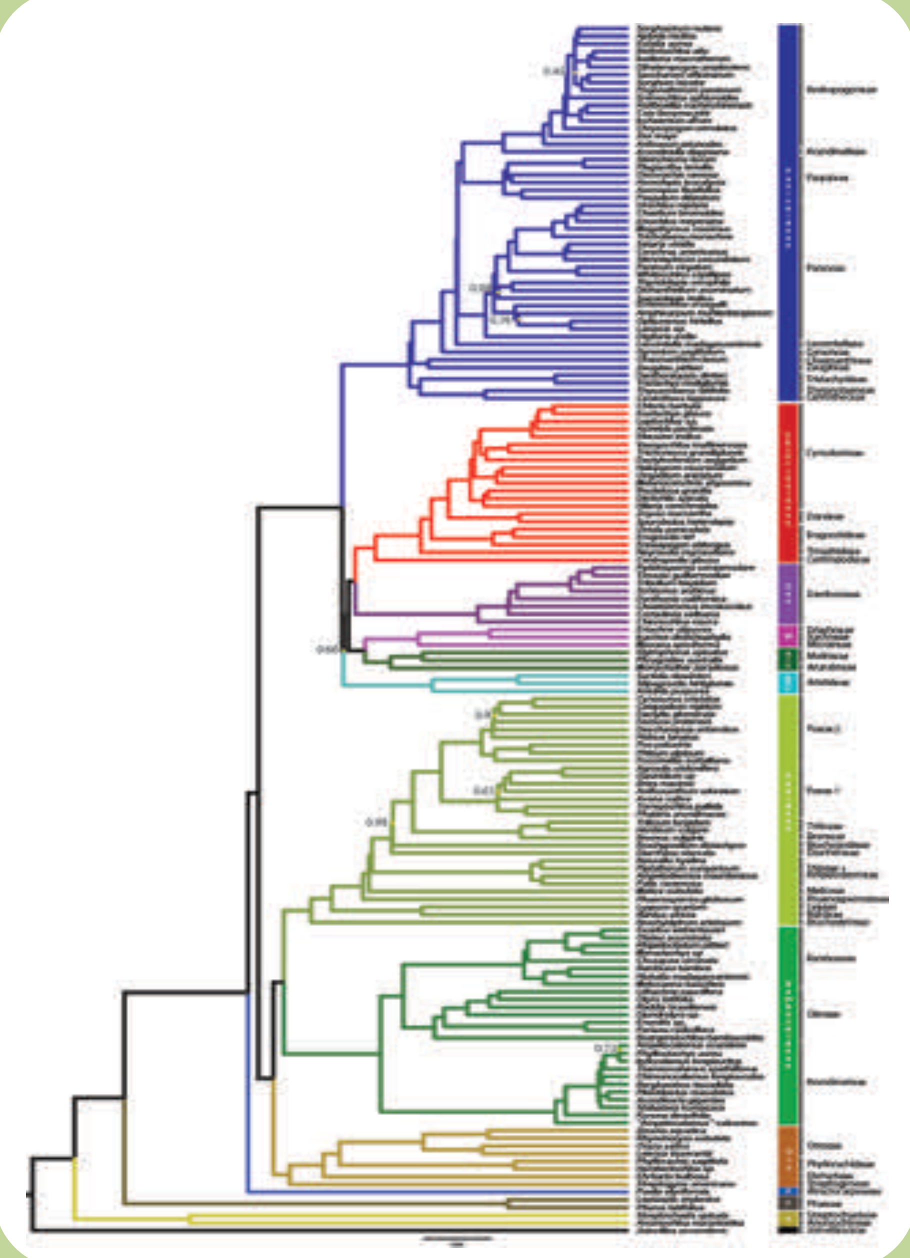
→ ***GSSCPs have to be studied*** **in situ**

Materials and methods

Taxa sampled:

