A high-throughput automated method for analyzing plant cuticle specimens

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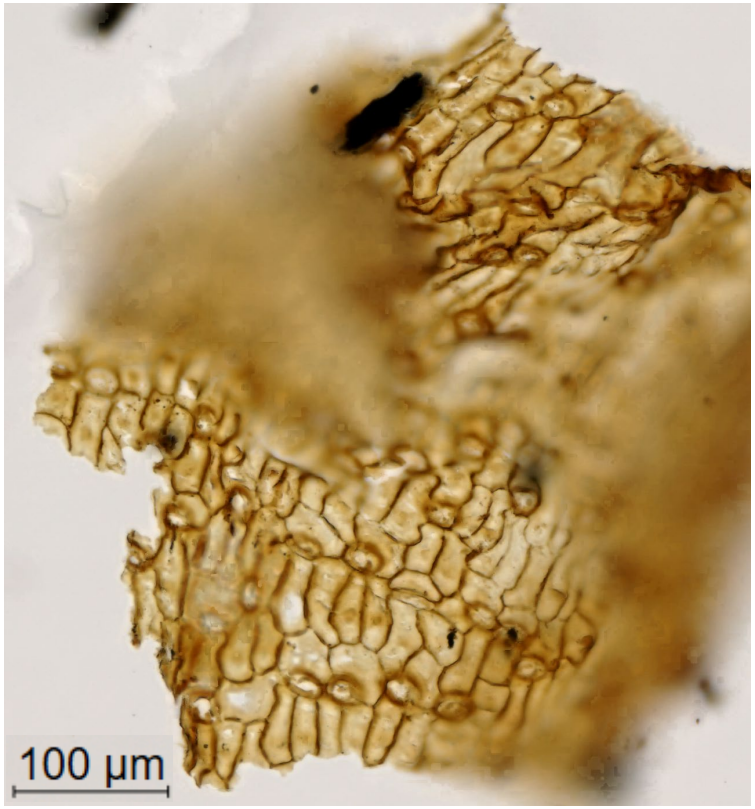
Potential of Fossil Cuticle

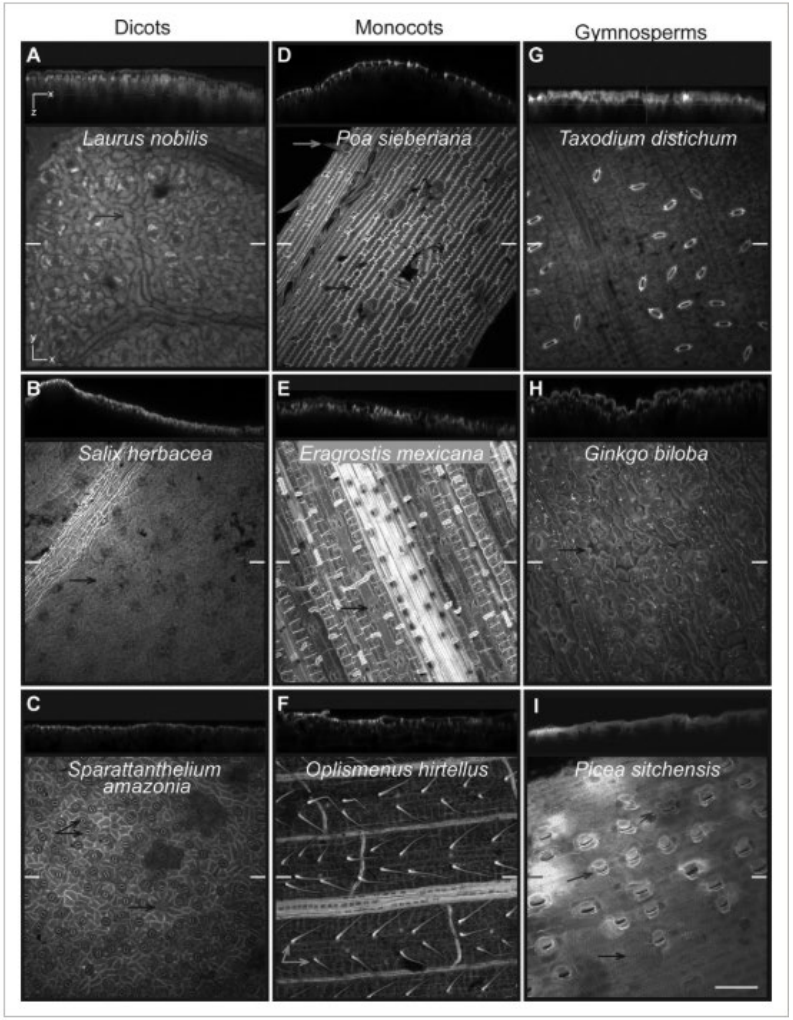
- Dispersed leaf cuticle is a forgotten fossil
- More abundant in paleo record than entire leaves



Carboniferous Dispersed Cuticle

Taxonomic Characteristics

- Epidermal cells capture taxonomic characteristics



Urban et al 2018

Importance of Epidermal Cells

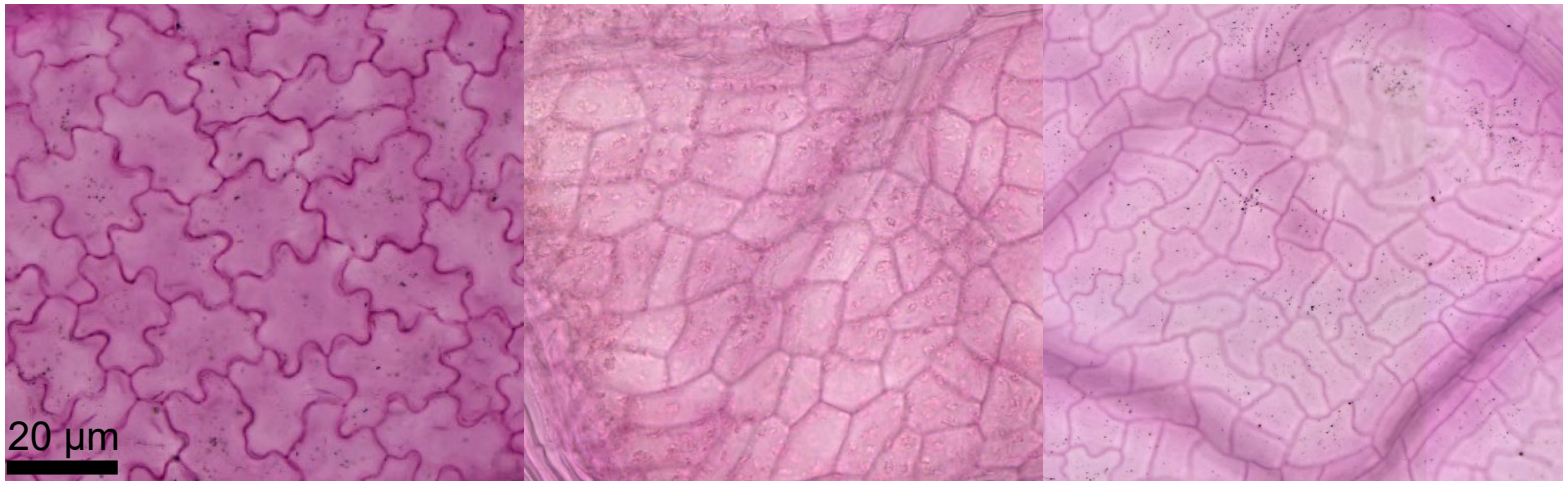
- Waxy layer that preserves imprint of epidermal cells
- Epidermal cells capture leaf development and reflect climatic conditions

Shutterstock

Epidermal Cells and Climate

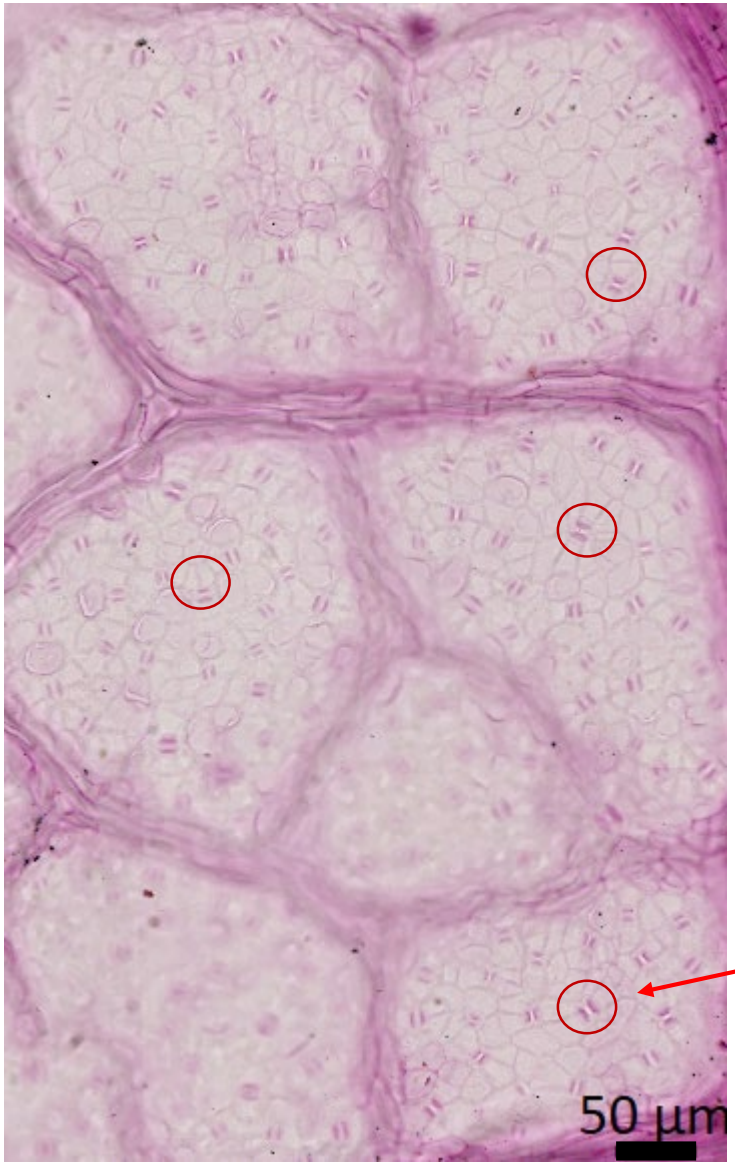
- Modern cuticle assemblages can help analyze the relationship between epidermal cell shape and climate

- Establishing a new paleoproxy for reconstructing paleoclimate could fill in gaps in environmental paleorecords



Vermont | Mississippi | West Virginia

Previous Research: Stomata



- Focused on stomata
- Reconstruct paleo CO_2 concentration
- Stomatal counting has been automated

Previous Research: Epidermal Cells

- Previous research conducted manually

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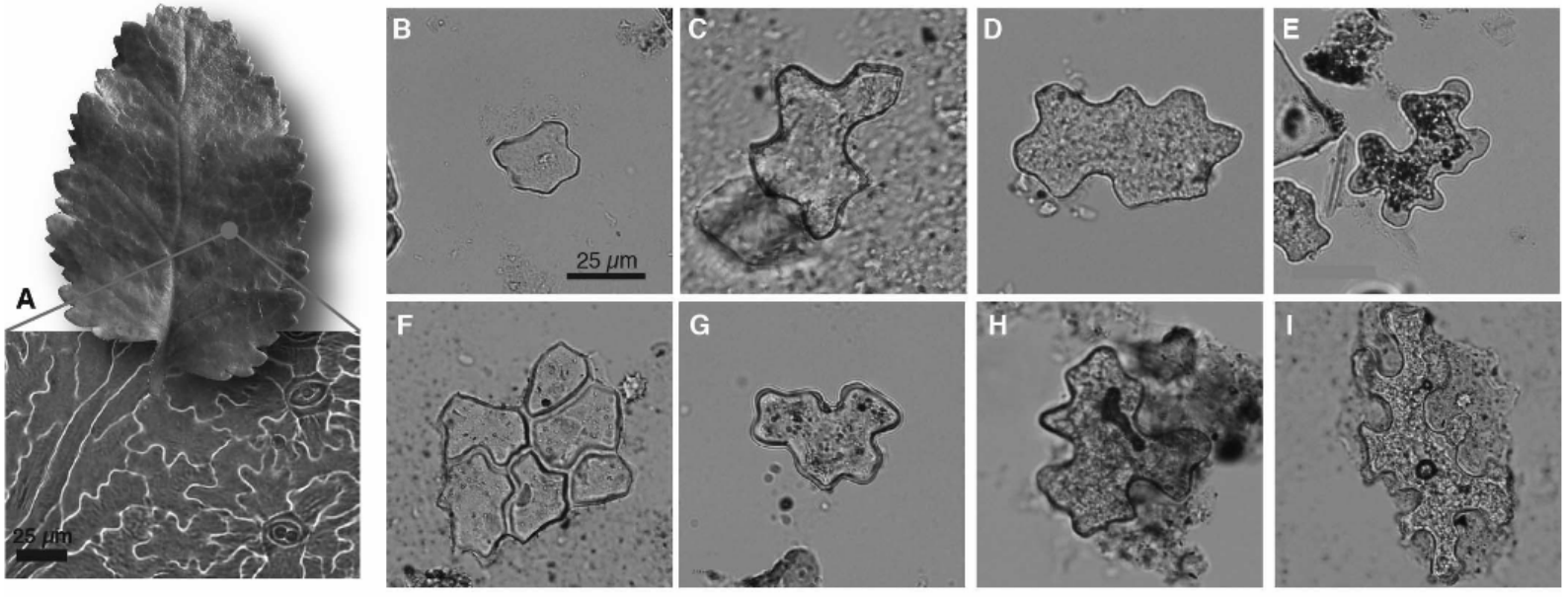
- Previous research conducted manually
- Leaf area index (LAI) used to reconstruct canopy openness in the Cenozoic of Patagonia



Dunn et al 2015

Research Question

How does epidermal cell morphology change in different climates?

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Hypothesis: Leaves grown in different climates will have different epidermal cell morphology.

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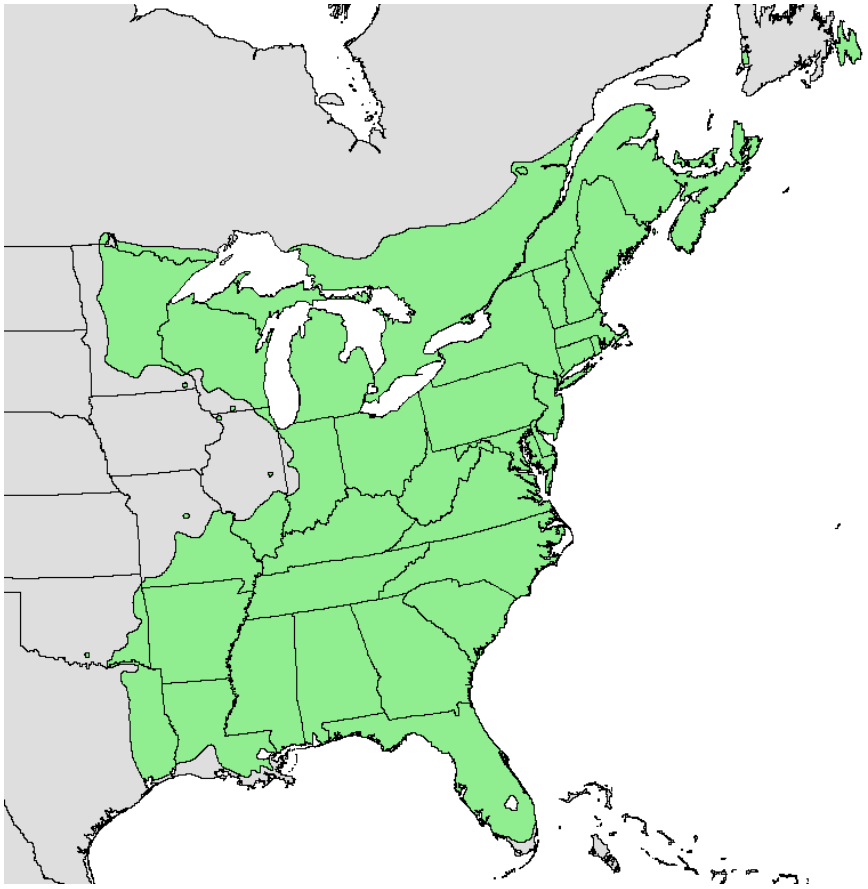
We used *Acer rubrum* to test this hypothesis

