

## **Complexities of modern leaf morphology, climate proxies, and applicability in the fossil record**

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### ***INTRODUCTION***

Predictions for the impacts of modern climate change will be significantly informed via quantitative understanding of historic patterns of the Earth's changing climate and will allow us to better predict modern patterns anticipated over the next century. This research investigates the development of new, continuous leaf margin traits in relation to their environment with the potential to better quantify existing proxy signal strength even within the restrictions employed by the fossil record. Specifically this work will focus on the quantification of environmental relationships of those traits to refine climatic interpretations, provide more detailed understandings of ecological contexts, and insights into the macro evolutionary response of plants to geologic (or anthropogenic) perturbations. In addition, quantitative proxies independent of taxa relationships and not limited by complete leaf margin analysis requirements will provide greater access to a currently limited paleobotanical record.

Since the early 1900's, paleontologists have been using the percentage of toothy leafed species based on categorical values to interpret paleoclimate with some success indicating a strong tie between toothiness and climate (Peppe et al. 2011, Royer et al. 2005, Johnson 2002). Current proxy literature also suggests consistent patterns of roundness, toothiness, and stomatal densities increasing with decreasing temperature and CO<sub>2</sub> concentrations (Peppe et al. 2011, Royer et al. 2005, McElwain and Chaloner 1995, Woodward 1987). However, these interpretations display wide error rates when compared to predictions of broadly accepted

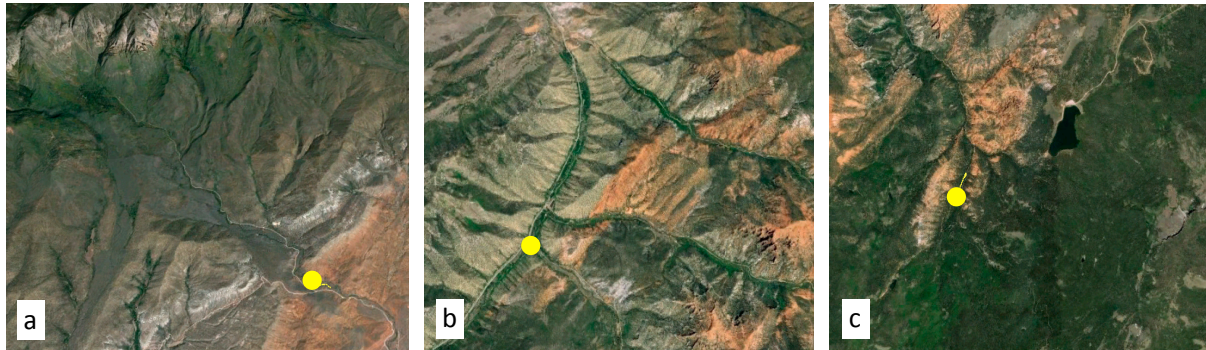
models based on abiotic proxies. Though new combinations of multiple leaf traits and the addition of limited continuous versus categorical traits have shown promise in improving the accuracy of paleoclimate interpretations to within 2°C of the abiotic models (Royer et al. 2005), complex physiological trade-offs in morphology present confounding errors and complexity (Holland and Richardson 2007). A number of modern proxy methods include chemical leaf content analysis and leaf mass area (LMA), but are not easily applied to the fossil record. When developing protocols for the paleontological record, it is particularly important to bear in mind constraints including likelihood of collecting whole leaf fossils and taphonomic biases for preservation. Leaf fossil preservation bias for flood planes, lakes, and streams is a result of the high potential for rapid burial in low energy flows. This often constrains fossil leaf records to mesic regimes within a region and may misrepresent interpretation of regional climates and ecosystems.

This research investigates a modern species analogue relevant to taphonomic bias in the fossil record and implications for application to the fossil record. *Betula occidentalis* (western water birch) was studied at three sites over an 1100 m elevation gradient in southwestern Utah to observe morphological changes in response to variations in temperature and CO<sub>2</sub> concentration. Specific attention is paid to the development of continuous trait variables for quantitative applications. Results are evaluated to better understand how toothiness, roundness, and stomatal density change in *Betula occidentalis* (western water birch) over an elevation gradient greater than 1000 m in southwestern Utah where samples are constrained to locations within the water table thereby holding water availability high and constant.