- >200 grass genera from all Poaceae subclades
- Leaf material

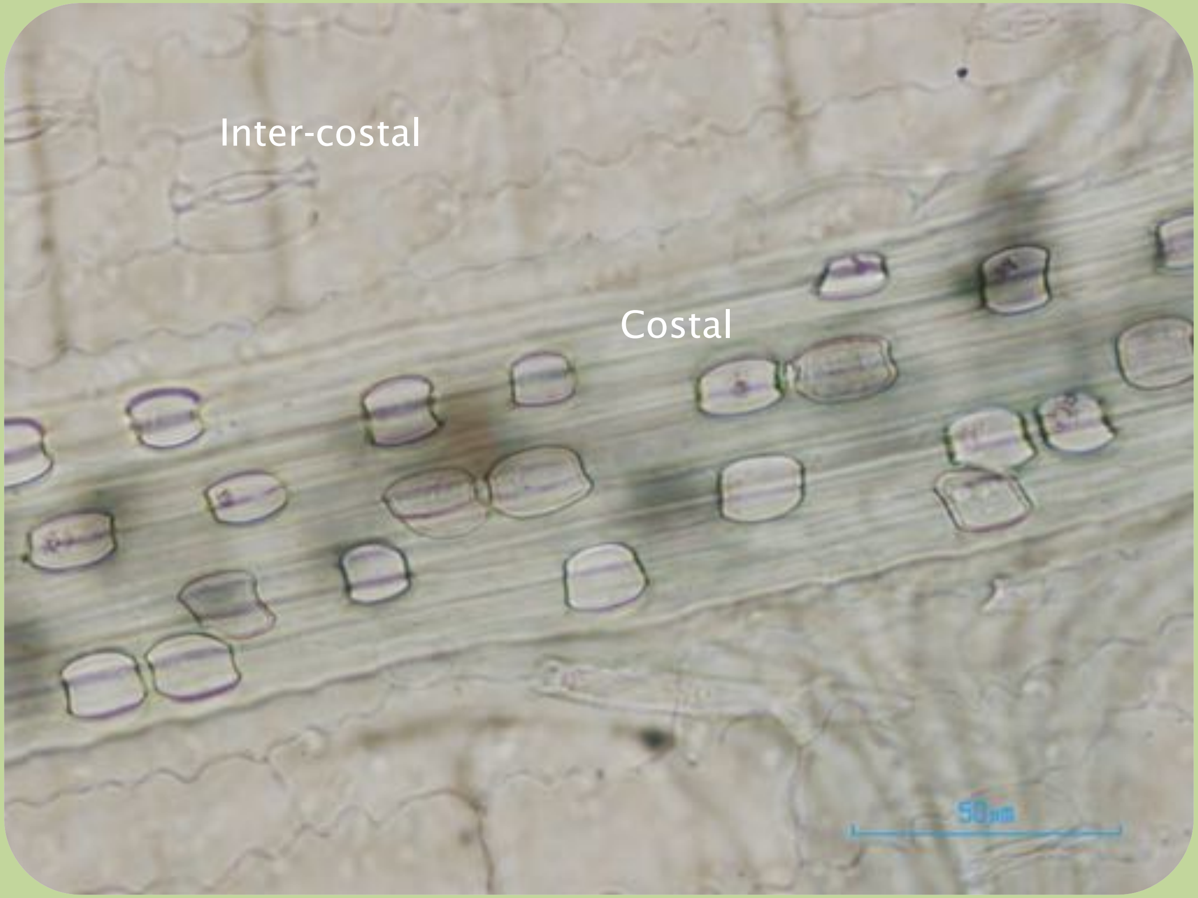
(Gallaher et al. in prep.)



Materials and methods

Data collected:

- Orientation and distribution of GSSCP shape

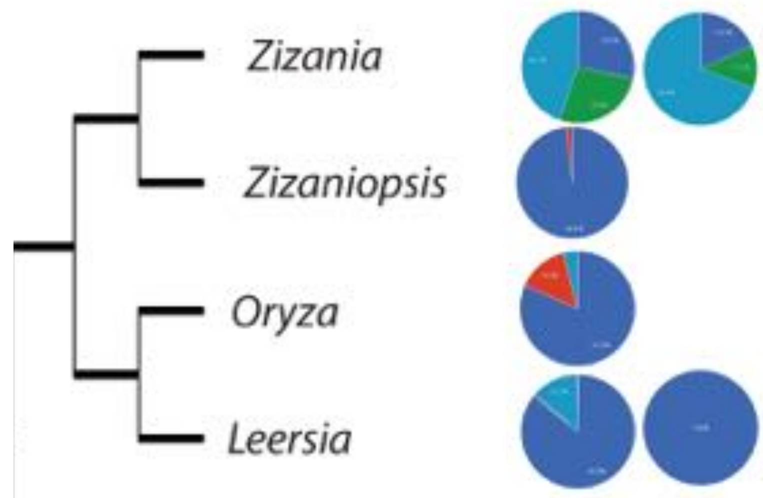


Materials and methods

Data collected:

- Orientation and distribution of GSSCP shape

- Relative abundances of GSSC types in GSSCP assemblages extracted from leaves

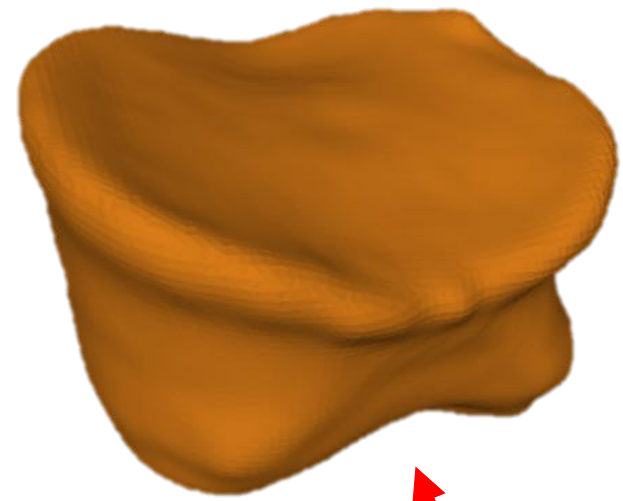


- >200 GSSCP /sample

Materials and methods

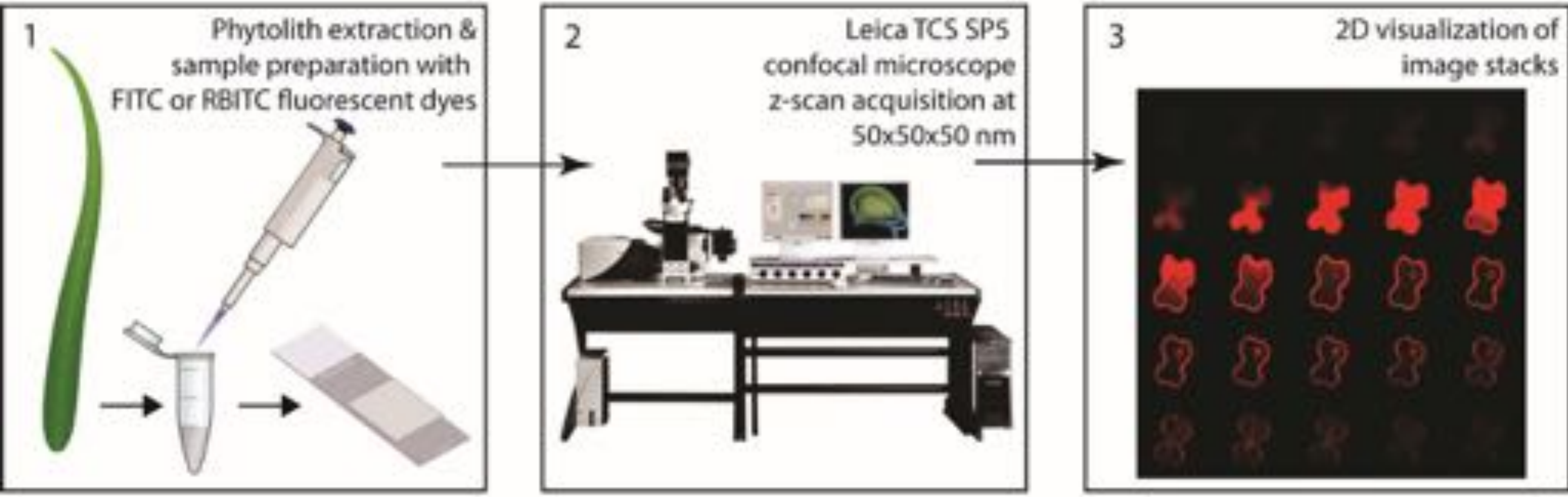
Data collected:

- 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

Protocols for imaging 3D Phytoliths - Stromberg Lab.
Updated: Feb. 8, 2017

Making Slides for Confocal Microscopy

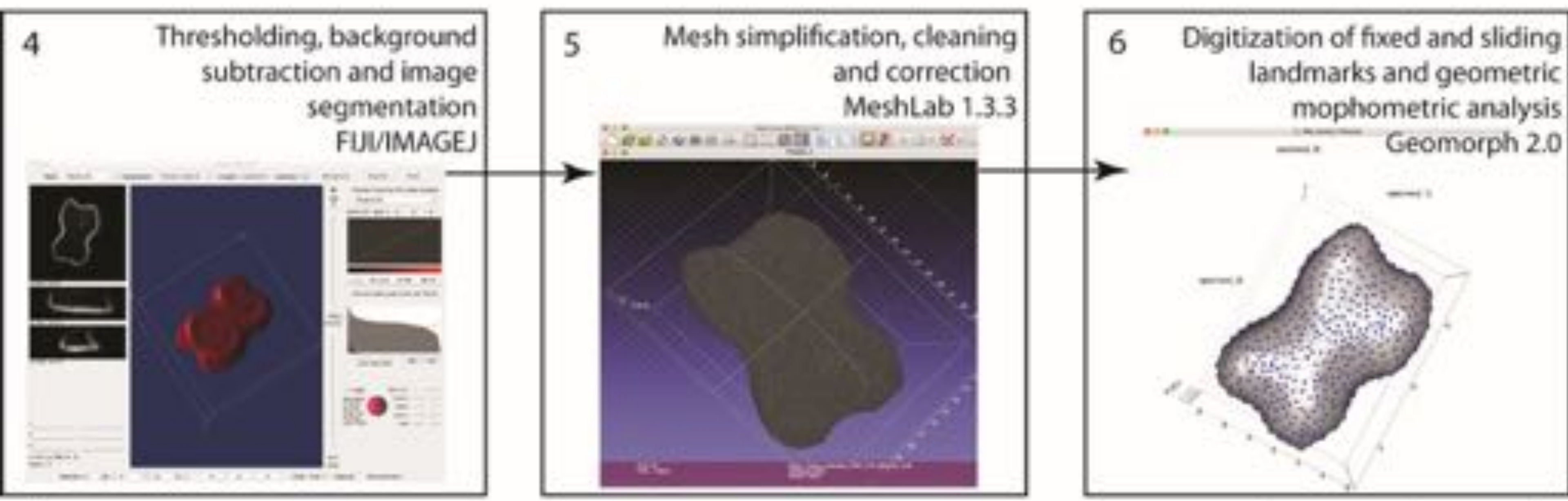
LABELING

- Label microcentrifuge tubes (1-8 or A-H, etc...) and write sample information in the project notebook

STAINING/MOUNTING

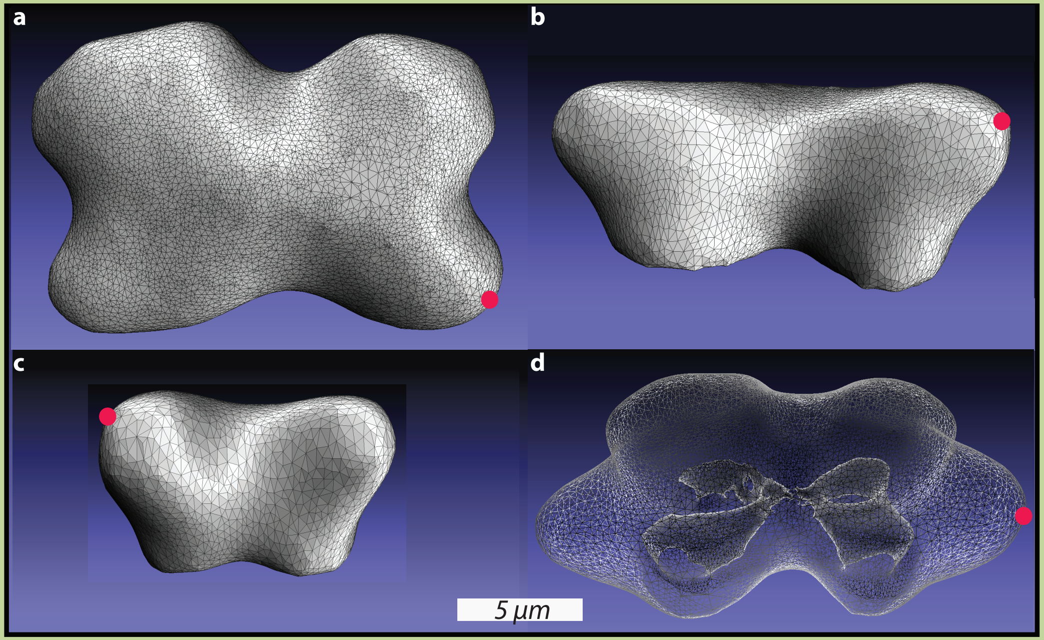
- **Gently vortex vials with extracted phytoliths (in 95% ETOH).**
  - Allow particles to settle **(about 5 min).**
  - With a (p1000) micropipette remove 200 microliters (ul) of solution from the bottom of the glass vial and add it to a 0.7 ml microcentrifuge tube.
  - Spin an even number of tubes in the microcentrifuge for 5 min.
  - Pipette out and discard supernatant.
- **Add 200 ul of 10% detergent solution (Wash1)**
  - Shake on vortexer (set at 6) with microtube attachment for 10 min.
  - Spin for 5 min. Pipette out and discard supernatant.