Acer rubrum

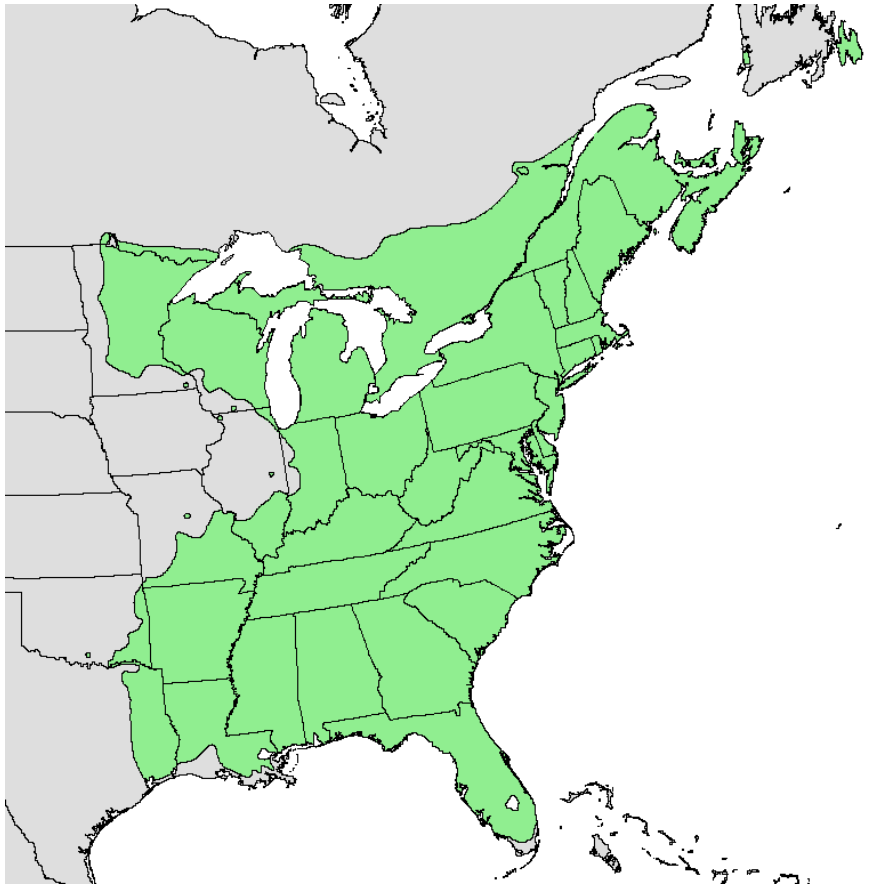
- *Acer rubrum* is a widespread species



Wikipedia

Acer rubrum

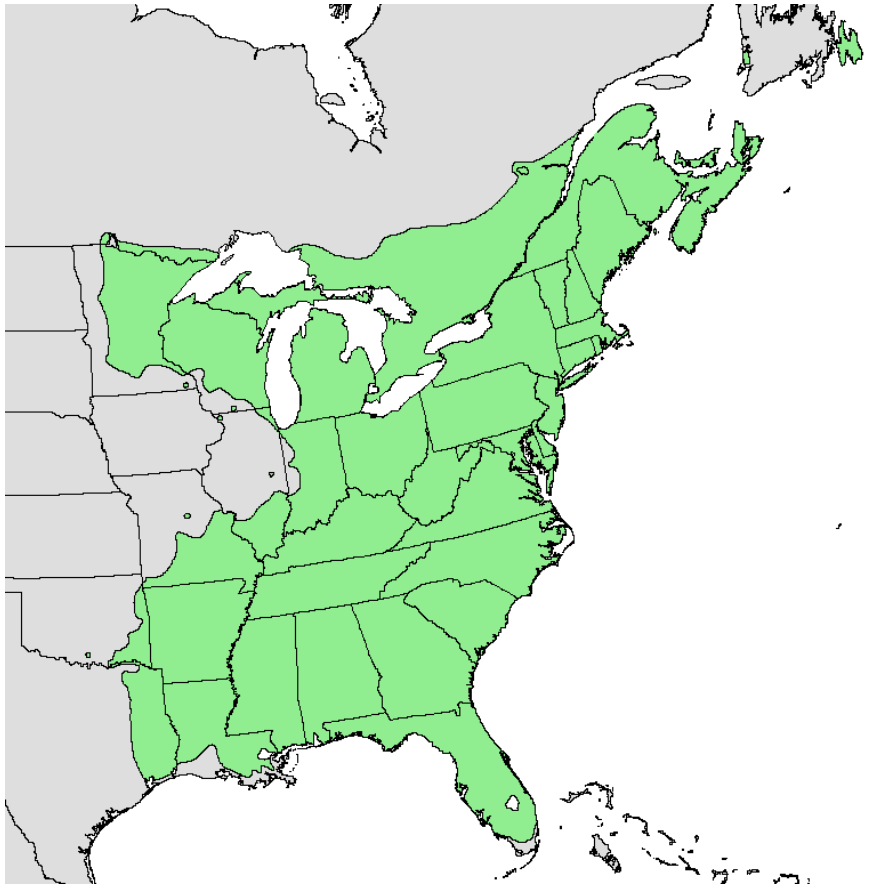
- *Acer rubrum* is a widespread species
- Tolerates a wide range of climatic conditions



Wikipedia

Acer rubrum

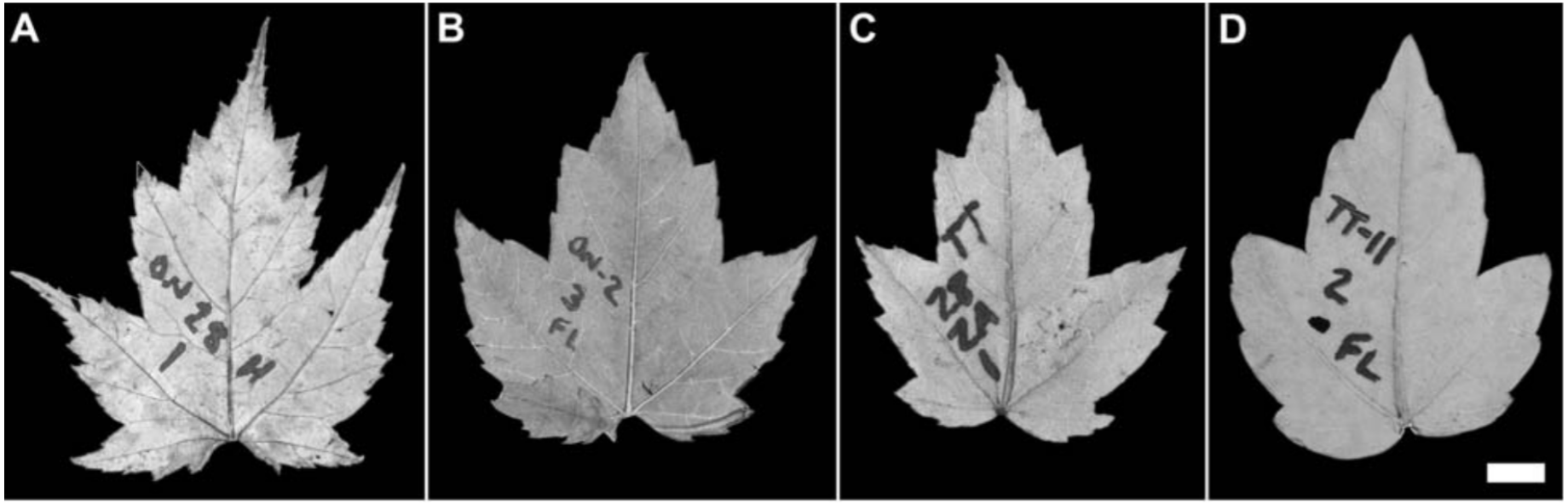
- *Acer rubrum* is a widespread species
- Tolerates a wide range of climatic conditions
- Ideal for studying relationship with climate



Wikipedia

Acer rubrum

- Known morphological relationship between leaf teeth and climate



Royer et al. 2009

Methods Summary

- *Acer rubrum* samples obtained from Smithsonian herbarium

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  - Filtering of noise in results

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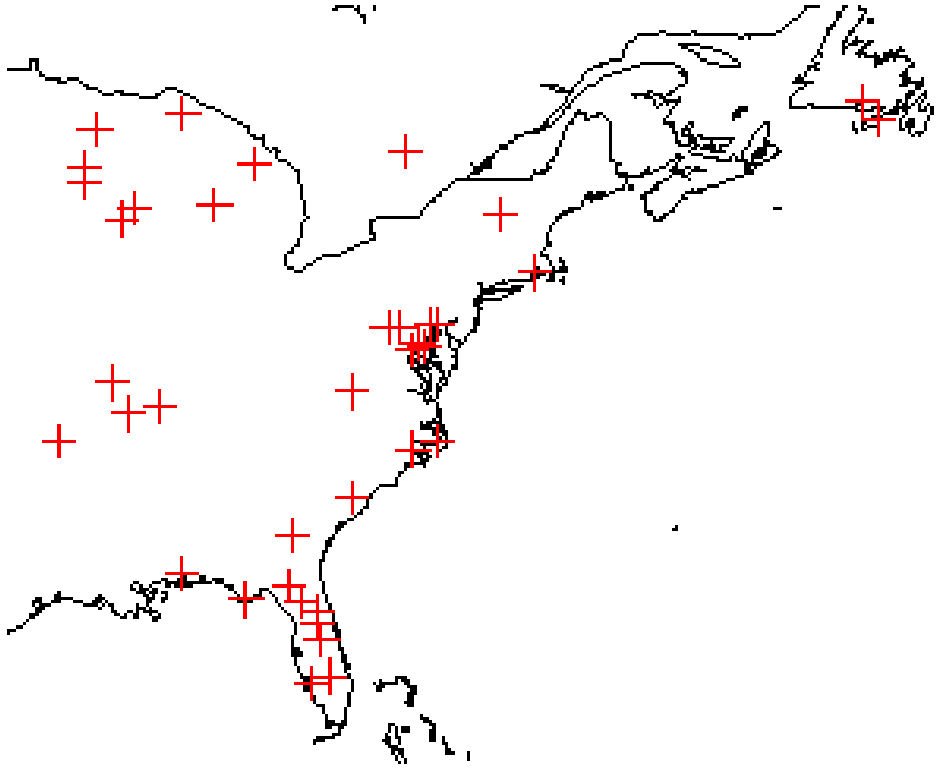

Acer rubrum Samples

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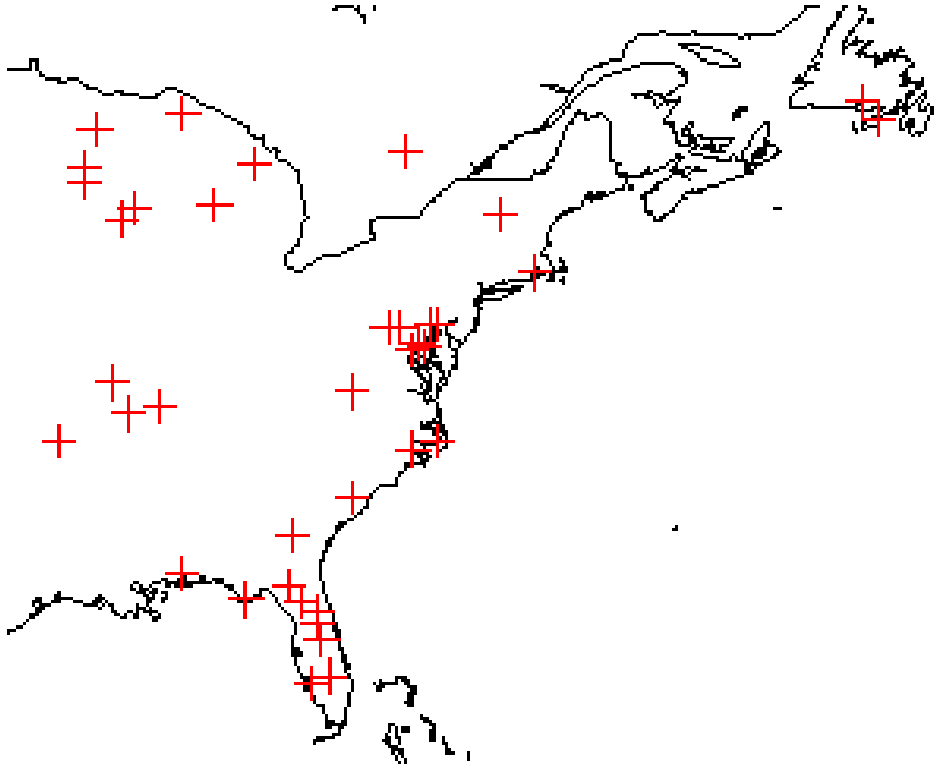
- These results are for a subset of 37 individuals (54 leaves)
- Sun and shade leaves could not be accounted for



Climate Data

Climate data was obtained from worldclim.org

Temperature Variables	Precipitation Variables
Annual Mean Temperature	Annual Precipitation
Mean Diurnal Range	Precipitation of Wettest Month
Isothermality	Precipitation of Driest Month
Temperature Seasonality	Precipitation Seasonality
Max Temperature of Warmest Month	Precipitation of Wettest Quarter
Min Temperature of Coldest Month	Precipitation of Driest Quarter
Temperature Annual Range	Precipitation of Warmest Quarter
Mean Temperature of Wettest Quarter	Precipitation of Coldest Quarter
Mean Temperature of Driest Quarter	
Mean Temperature of Warmest Quarter	
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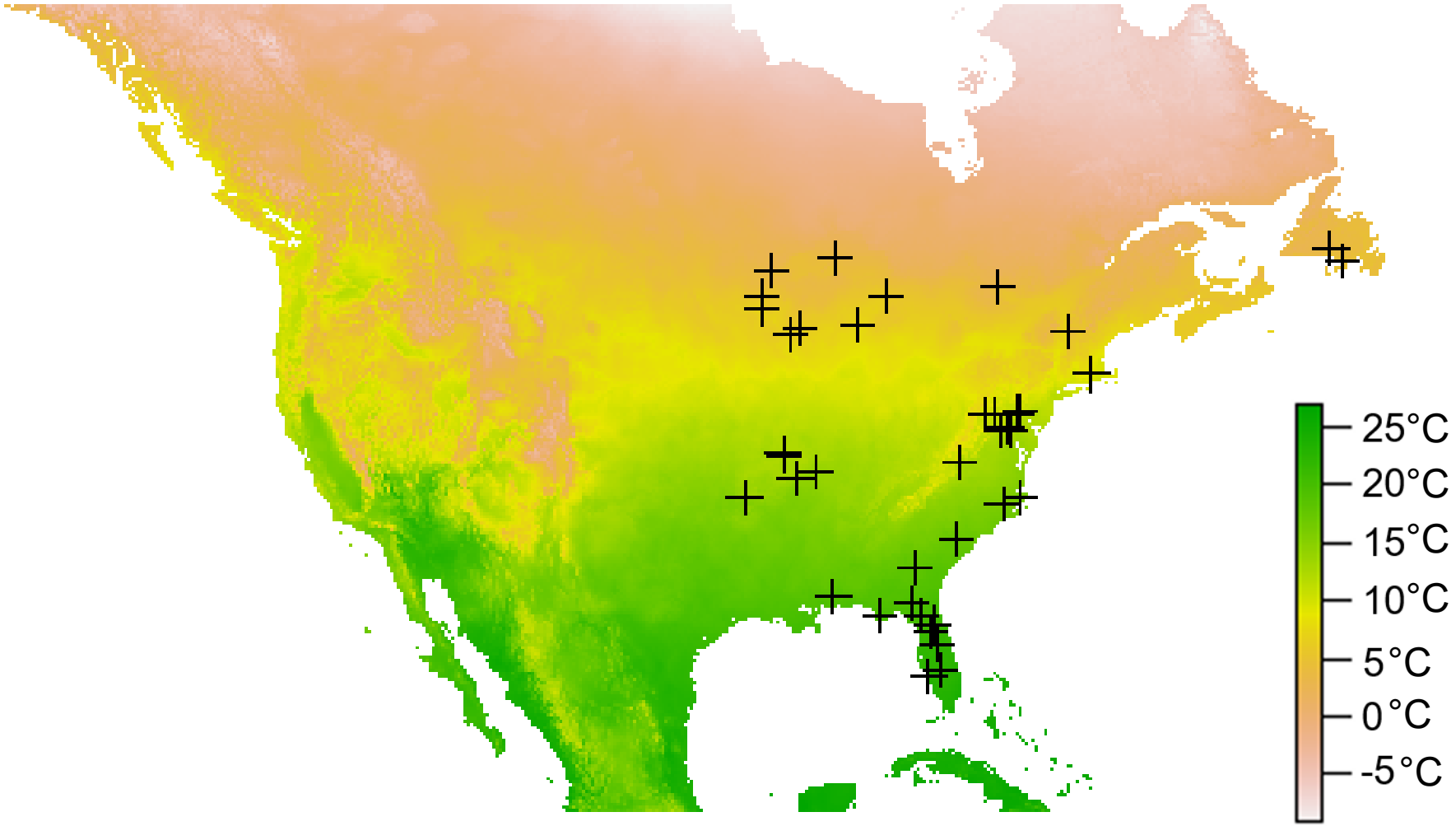
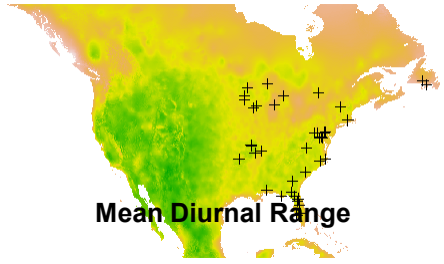
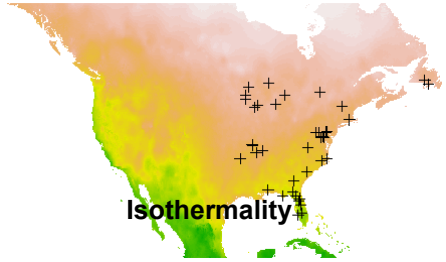
Temperature Gradient Maps