### ***METHODOLOGICAL DESIGN***

Random sampling of *B. occidentalis* was completed at three localities over an 1100 m gradient. Locations were selected such that watersheds be independent of one another, but of similar surrounding terrain, montane canyons. Center Creek, the high-elevation site descending from 2459 m, is located southeast of Parowan, Utah in the Dixie National Forest at 37° 44' 38.9" latitude and 112° 47' 31.8" longitude. The mid-level elevation site descending from 2037 m, Parowan Canyon Creek, is also located south of Parowan, Utah in the Dixie National Forest at 37° 47' 33.9" latitude and 112° 49' 06.4" longitude. Located west of Leeds, Utah in the Dixie National Forest, is the low-elevation site, Leeds Creek. This site descends from 1314 m and is

located at 37° 16' 21.9" longitude and 113° 23' 12.9" latitude. Figure 1 illustrates the regional terrain of each site location.



**Figure 1.** Field site locations via Google earth ® v. 7.1.2.2041 at 14,600 m eye altitude. The three stream sites are watershed canyon terrains originating from montane areas of southwestern Utah. A. Leeds Creek, the low-elevation site, descends from 1314 m elevation. B. Parowan Canyon Creek, the mid-elevation site, descends from 2037 m elevation. C. Center Creek, the high-elevation site, descends from 2459 m elevation.

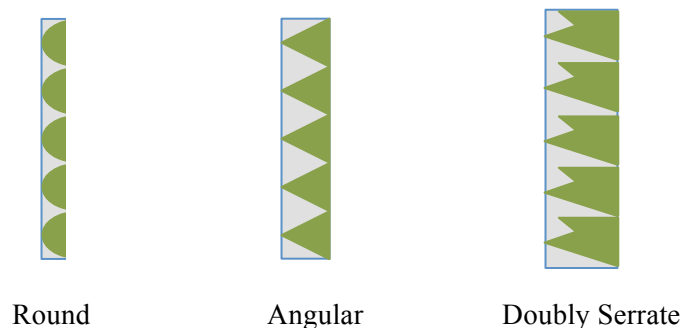
Random *B. occidentalis* leaves were collected at each location continuously such that every independent shrub along the stream channel (within 1.5 m of the thalweg) was sampled three times until 30 shrubs had been sampled and 90 leaves collected per site. Although shrub densities differed at the three sites, each site's samples were collected within a 20 m elevation range. Random sampling techniques required that small shrubs less than 1.5 m in height or with maximum trunk diameters less than 2.5 cm be excluded. Additionally, only leaves with less than 10% damage were collected. By starting at the branch located nearest to 2 m above the base of the randomly selected trunk and working backwards from its leader tip for leaf sampling, collection of leaves at the base of the shrub which are frequently abnormally large in size was prevented.

Post-collection samples were transported by cooler to the lab, individually labeled, and scanned against quadrille rule paper background for scale. In preparation for stomatal counts, leaf peels were conducted using quick dry nail polish. Polish was applied to the abaxial side of each leaf from the midrib to the right margin covering each leaf's widest area. The polish was allowed to dry for at least 20 minutes and then peeled using clear packing tape. The packing tape with stomatal peel attached was easily fixed to the microscope slide for viewing. If not able to complete leaf peels immediately, samples were stored in a refrigerator at 5° C. Due to

abundance of trichomes, on some leaves, a few samples were damaged during the stomatal peel process.