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*



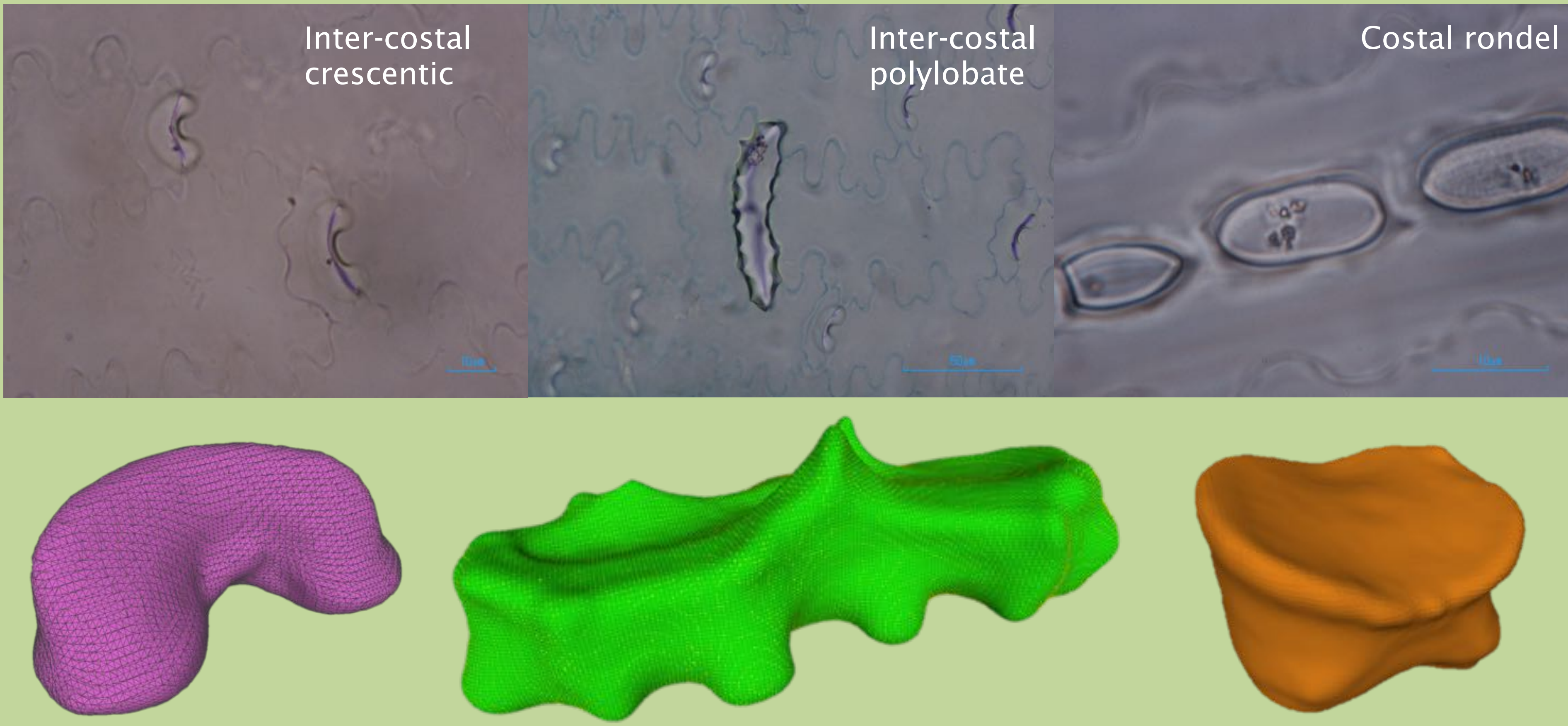
- Use 3-D surface meshes to calculate measures of size such as length, width, height, surface area and volume