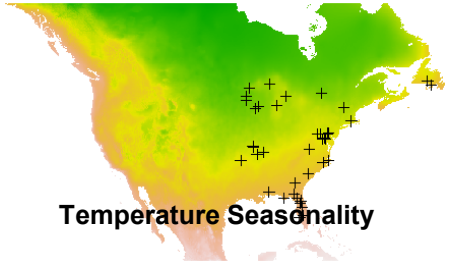


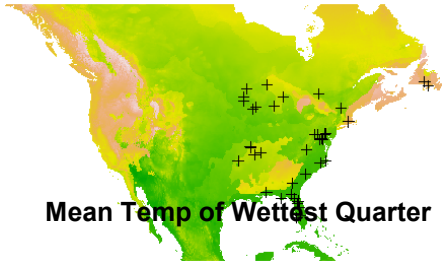
Annual Mean Temperature

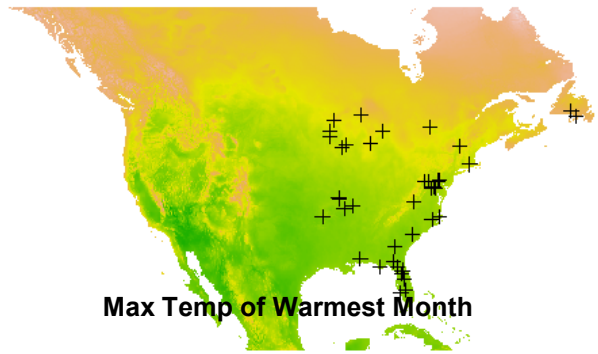
Temperature Gradient Maps

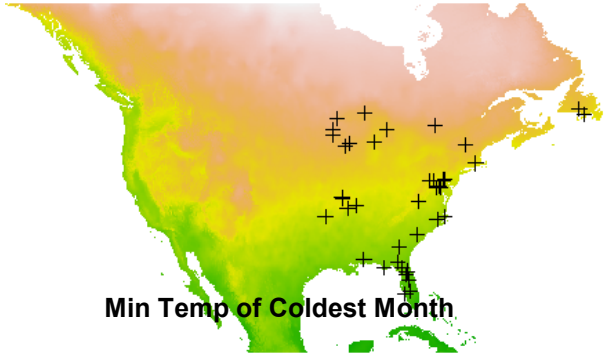

Mean Diurnal Range

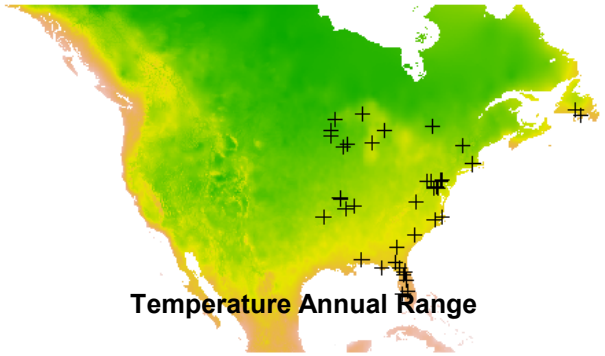

Isothermality

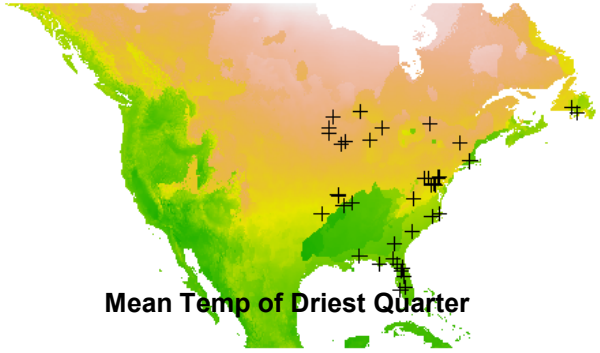

Temperature Seasonality

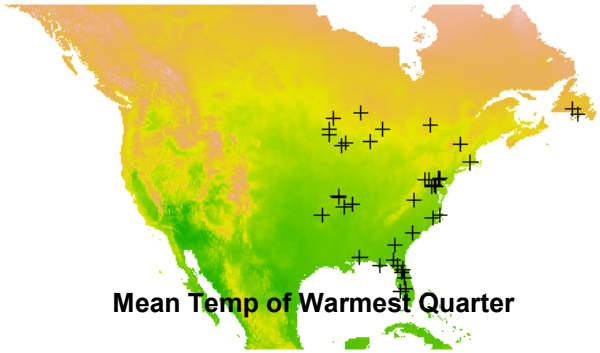

Mean Temp of Wettest Quarter

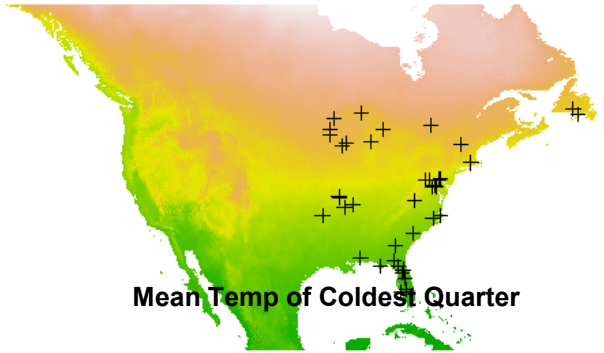

Max Temp of Warmest Month


Min Temp of Coldest Month

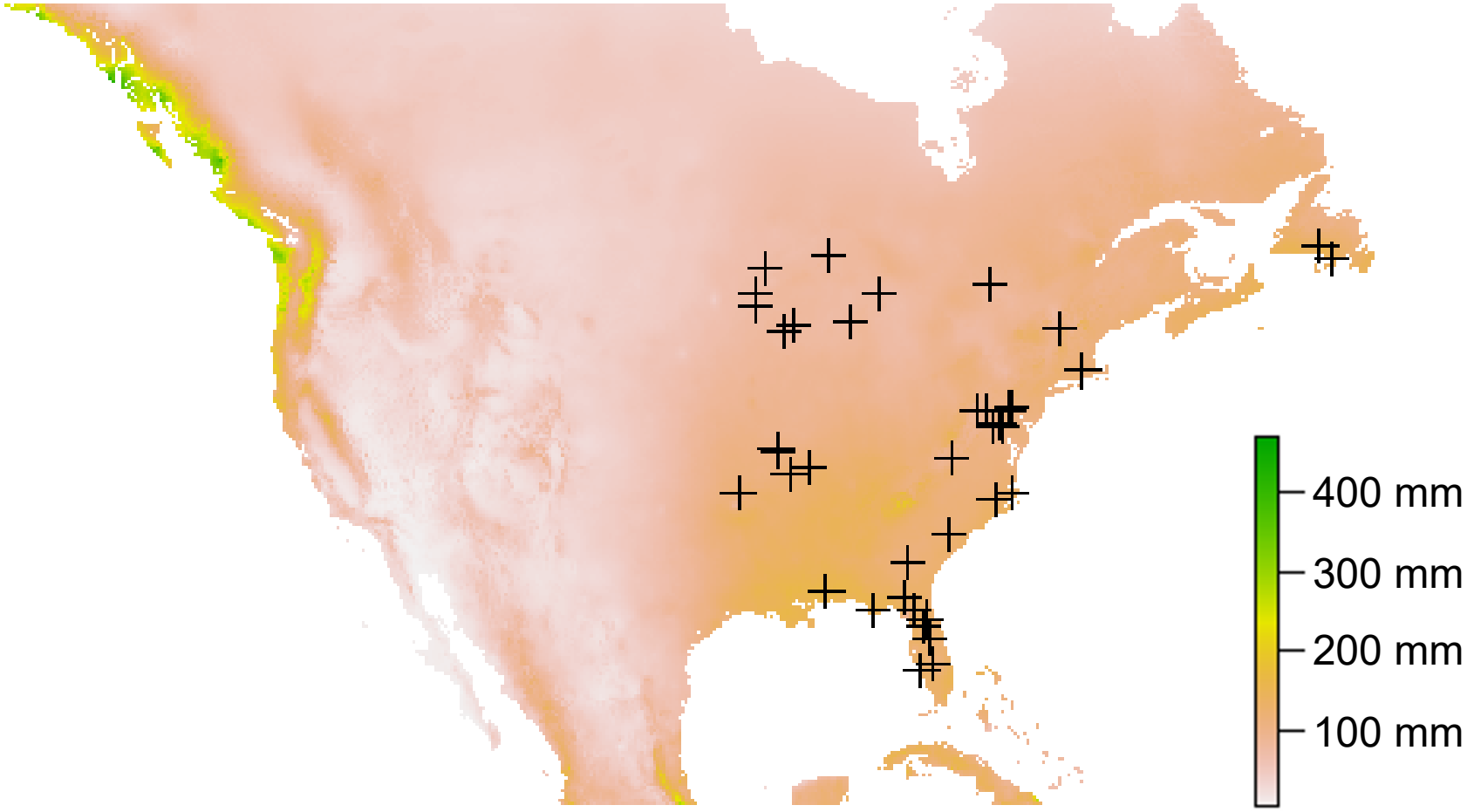

Temperature Annual Range


Mean Temp of Driest Quarter


Mean Temp of Warmest Quarter

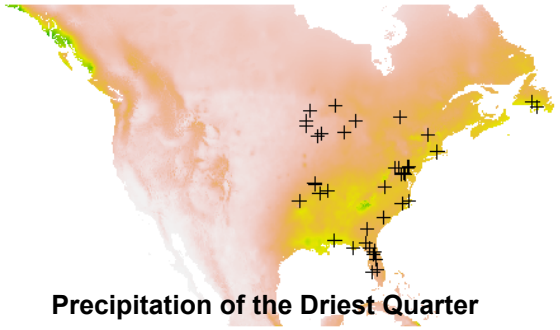

Mean Temp of Coldest Quarter

Precipitation Gradient Maps



Annual Precipitation

Precipitation Gradient Maps



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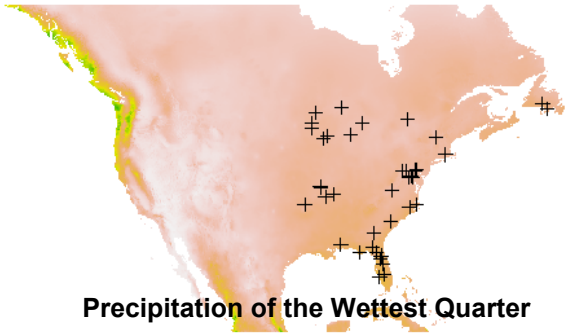
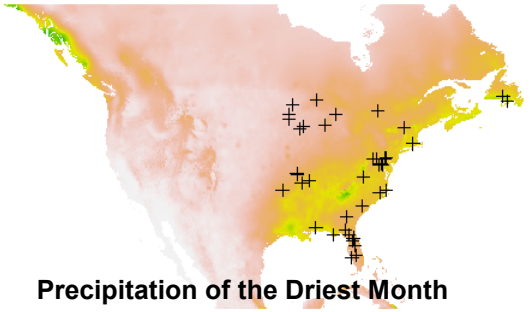
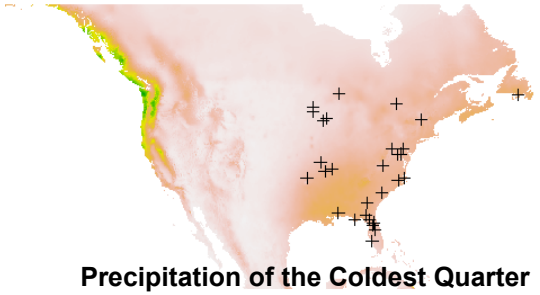


Precipitation of the Wettest Quarter



Precipitation of the Driest Month



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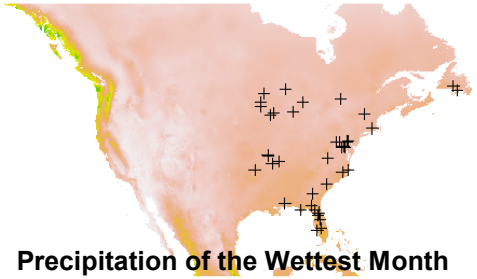

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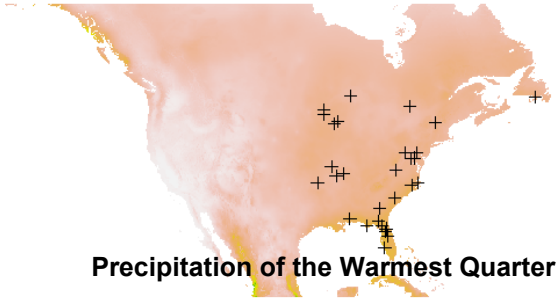

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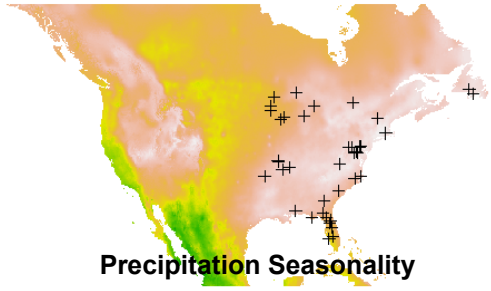

Precipitation Seasonality

Slide Preparation and Imaging Methods

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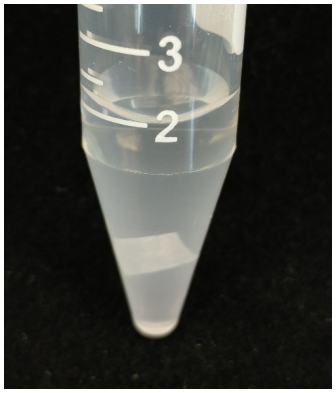

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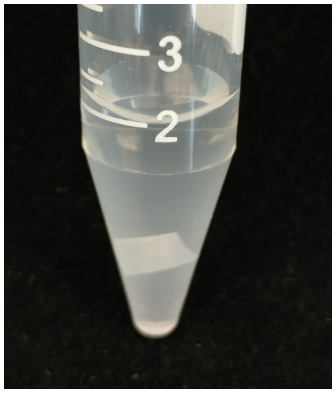

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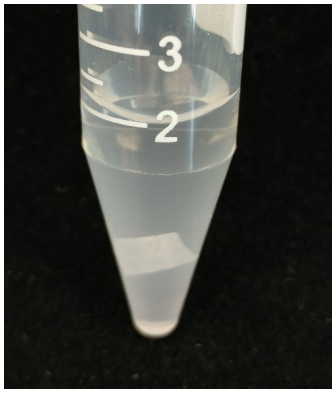

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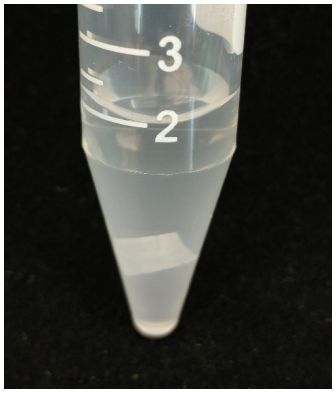
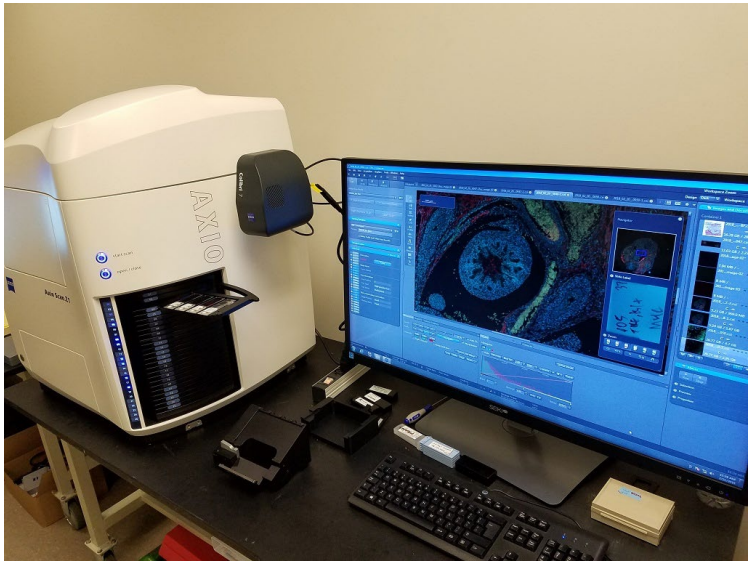
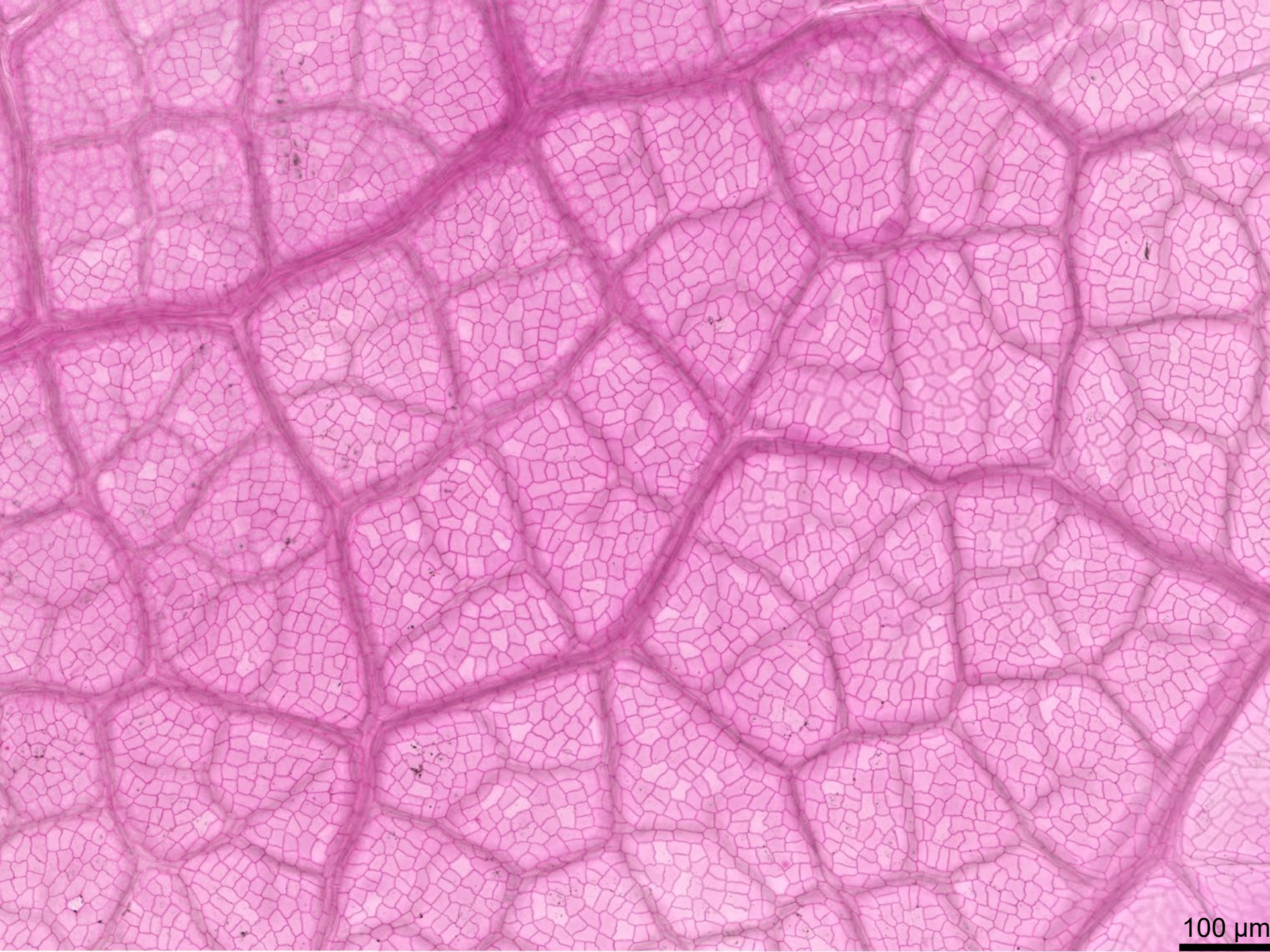
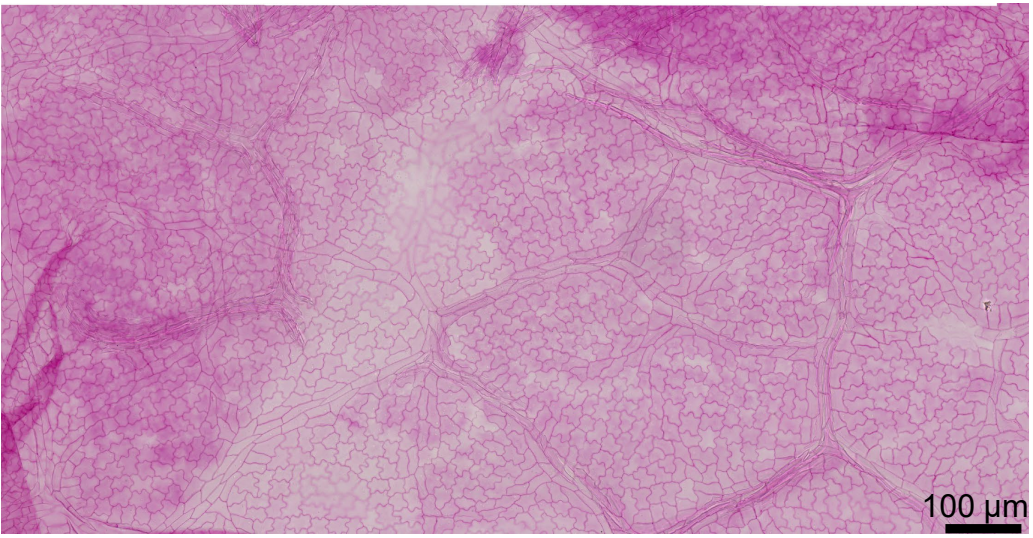