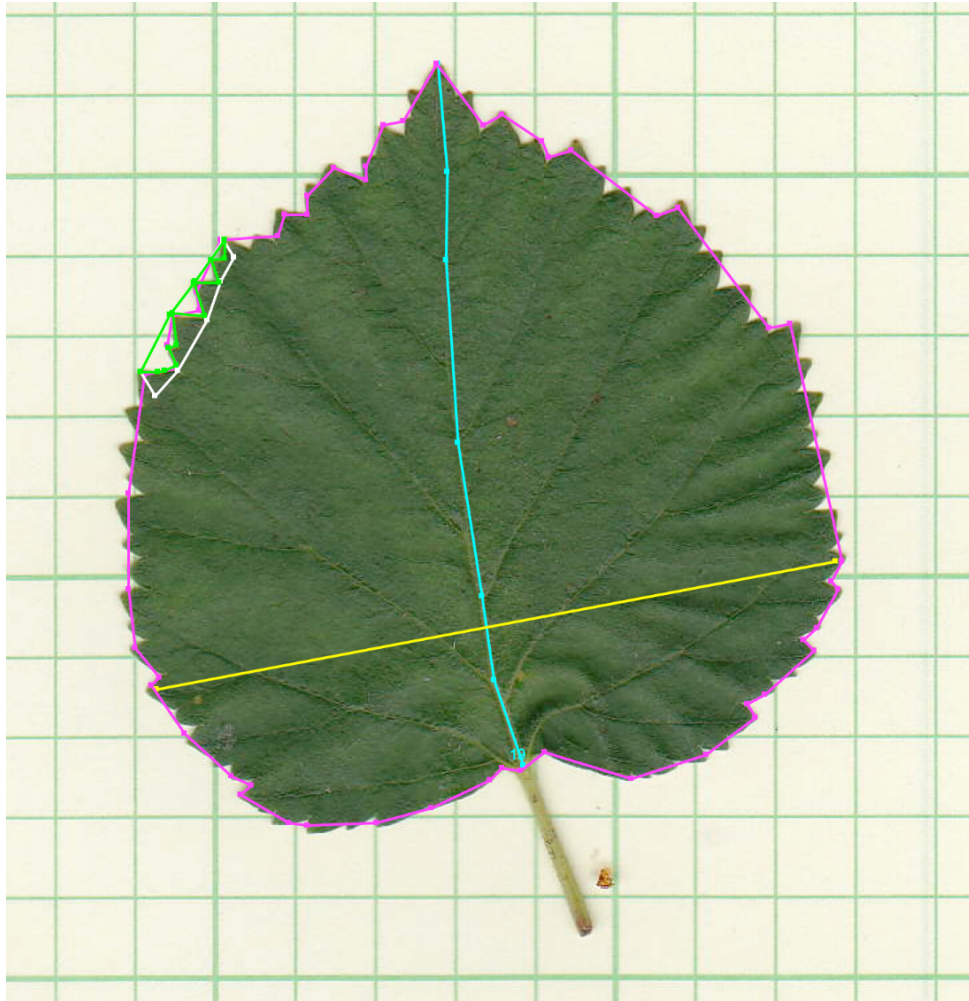
Until recently, the literature has defined toothiness using categorical variables through simple visual observation of leaf margin and identifying the margin as toothy or not toothy. Differentiation between lobiness and toothiness has been described by Hickey, 1973 and further updated by the Working Group of the Smithsonian Institution, 1999. More recently, number of teeth and perimeter to leaf blade area ratios have been used to quantify leaf toothiness (Royer et al. 2012). Though perimeter to leaf blade area ratios do provide quantitative analyses, the analysis are constrained by limited variation, particularly when considering small teeth such as found in *B. occidentalis*. Additionally, number of teeth per perimeter length does not take into account the length or relative area of each tooth.

Here we suggest a new quantitative protocol, which compares additive vs. absence margin spaces (Fig. 2). Past measures of leaf toothiness have assumed that leaf teeth are additive tissue providing additional function yet higher material costs. We suggest that leaf teeth are actually indicative of absence space from which tissue was lost and material costs reduced. To measure absence space, a pseudo entire leaf margin is defined across the teeth points and loss area defined through teeth vertices. An absence space ratio is computed as the ratio of absence space vs. the total potential area of leaf margin space defined between the teeth points and teeth vertices. The benefit of this quantification is that toothiness is not diminished by total leaf area as in examples of calculating ratios of leaf perimeters to areas, and increased variance between samples provides significance in statistical analysis otherwise lost.



**Figure 2.** Additive space is defined in the examples above by regions indicated in green where absence space is indicated by light gray regions. These spaces are contextualized as either tissue added to the entire leaf area as in additive space or tissue missing from the entire leaf area described as absence space.

Established protocols including Feret's diameter and stomatal count per area are used for quantitative comparison of leaf roundness and stomatal density respectively. Leaf roundness is calculated as the ratio of long and short axes of the leaf blade based on the assumption that a ratio of 1 is indicative of a perfectly round leaf. Ratios larger than one indicate leaf shapes that are increasingly elongate. Dimensional data for toothiness and Feret's diameter were collected using ObjectJ plugin v. 1.03p with ImageJ v. 1.49d (Fig. 3).