- Transform and align meshes using Procrustes superimposition to remove size

3-D data workflow: *Outcomes*

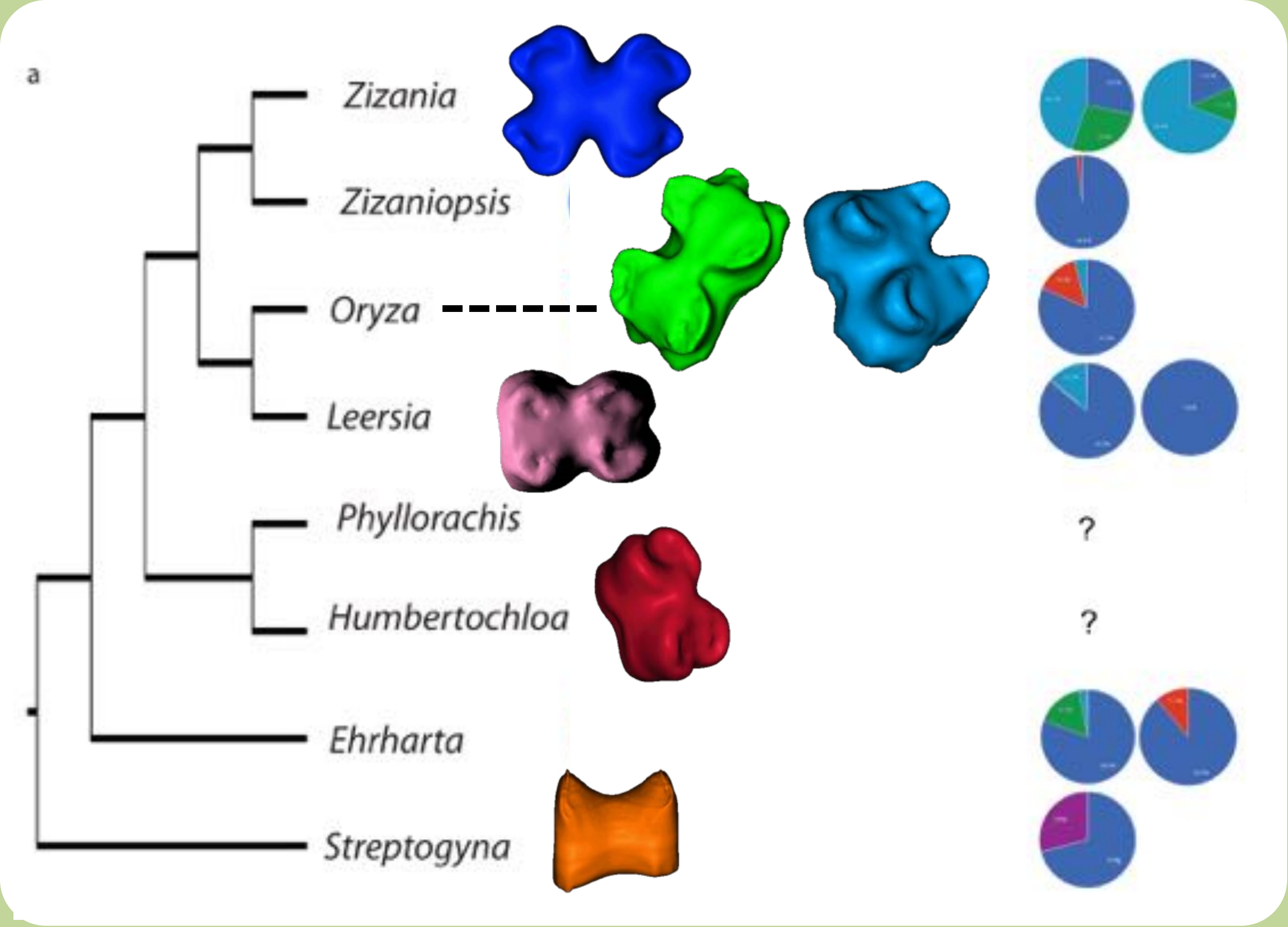
- Quantified shape for morphometric analysis and phylogenetic mapping
- Animations and 3-D printable objects