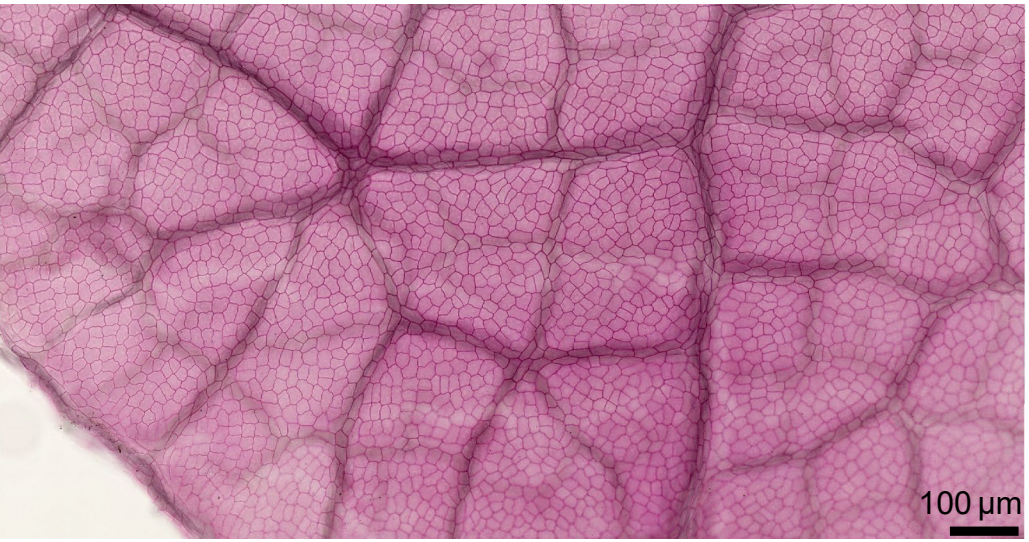


IGB


100 µm

Data Analysis





- Previous cuticle research analyzed by hand

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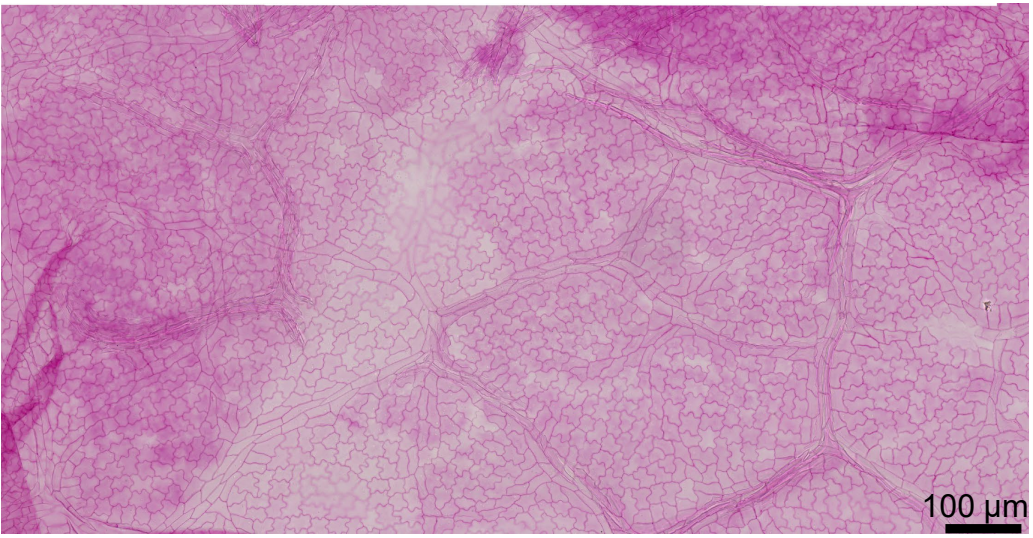

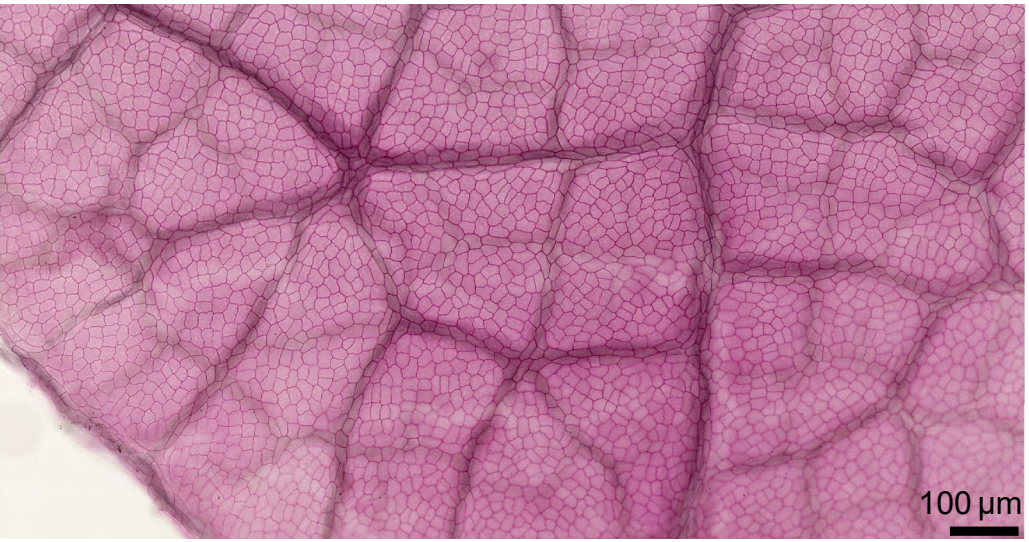

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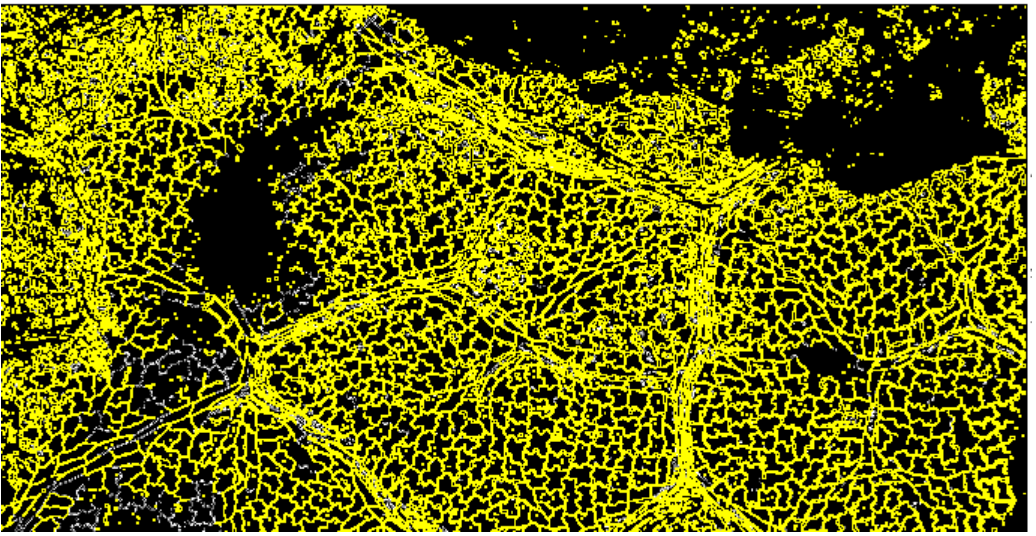

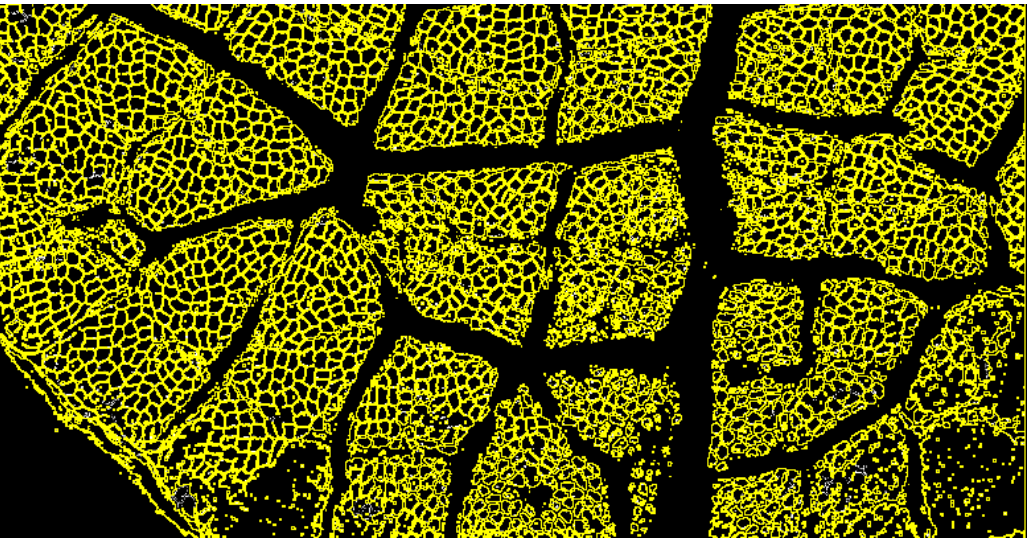

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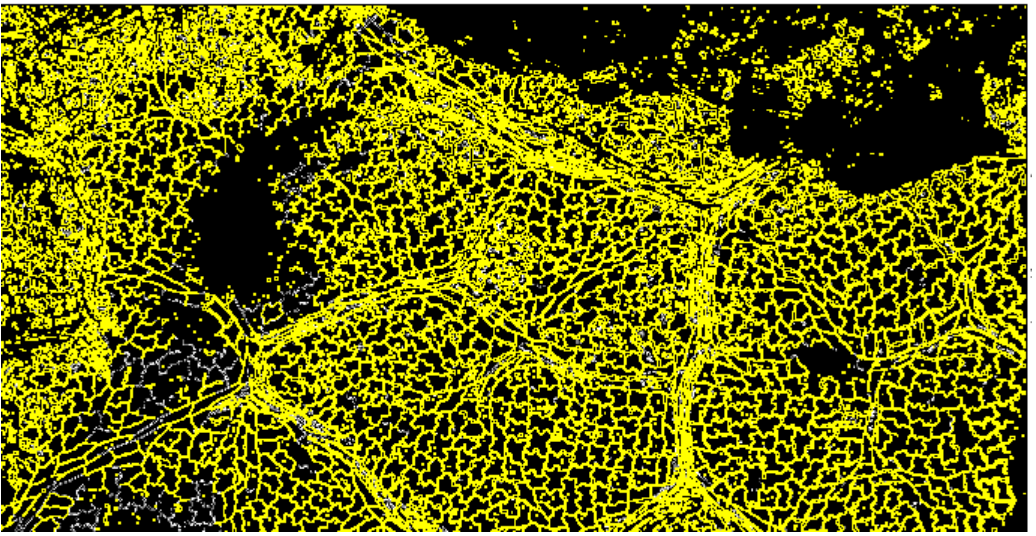
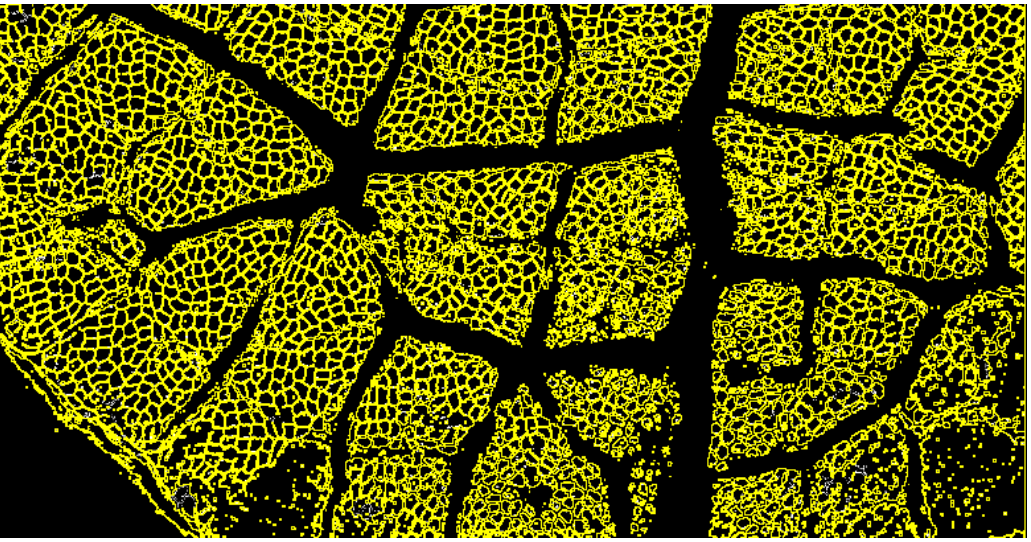
- Used Fiji, distributed by ImageJ