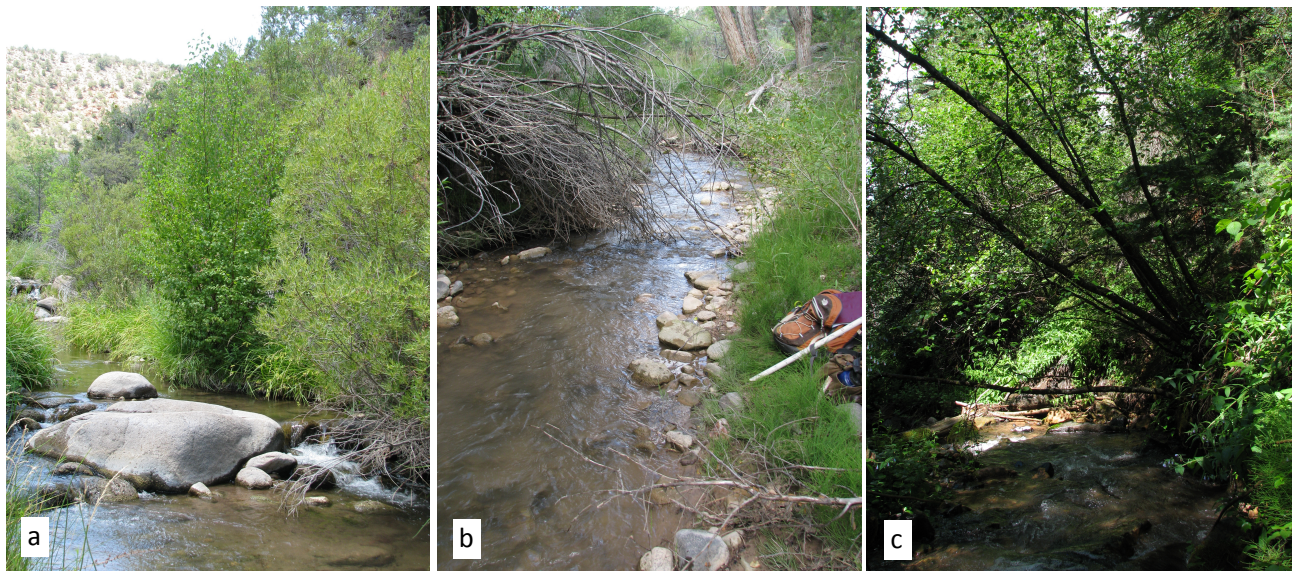
**Figure 3.** ObjectJ is used to calculate Feret's diameter, total leaf blade area, and toothiness as described by absence space. Feret's diameter is calculated using the blue (long axis) and yellow (short axis) lines. Total leaf area is estimated by the pink line. Absence space is the area calculated within the green outlined space, versus a pseudo entire leaf margin area outlined in white.

Stomatal counts were observed using a compound microscope with mechanical stage at 400x magnification. Total area of the viewing lens is 0.170 mm calculated using a micrometer

slide. Random counts were replicated three times at three locations across the widest part of the leaf blade and perpendicular to the midrib: near the midrib, along the toothy margin, and at the midpoint between the midrib and toothy locations. This provides three replicates of three count locations for each leaf or 270 stomatal counts per site. Only those stomata with complete inclusion of the aperture within the viewing area were counted. To provide a quantitative description of change in morphological traits, statistical analyses were applied utilizing R v. 3.1.0.

## **DATA**

To contextualize the difference in ecotones at each of the sites, a brief summary of floral composition is provided (Fig. 4). The low-elevation ecotone at 1314 m is dominated by *Juniperus osteosperma* (Utah juniper), *Salix exigua* (narrowleaf willow), *Carex* sp. (sedges), *Yucca* sp. (yucca), and *Opuntia* sp. (prickly pear cactus). The ecotone of the mid-elevation site is defined by the significant presence of *Populus angustifolia* (narrowleaf cottonwood), *B. occidentalis*, *Equisetum arvense* (common horsetail), *Equisetum hymale* (scouring rush), and *Maianthemum racemosum* (false Solomon's seal). The high-elevation ecotone is dominated by *Pinus ponderosa* (ponderosa) with densities of *Cornus sericea* (red osier dogwood), *Martensia arizonica* (aspen bluebells), and *Bryophyta* sp. (moss) along and within stream channel.