Anomochloa marantoidea



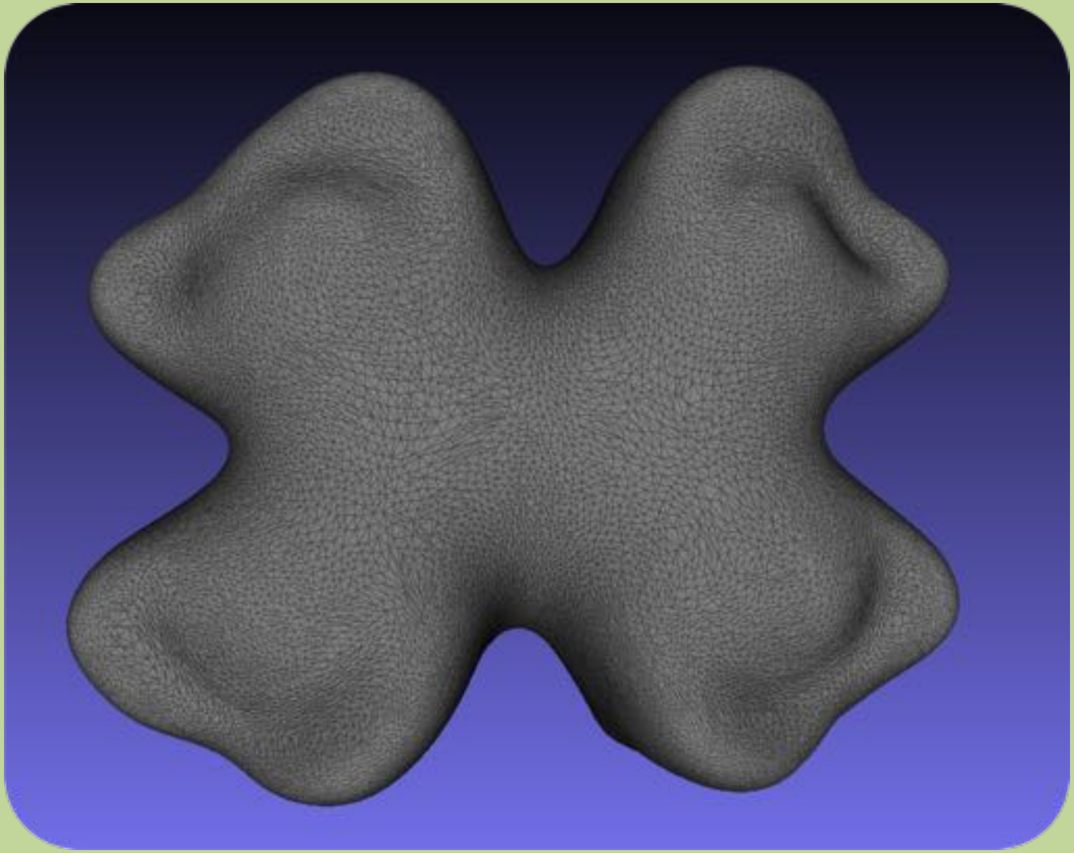
Preliminary results

- Counts and confocal images of 3-D shape of GSSCPs for the Oryzoideae, Bambusoideae and early-diverging grasses



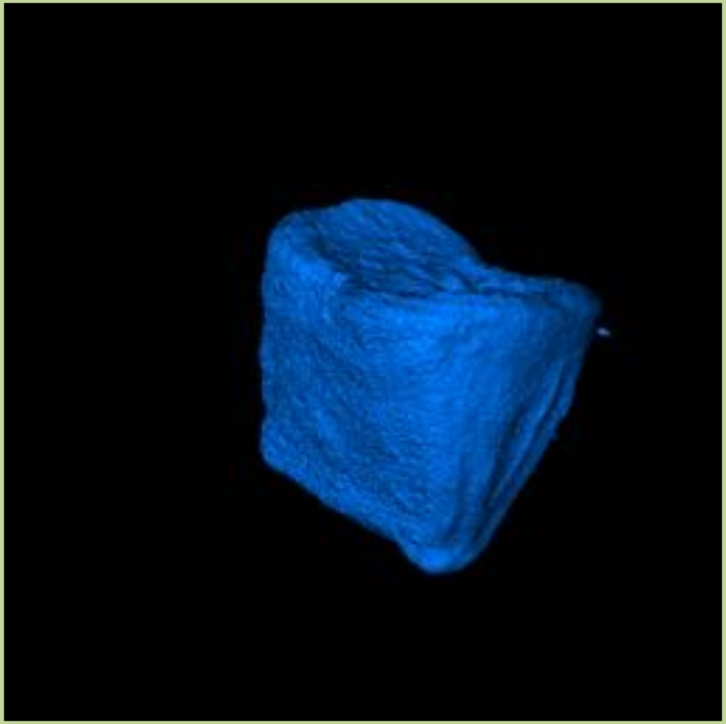
Problems encountered so far

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



Oryza

Rough mesh before smoothing algorithms have been applied



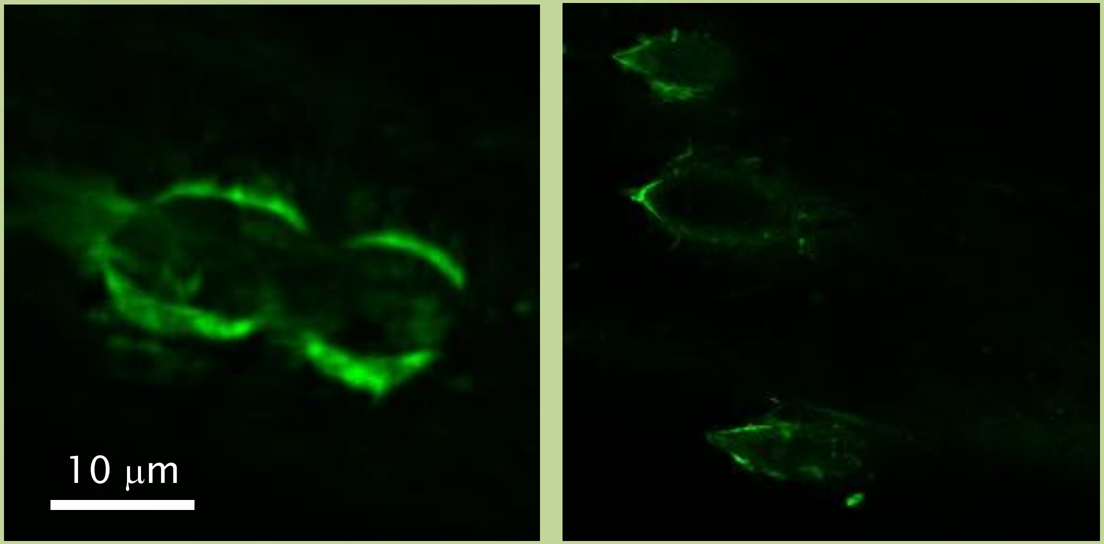
Pharus

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
2. Manual removal of “debris”
3. Development of a new stains in the form of hybridization probes specific to silica



Mehmet Sarikaya
Materials Sciences & Engineering
University of Washington

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)!