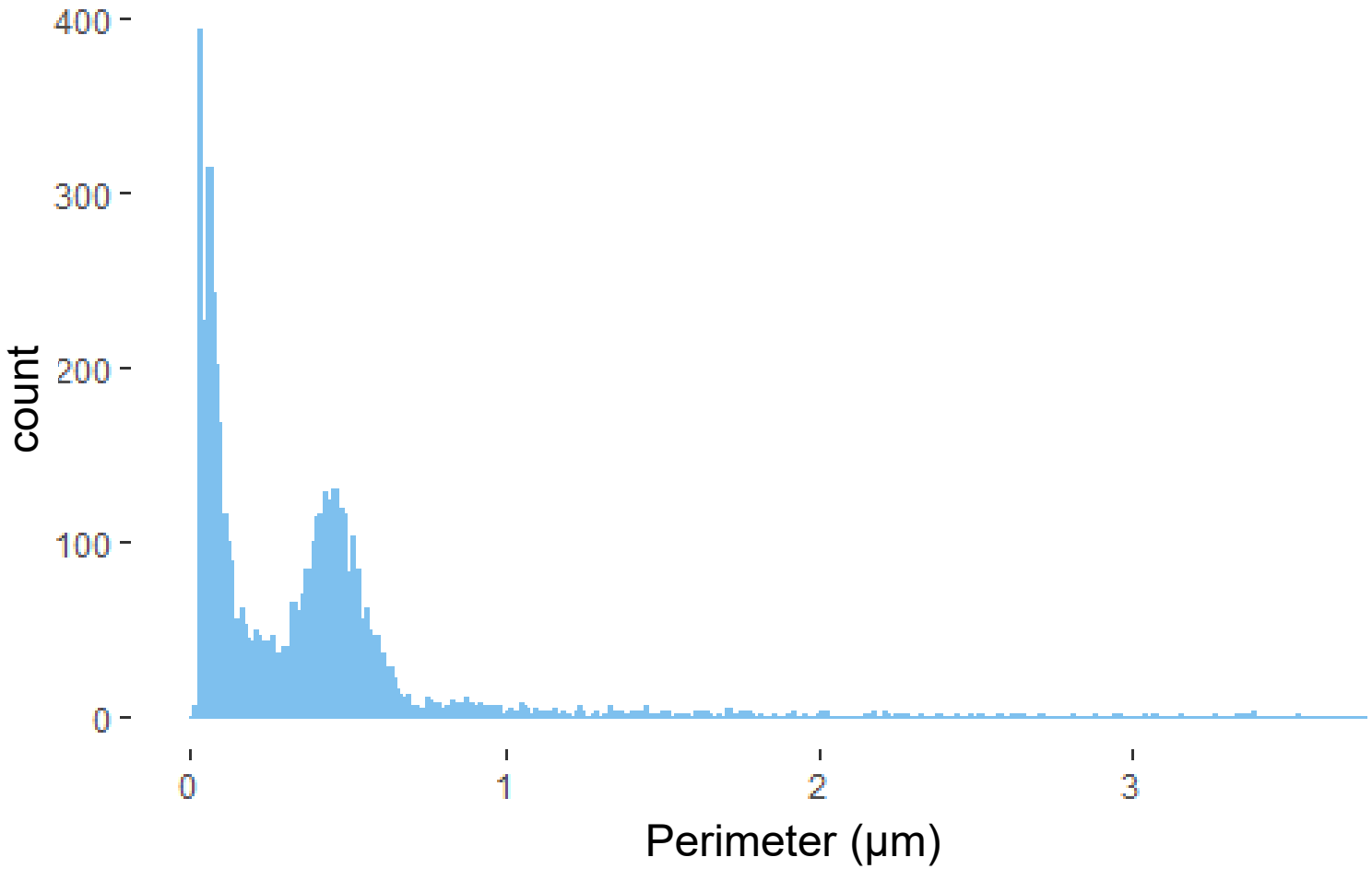
Data Analysis





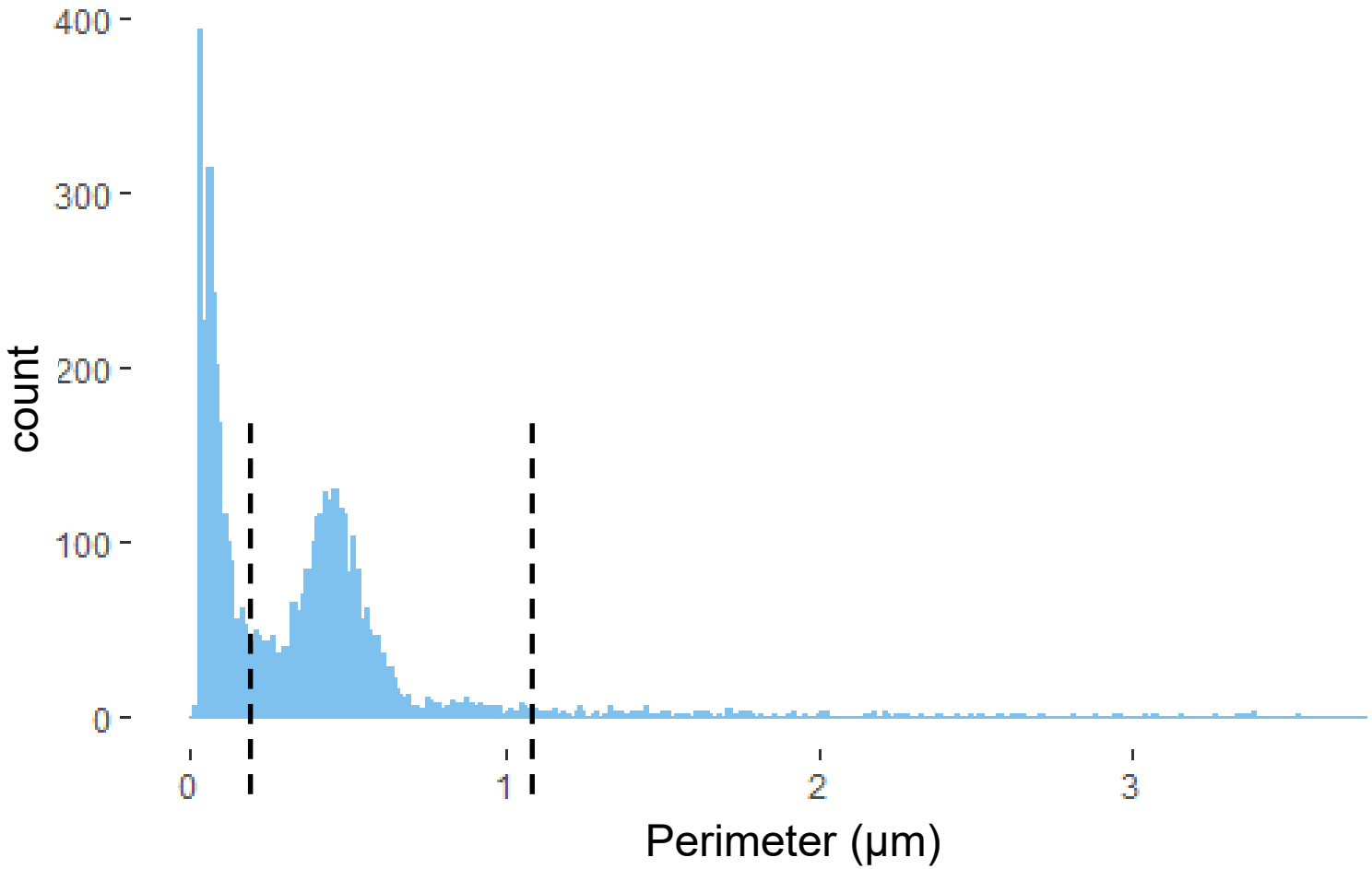
- Previous cuticle research analyzed by hand
- This method and analysis are automated
- Used Fiji, distributed by ImageJ
- Selected areas not epidermal cells

Data Filtering



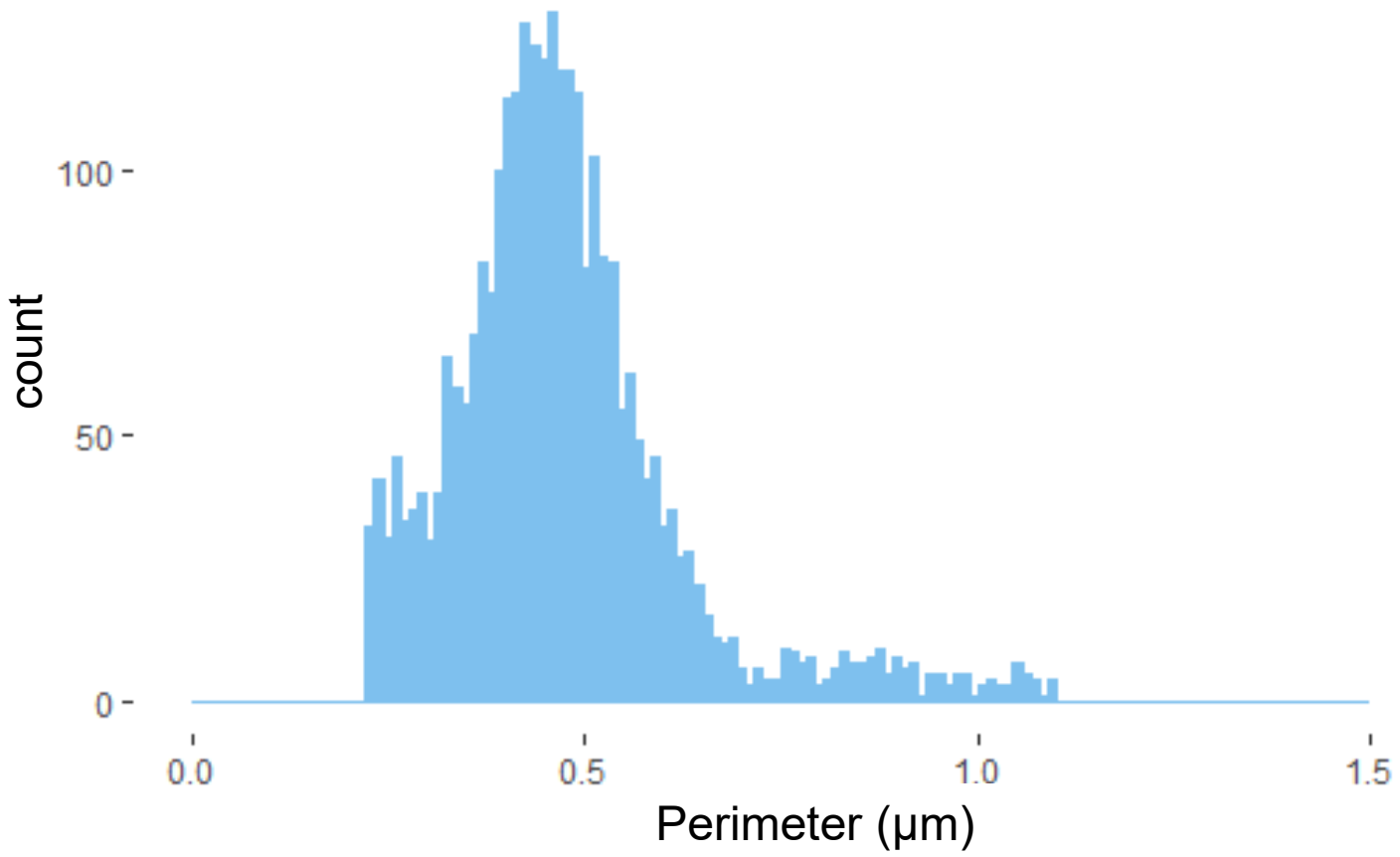
- Used perimeter to filter the data

Data Filtering



- Used perimeter to filter the data
- Set initial boundary of 0.22 microns and 1.10 microns

Data Filtering



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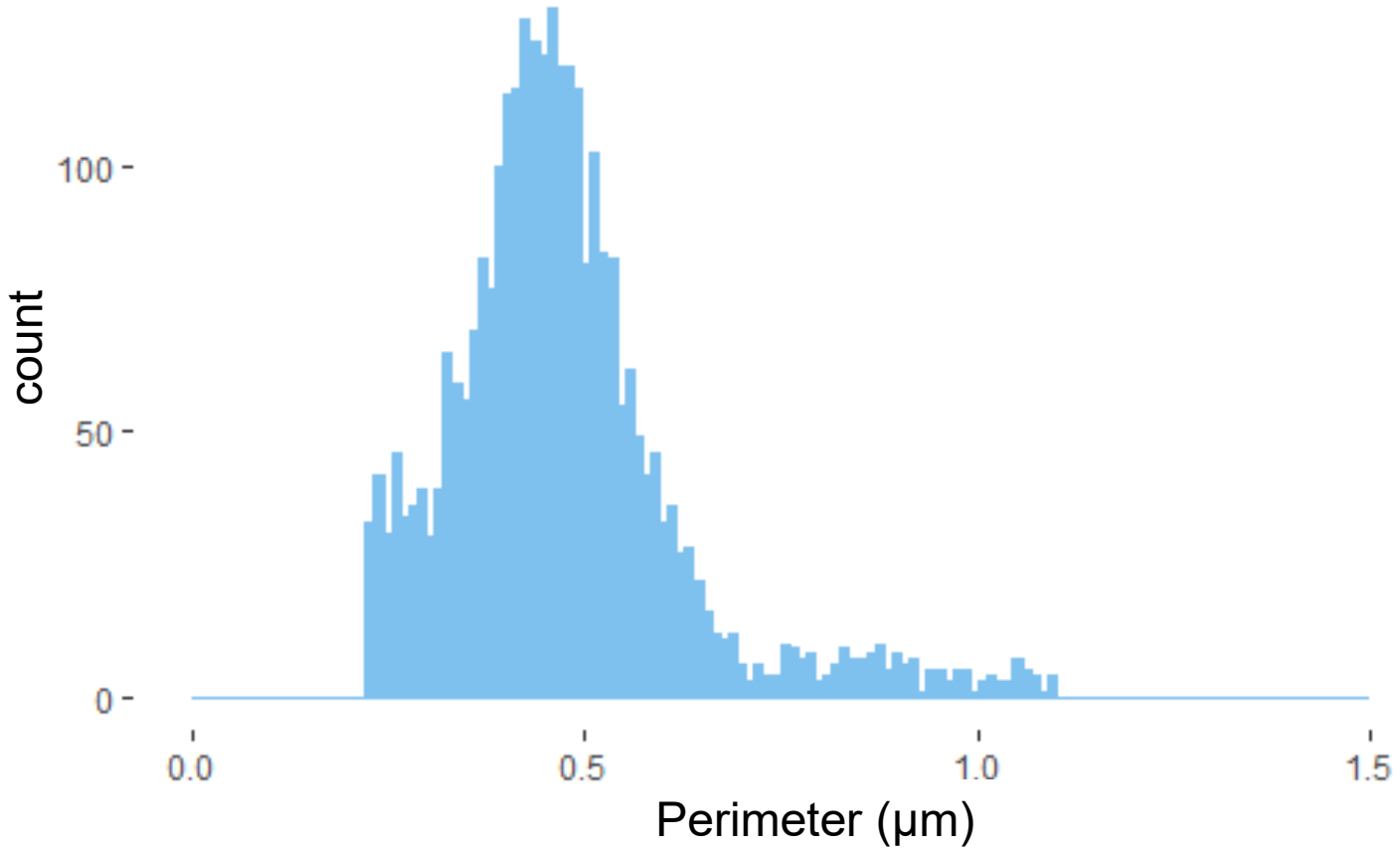
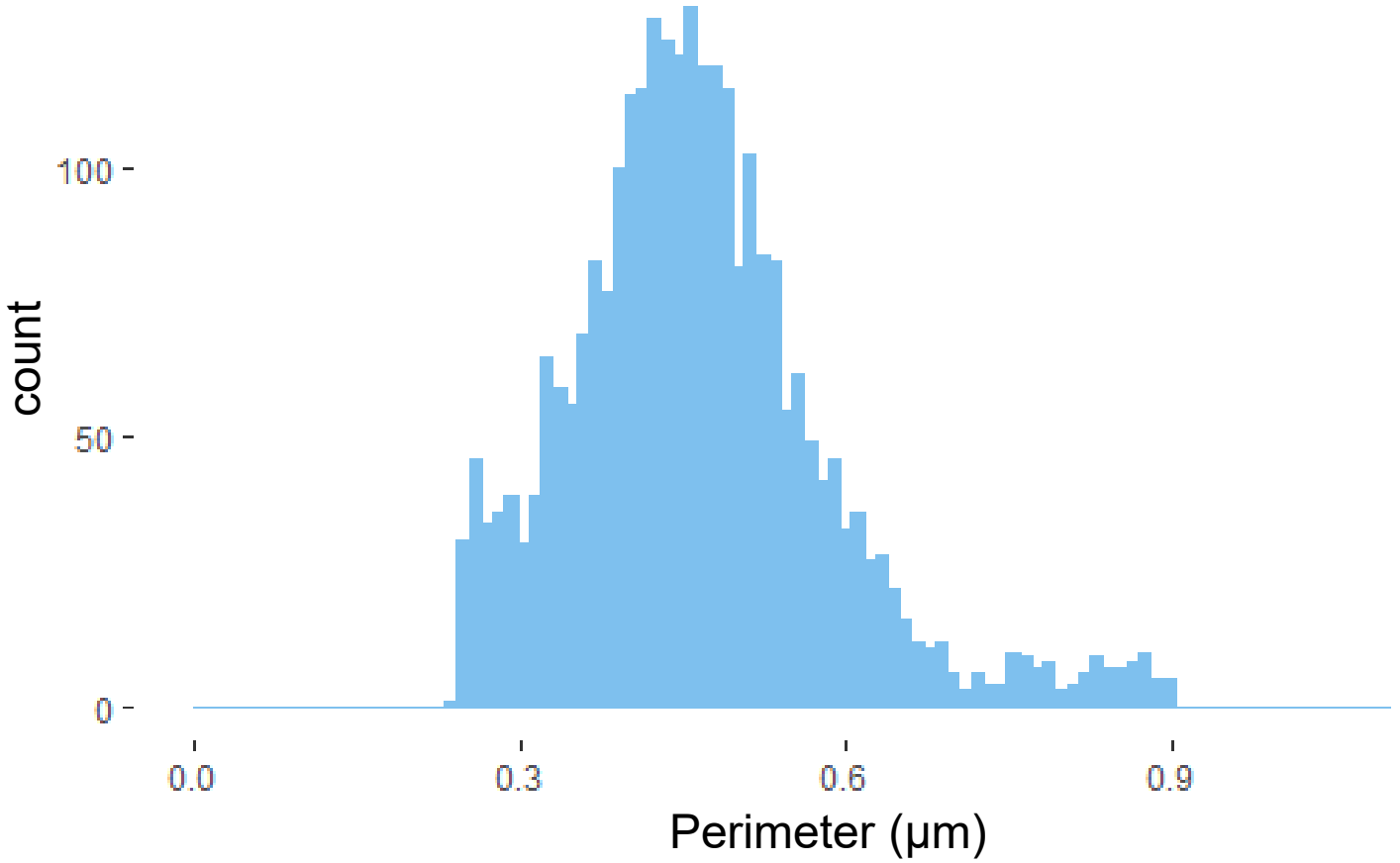