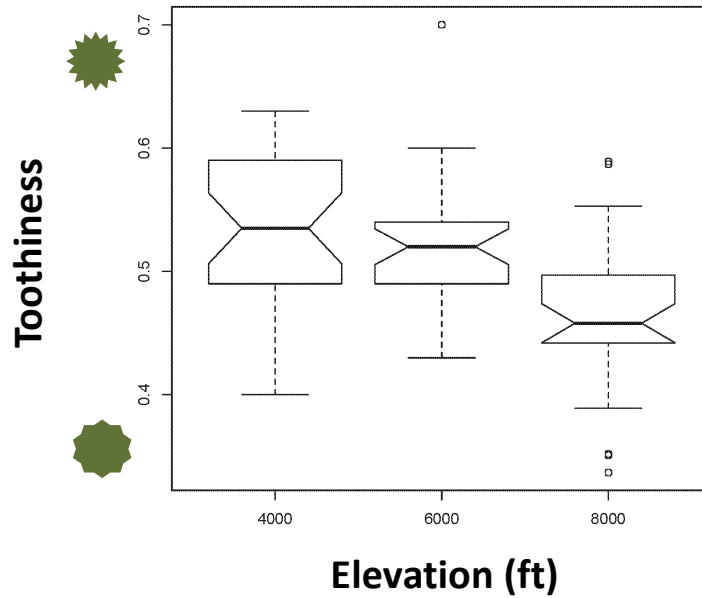
**Figure 4.** Photos of field site locations illustrating floral ecotones. A. Leeds Creek, the low-elevation site, is dominated by *Juniperus osteosperma* (Utah juniper) and *Carex* sp. (sedges). B. Parowan Canyon Creek, the mid-

elevation site, includes dense populations of *Populus angustifolia* (narrowleaf cottonwood), *Equisetum arvense* (common horsetail), and *Equisetum hymale* (scouring rush). C. Center Creek, the high-elevation site, is dominated by *Pinus ponderosa* (ponderosa) with high densities of *Cornus sericea* (red osier dogwood).

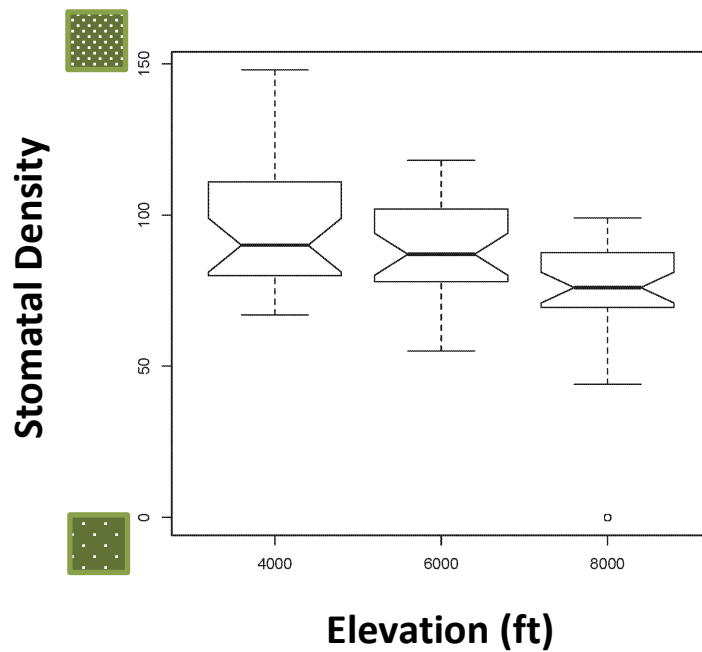
Mean values for each site were calculated for each of the traits investigated and are provided in Table 1. Statistical analyses of the relationship between toothiness (defined by new protocols suggested here) and elevation support a negative relationship of decreasing toothiness with increasing elevation ( $p=0.00004$ ) illustrated in figure 5. A statistically significant negative correlation was also observed in the relationship between stomatal density and elevation ( $p=0.0002$ ) as shown in figure 6. However, figure 7 indicates a positive relationship between roundness and elevation ( $p=0.001$ ).

**Table 1.** Mean values of investigated traits by site location (n=30).

	Blade Area (mm)	Circular Diameter of Area (mm)	Feret's Diameter	Toothiness	Inner-Leaf Stomatal Density (mm <sup>-1</sup> )	Mid-Leaf Stomatal Density (mm <sup>-1</sup> )	Outer-Leaf Stomatal Density (mm <sup>-1</sup> )	Leaf Stomatal Density (mm <sup>-1</sup> )
1314 m Leeds Creek	1945.33	49.03	1.14	0.53	101.35	95.29	95.03	96.04
2037 m Parowan Canyon Creek	1124.09	37.46	1.15	0.52	85.49	86.47	91.44	87.80
2449 m Center Creek	1206.32	38.26	1.09	0.46	77.78	75.82	76.41	76.67