Acknowledgements

Burke Museum, University of Washington

UW Biology

Strömberg Lab

Grad students:
Camilla Crifo
Will Brightly

Postdocs:
Georgina Erra
Tim Gallaher

Undergrads & volunteers:
Thien-Y Le
Kim Smith
Ryan Thummel
Nik Pershing
Anna Schorr
Elie Aboulafia
Brittany McManus
Casey O'Keefe
Ashly Senske
Erin Sofonowski
Claire Marvet
Sultan Akbar

Collaborators

Vandana Prasad
Dhananjay Mohabey
Ashok Sahni
Adam Leaché
Sue Hartley
Erika Edwards
Colin Osborne
Pascal-Antoine Christin
Elizabeth Kellogg
C4 Grasslands Working Group
Doris Barboni
Rosa Maria Albert
Katharina Neumann
Luc Vrydaghs
Rich Kay

Funding

NSF

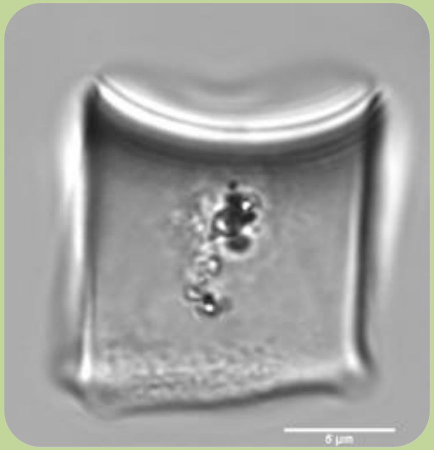
NESCent (National Evolutionary Synthesis Center)

University of Washington 1861 — RRF

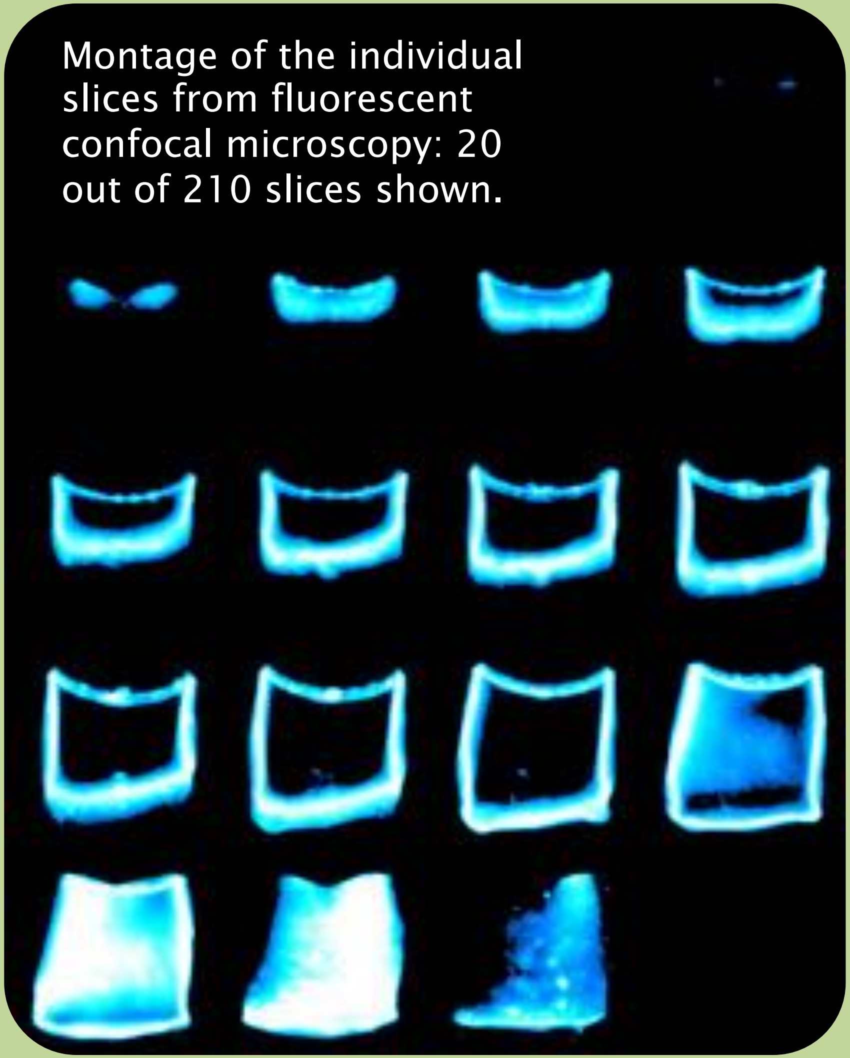

Thank you for your attention!

Part II: Problems encountered so far

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



Isolated GSSCP



Pharus