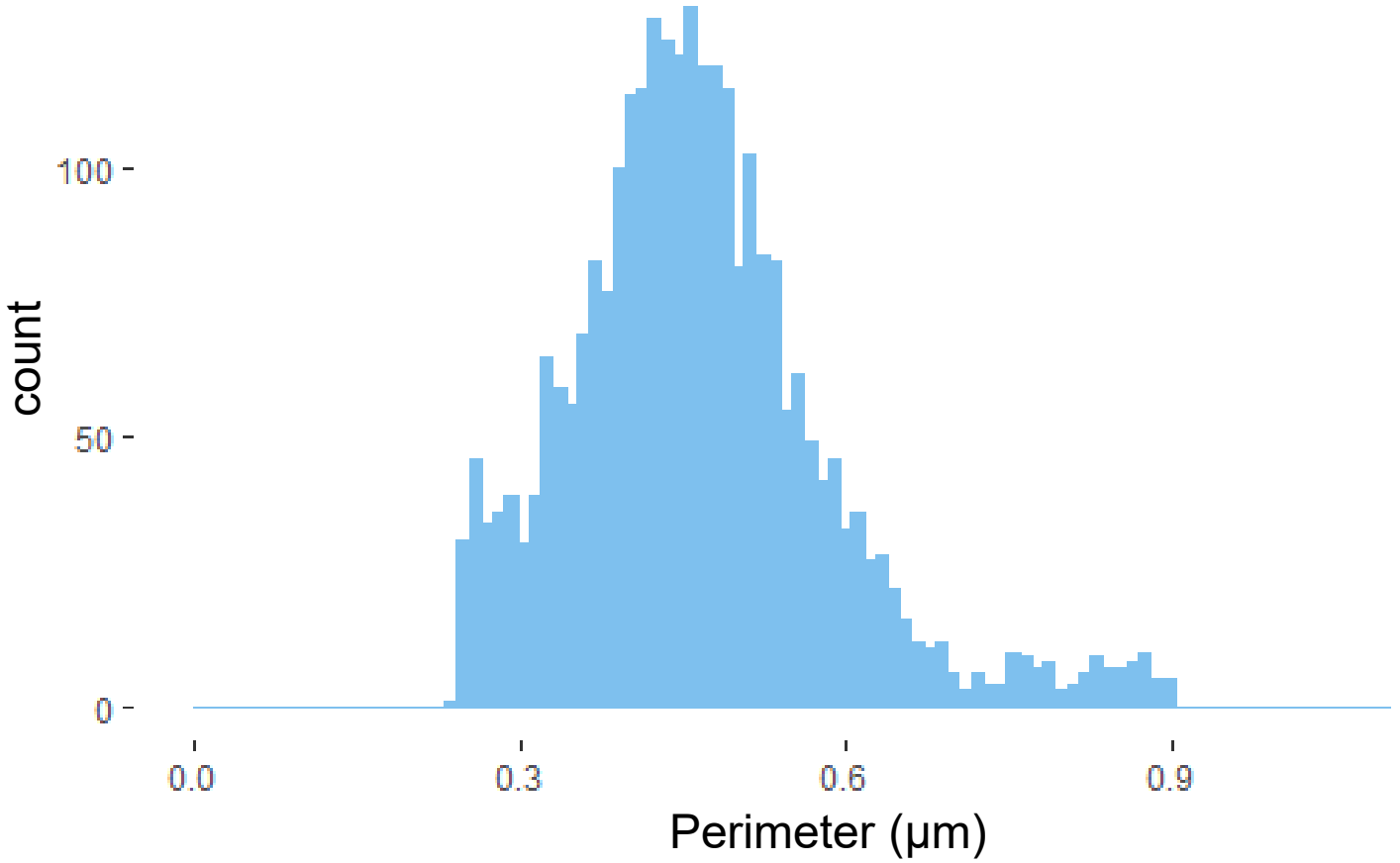
- Applied 97.5% confidence interval

Data Filtering



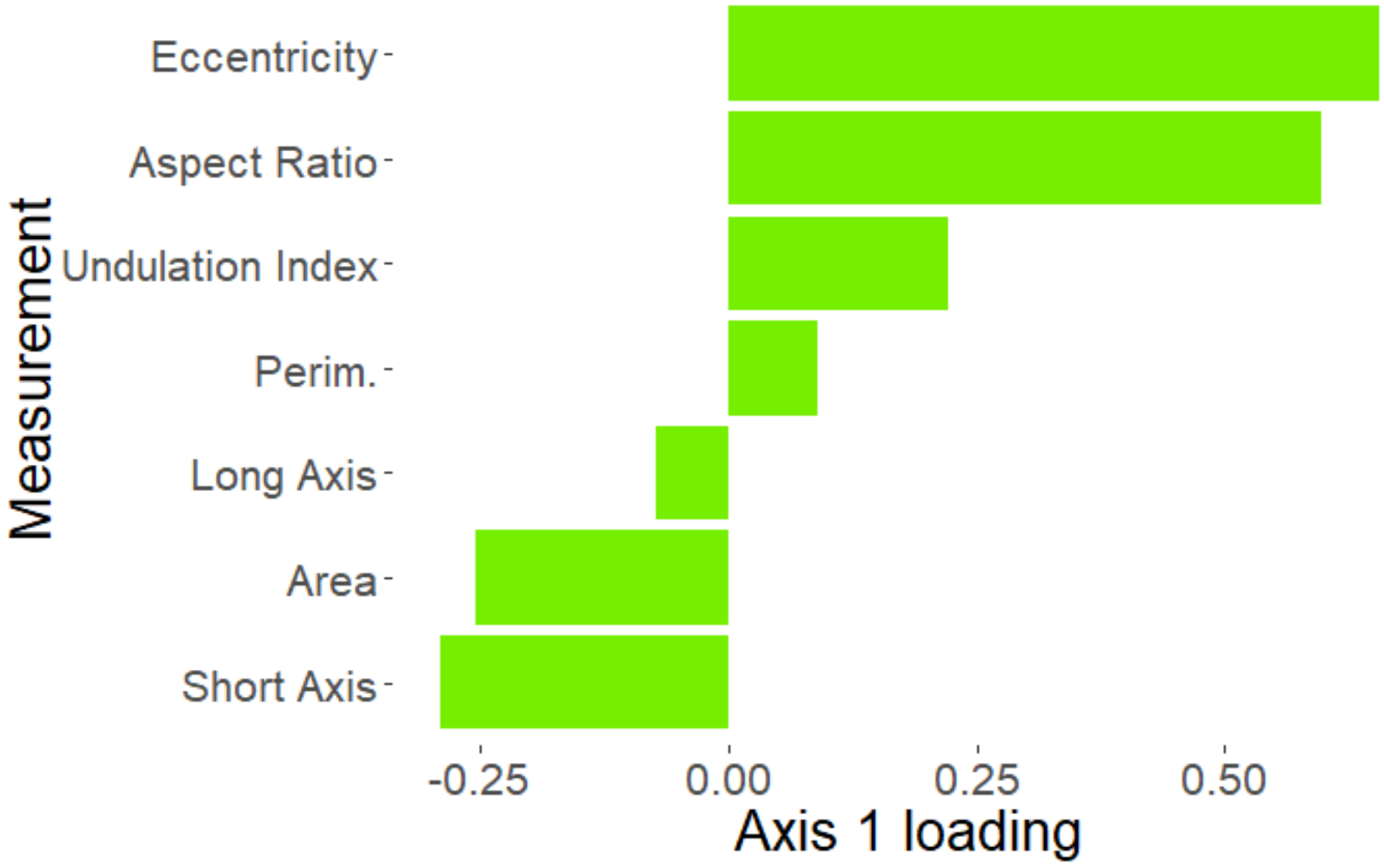
- Final data has relatively normal distribution

Data Filtering

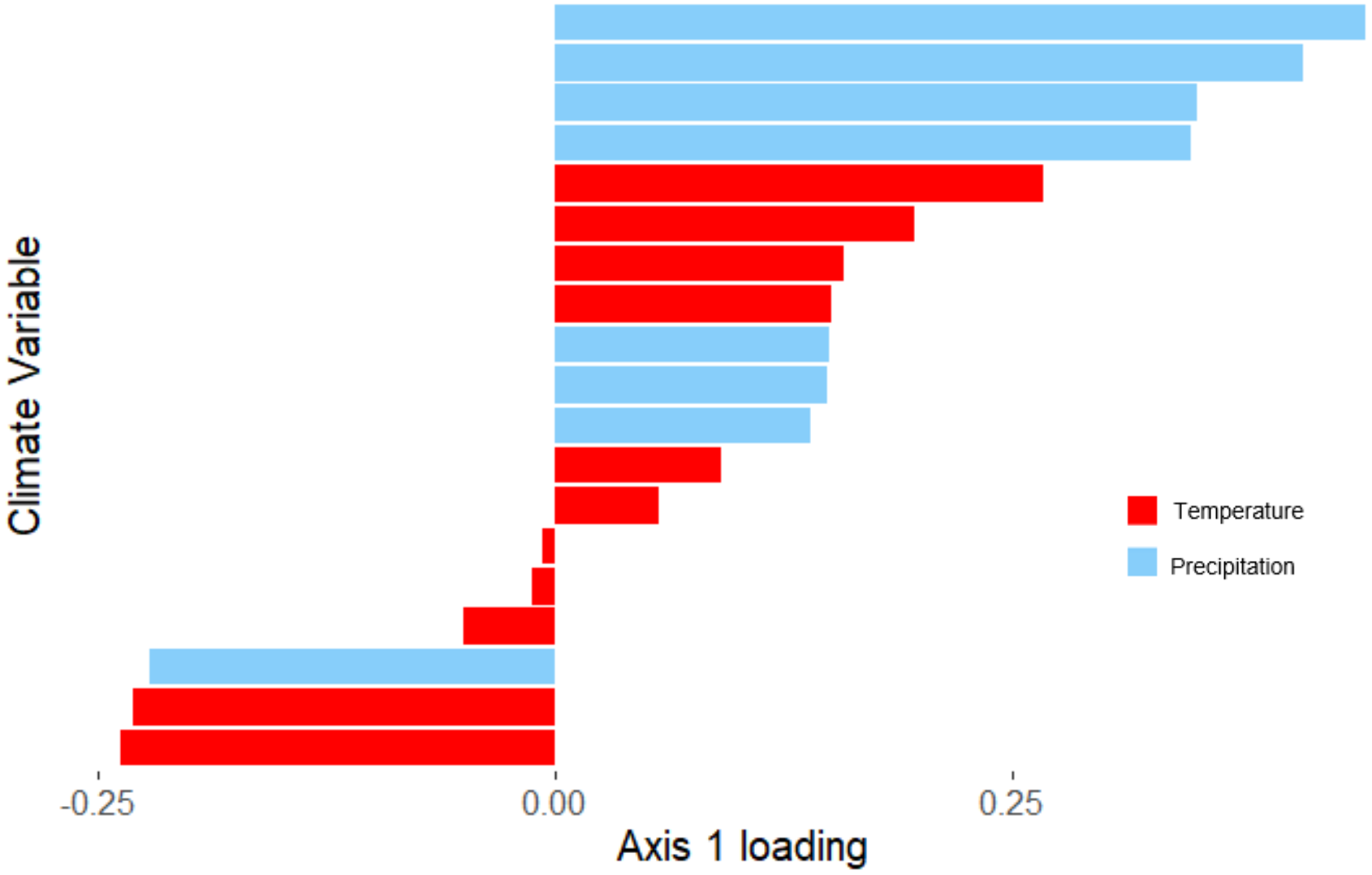


- Final data has relatively normal distribution
- Final measurements went into SVD analysis

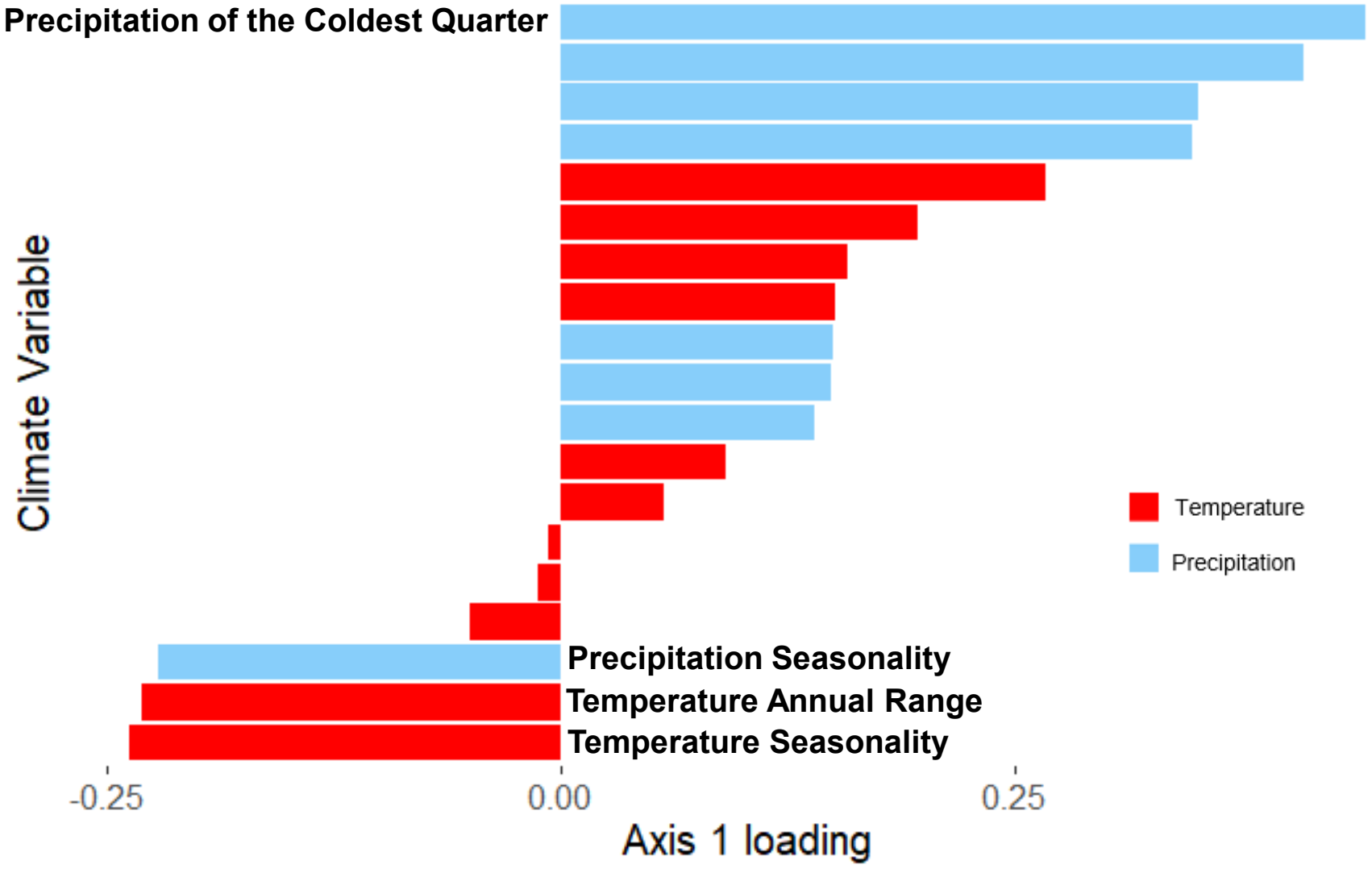
Results


Eccentricity
Aspect Ratio
Undulation Index
Perim.
Long Axis
Area
Short Axis
Measurement
-0.25
0.00
0.25
0.50
Axis 1 loading

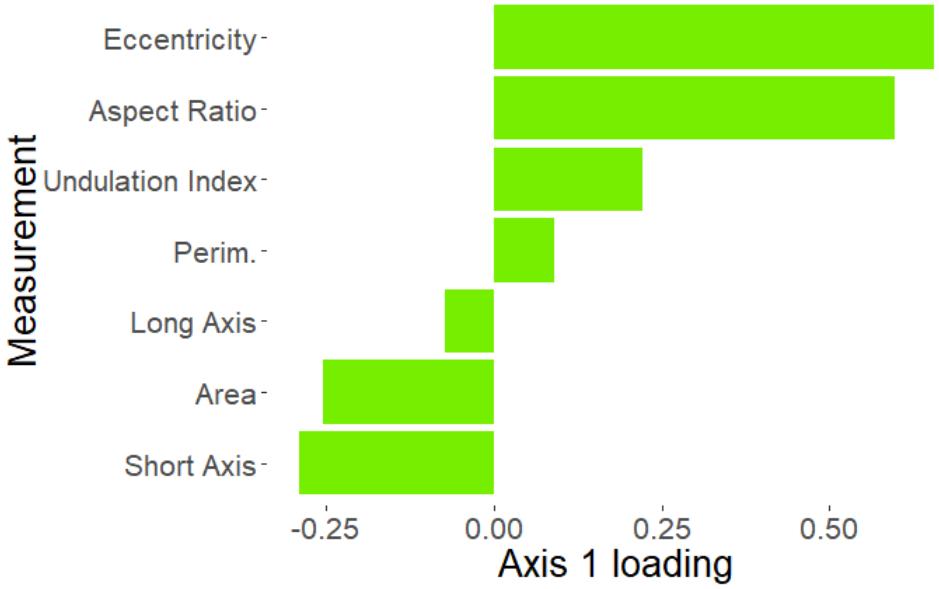
Results

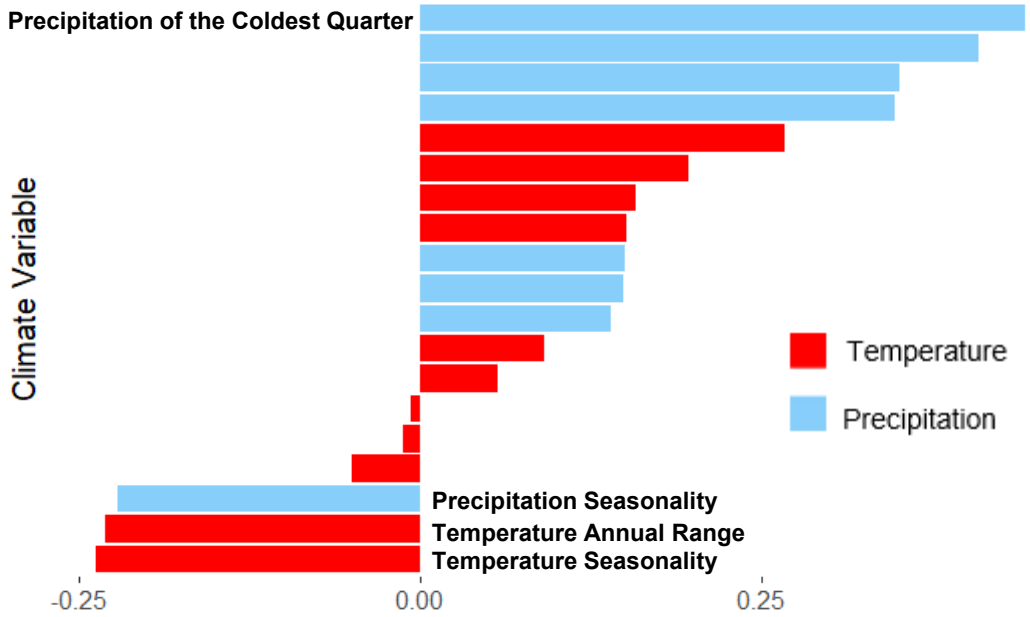

Climate Variable
Temperature
Precipitation
-0.25
0.00
0.25
Axis 1 loading

Results

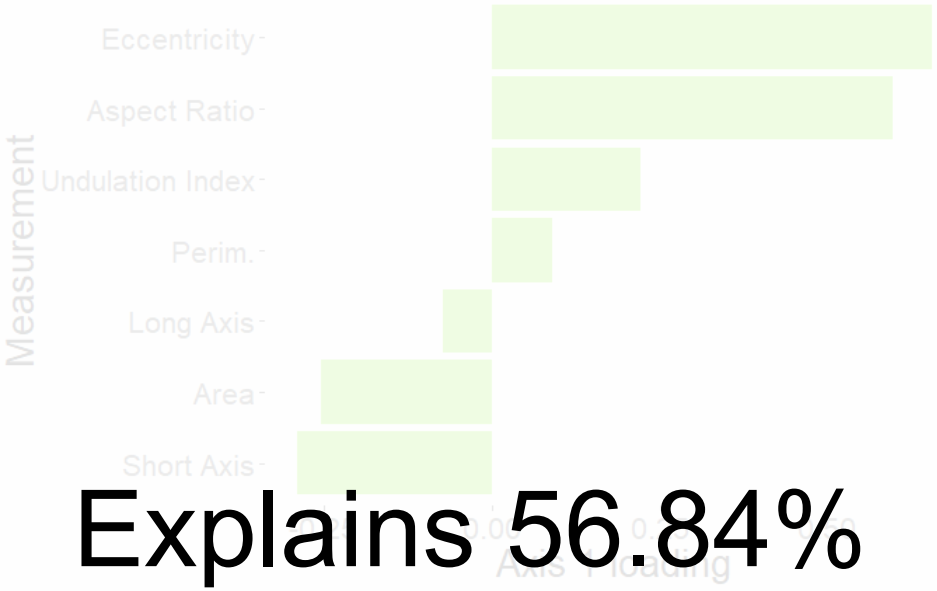

Precipitation of the Coldest Quarter
Climate Variable
Temperature
Precipitation
Precipitation Seasonality
Temperature Annual Range
Temperature Seasonality
-0.25
0.00
0.25
Axis 1 loading

Results


Eccentricity
Aspect Ratio
Undulation Index
Perim.
Long Axis
Area
Short Axis
Measurement
-0.25
0.00
0.25
0.50
Axis 1 loading

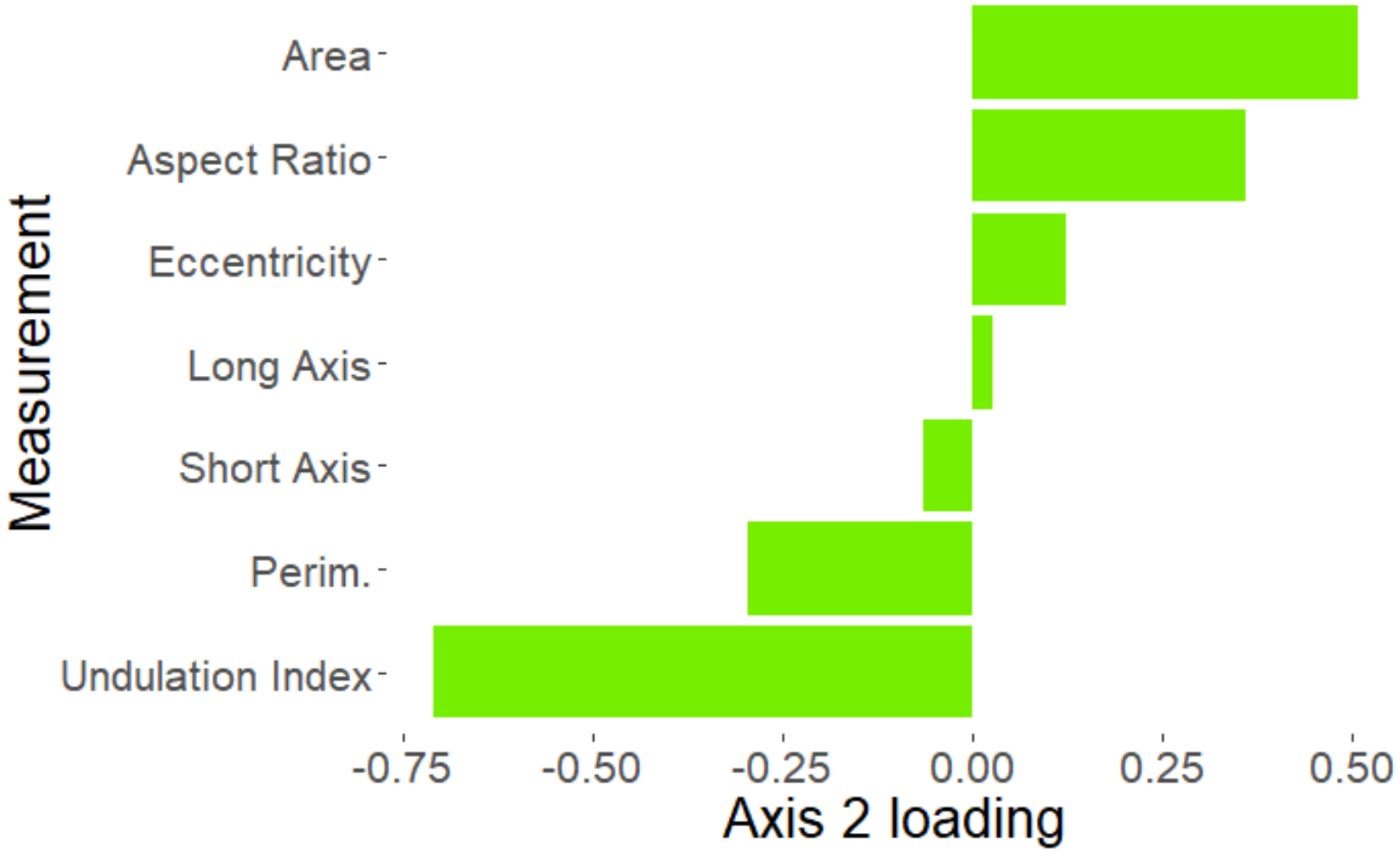

Precipitation of the Coldest Quarter
Climate Variable
Temperature
Precipitation
Precipitation Seasonality
Temperature Annual Range
Temperature Seasonality
-0.25
0.00
0.25

Results

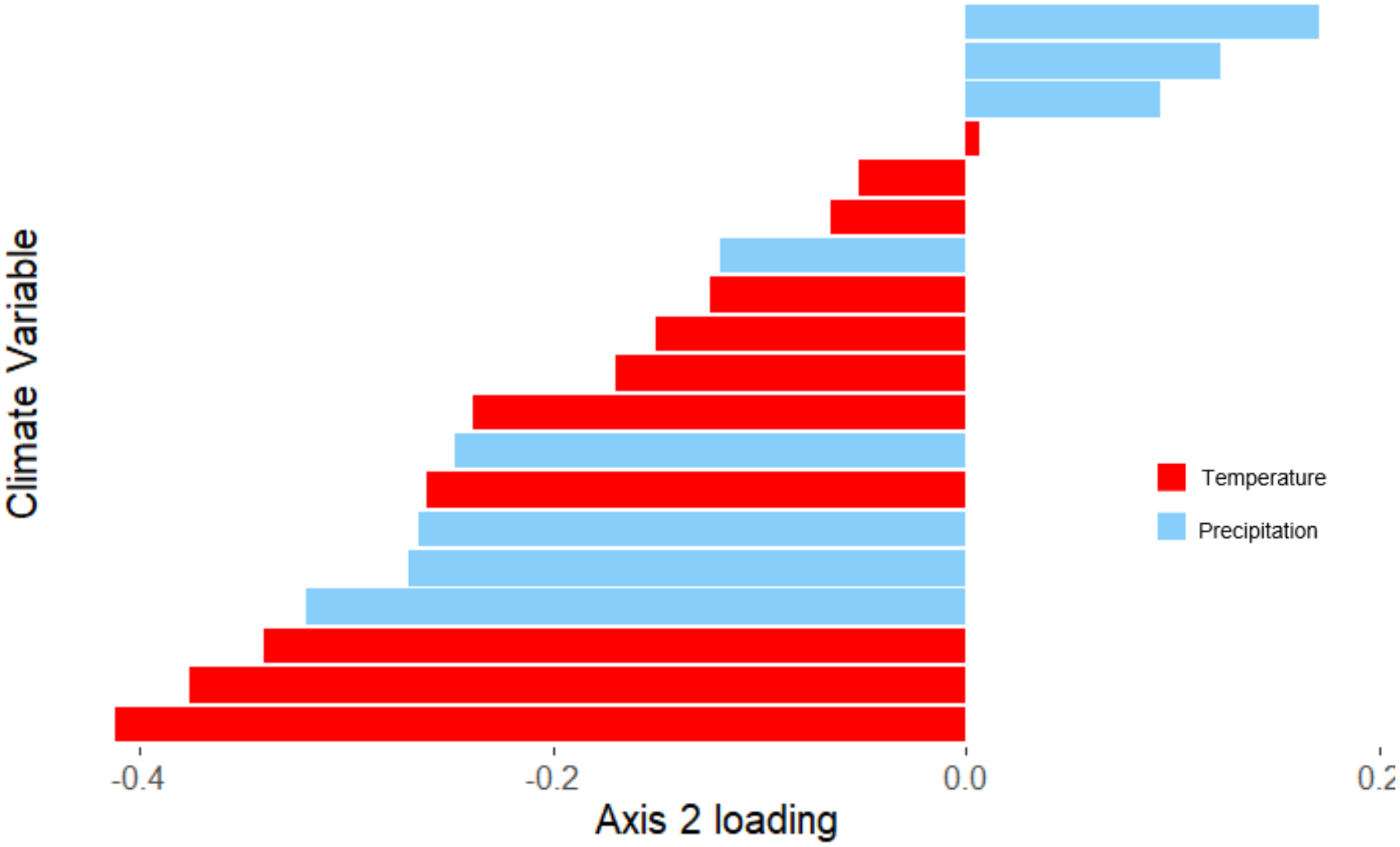


Explains 56.84%

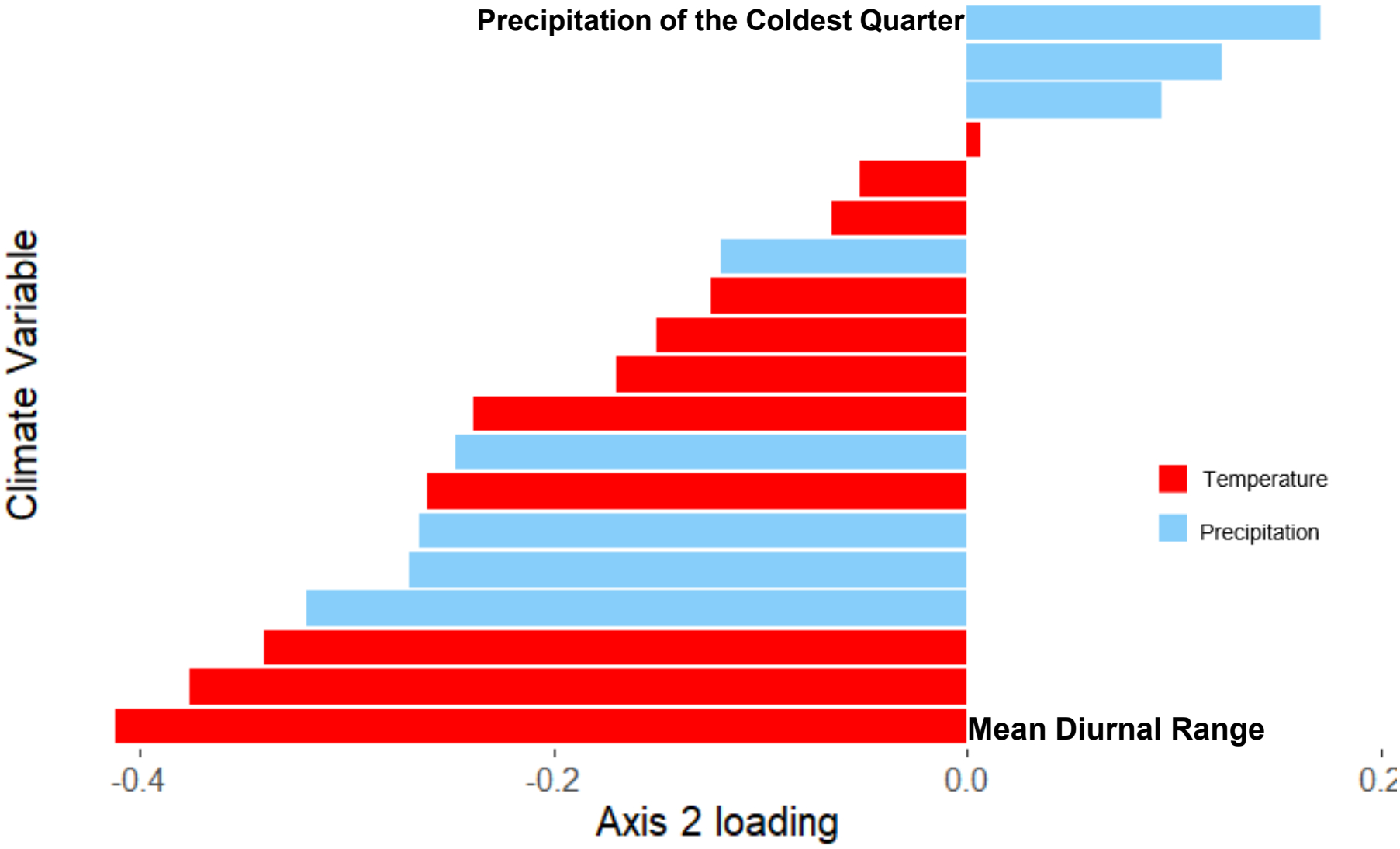
Results


Measurement
Area
Aspect Ratio
Eccentricity
Long Axis
Short Axis
Perim.
Undulation Index
-0.75
-0.50
-0.25
0.00
0.25
0.50
Axis 2 loading

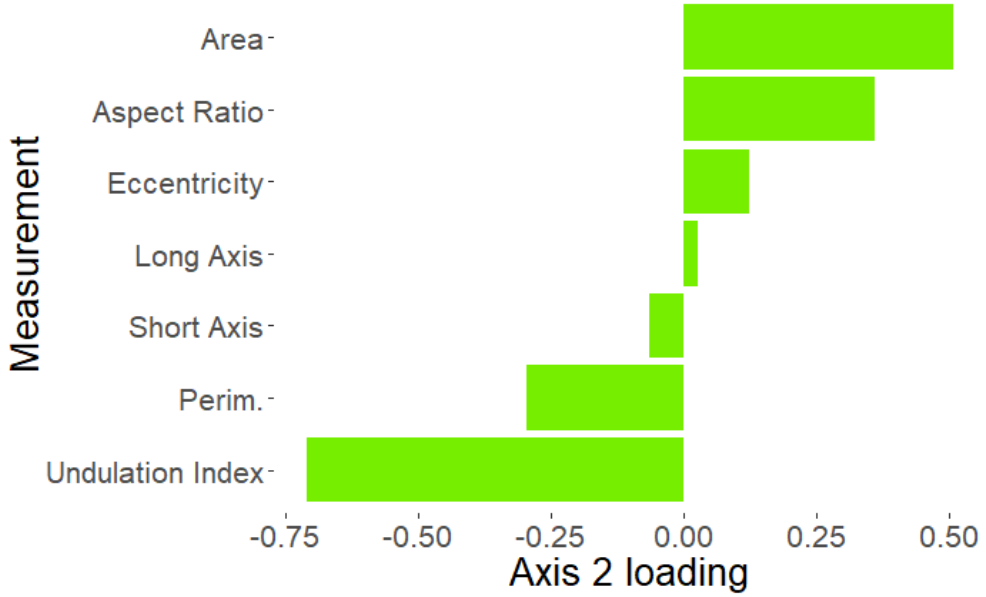
Results

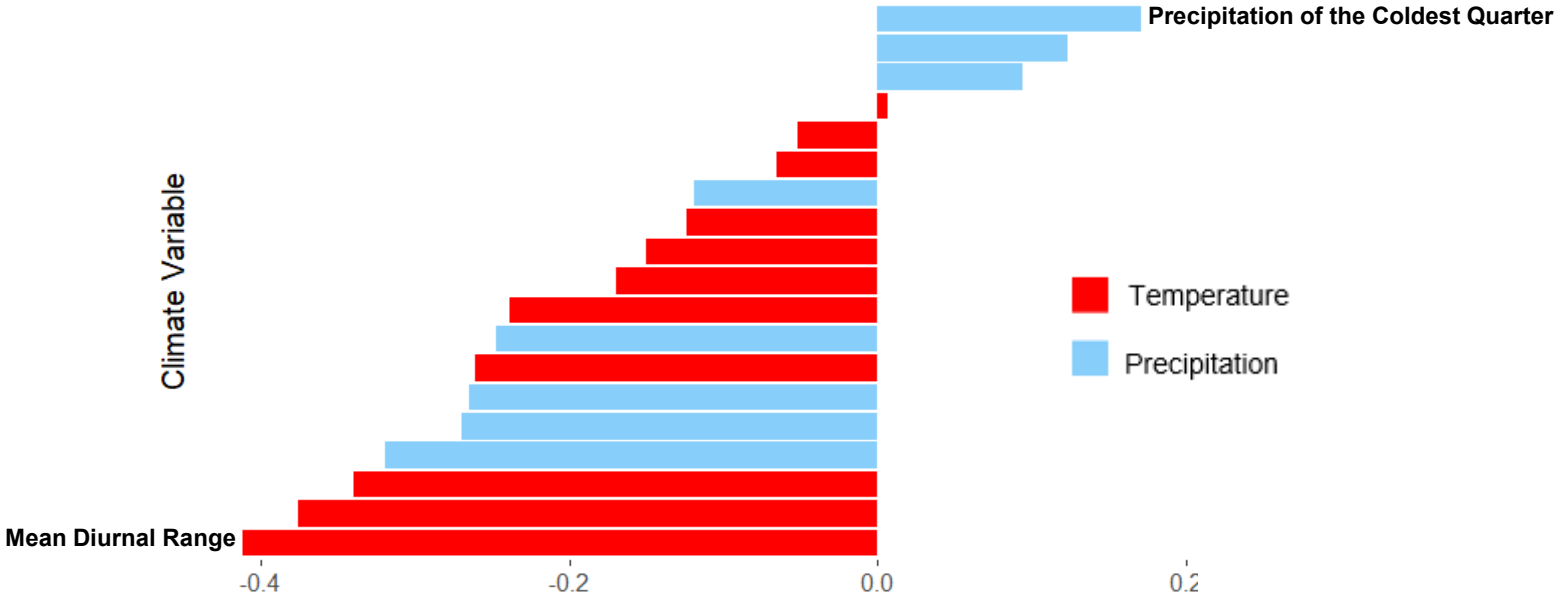

Climate Variable
Temperature
Precipitation
-0.4
-0.2
0.0
0.2
Axis 2 loading

Results

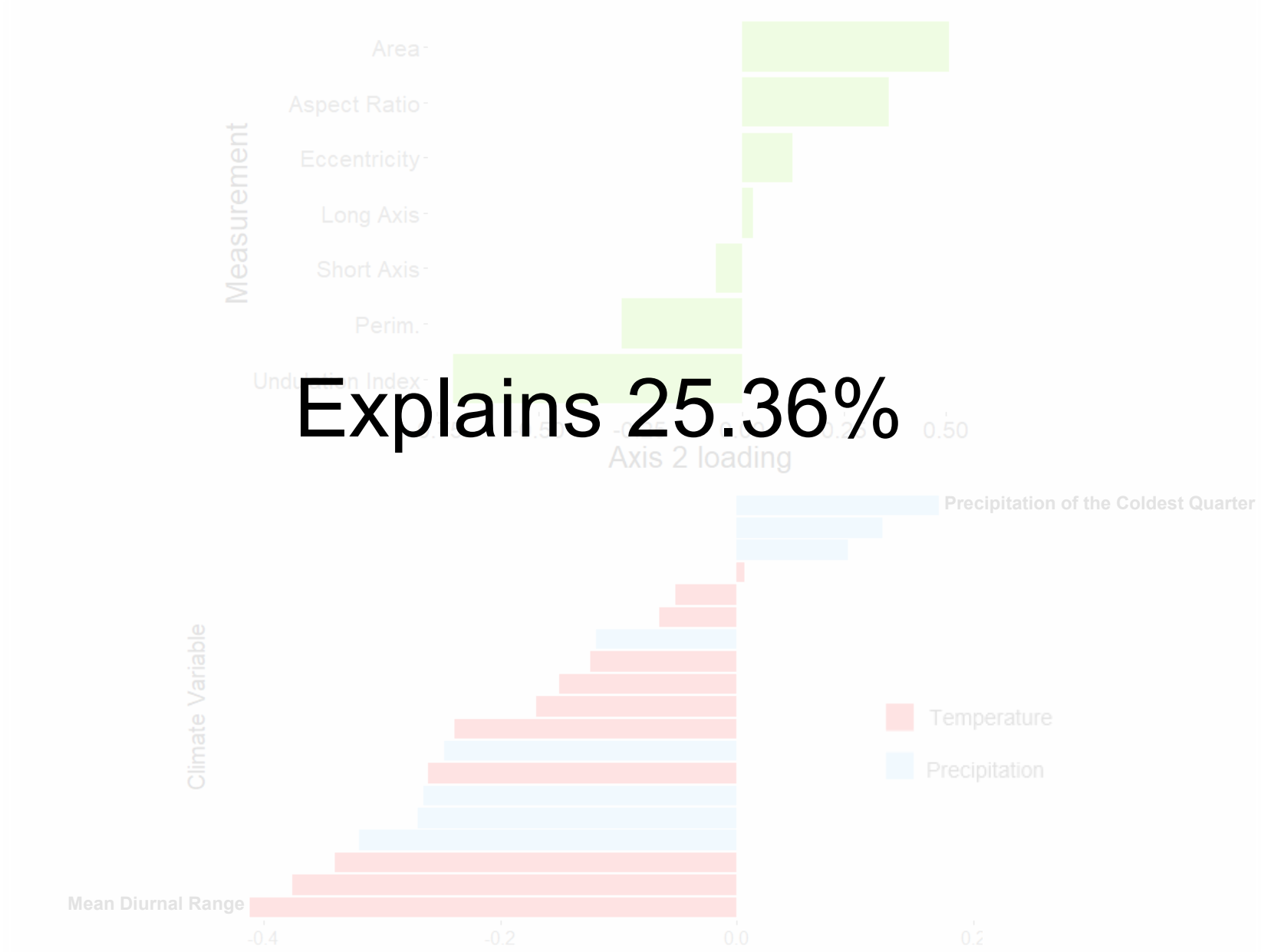

Precipitation of the Coldest Quarter
Mean Diurnal Range
Temperature
Precipitation
Climate Variable
-0.4
-0.2
0.0
0.2
Axis 2 loading

Results


Area
Aspect Ratio
Eccentricity
Long Axis
Short Axis
Perim.
Undulation Index
Measurement
-0.75
-0.50
-0.25
0.00
0.25
0.50
Axis 2 loading


Precipitation of the Coldest Quarter
Mean Diurnal Range
Climate Variable
Temperature
Precipitation
-0.4
-0.2
0.0
0.2

Results



Explains 25.36%

Conclusions

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- This is also important because replication for this study is made easier by automation
- Knowledge of paleoclimate is the only way in which we can begin to understand the climate systems of the future, especially the unknown factors associated with anthropogenic climate change.

Future Directions

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- This method can be applied to taxonomic identification based on cuticle characteristics

Acknowledgements

- Lab mates: Michael Urban, Ingrid Romero, and Derek Haselhorst
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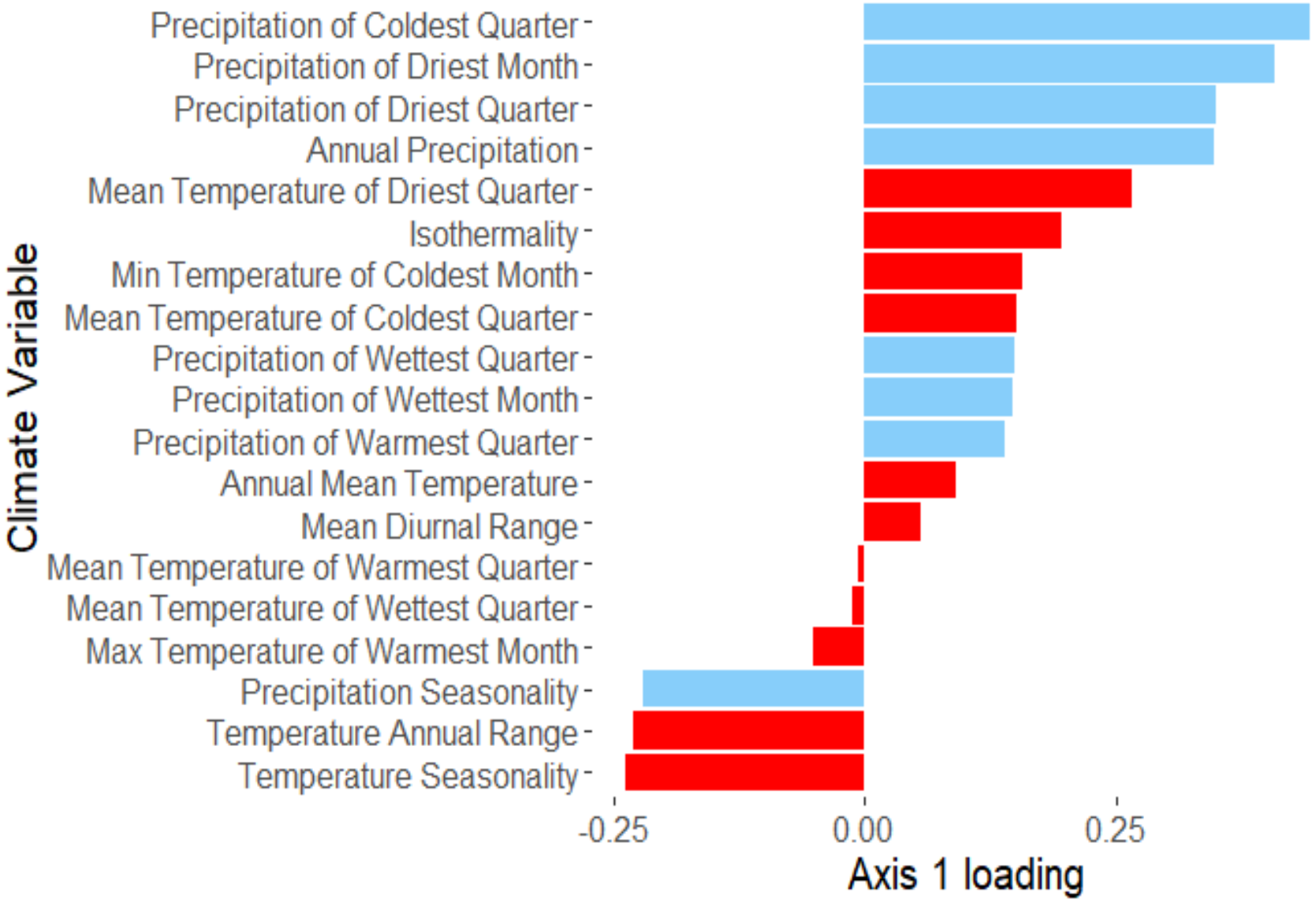
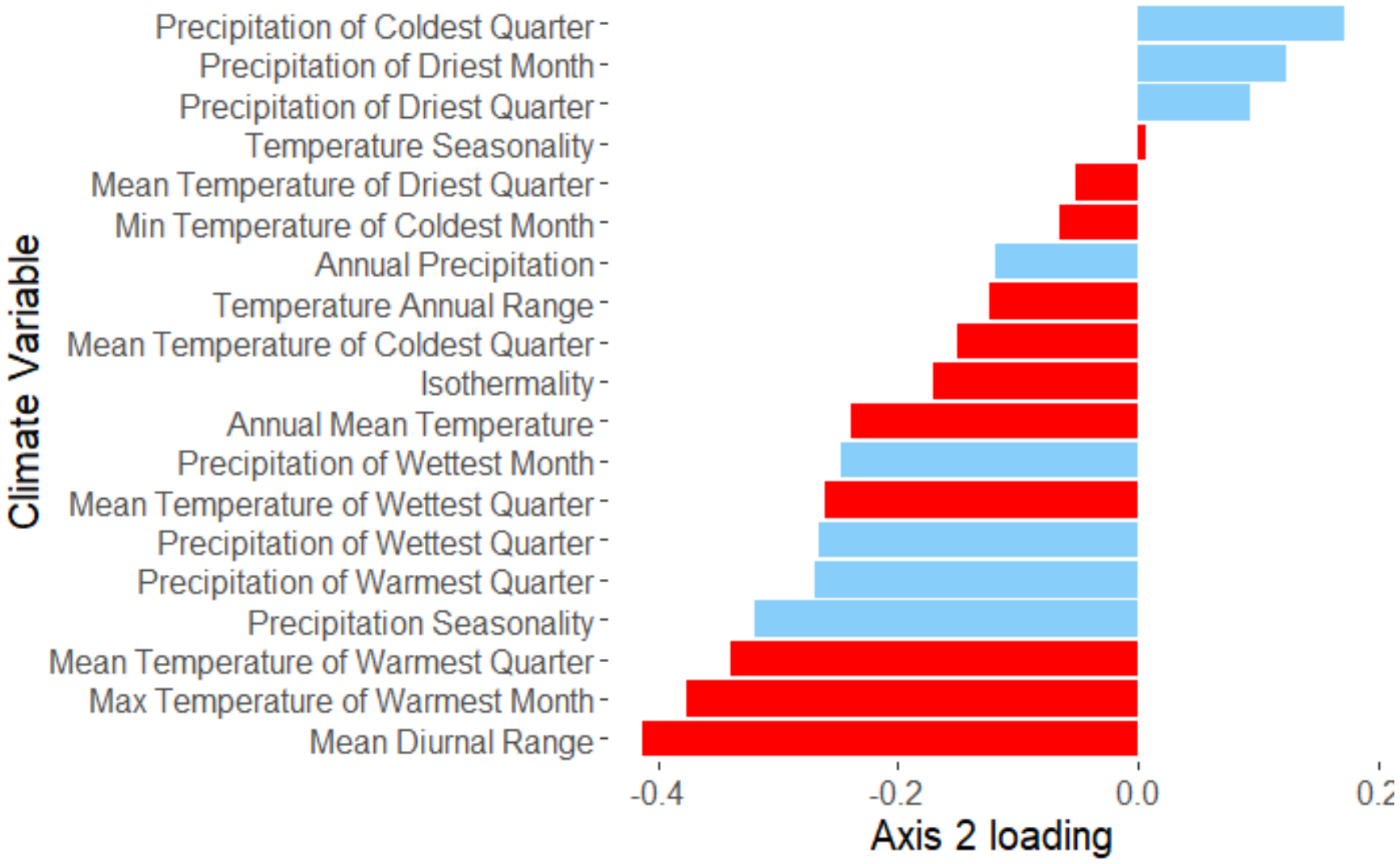
- Microscopy support: Advances in Biological Informatics, DBI-1262561


Climate Variable
Precipitation of Coldest Quarter
Precipitation of Driest Month
Precipitation of Driest Quarter
Annual Precipitation
Mean Temperature of Driest Quarter
Isothermality
Min Temperature of Coldest Month
Mean Temperature of Coldest Quarter
Precipitation of Wettest Quarter
Precipitation of Wettest Month
Precipitation of Warmest Quarter
Annual Mean Temperature
Mean Diurnal Range
Mean Temperature of Warmest Quarter
Mean Temperature of Wettest Quarter
Max Temperature of Warmest Month
Precipitation Seasonality
Temperature Annual Range
Temperature Seasonality
-0.25
0.00
0.25
Axis 1 loading


Climate Variable
Precipitation of Coldest Quarter
Precipitation of Driest Month
Precipitation of Driest Quarter
Temperature Seasonality
Mean Temperature of Driest Quarter
Min Temperature of Coldest Month
Annual Precipitation
Temperature Annual Range
Mean Temperature of Coldest Quarter
Isothermality
Annual Mean Temperature
Precipitation of Wettest Month
Mean Temperature of Wettest Quarter
Precipitation of Wettest Quarter
Precipitation of Warmest Quarter
Precipitation Seasonality
Mean Temperature of Warmest Quarter
Max Temperature of Warmest Month
Mean Diurnal Range
-0.4
-0.2
0.0
0.2
Axis 2 loading