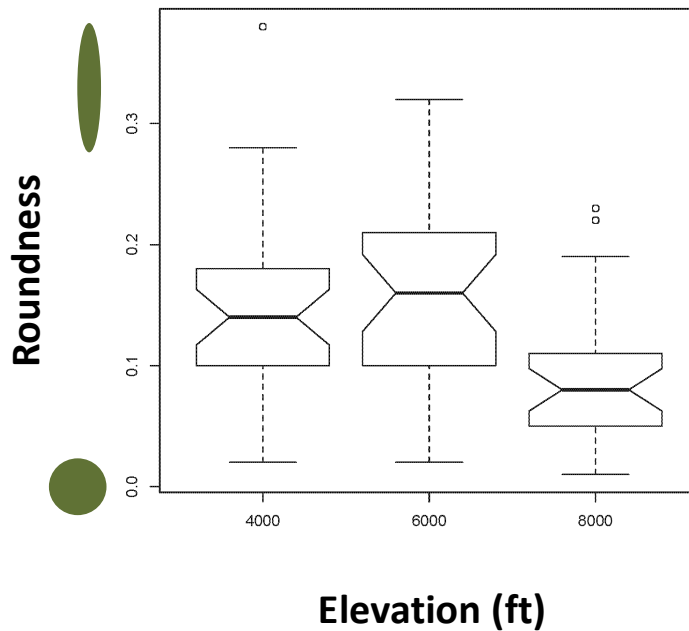


**Figure 5.** Statistical analyses of the relationship between toothiness and elevation support a negative relationship of decreasing toothiness with increasing elevation (n=90, p=0.00004).



**Figure 6.** A statistically significant negative correlation was observed in the relationship between stomatal density and elevation (n=90, p=0.0002).





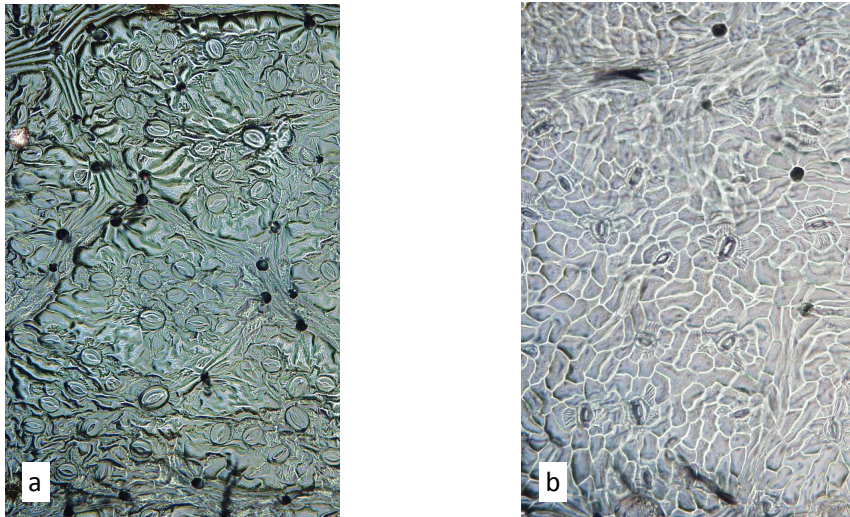
**Figure 7.** A statistically significant positive relationship between roundness and elevation ( $n=30$ ,  $p=0.001$ ) is observed.

## ***RESULTS/CONCLUSIONS***

As expected through the literature, leaf roundness increased with increasing elevation; however, toothiness and stomatal density decreased with increasing elevation. This evidence suggests that complex environmental interactions may complicate interpretations from morphological proxies and that further study may reveal a combination of traits that will quantify the strength of signal from existing proxies to increase the accuracy of paleoclimate interpretations. What distinguishes our research from earlier investigations is that we define toothiness by the absence space within an otherwise entire leaf margin providing not only a continuous variable, but also one that is independent of leaf size.

Future studies (prior to manuscript preparation) will include the comparison of our data with protocols provided to evaluate toothiness through additive space. A presumed inverse correlation may explain the above contradictions between our data and existing literature. However, we do not yet have a hypothesis to explain contradictions in stomatal density (Fig. 8). Based on our results, we propose that it is not necessarily essential to identify new, more

accurate proxies, but identify a combination of proxies that allow the established proxies to be further constrained and informed to provide more detailed paleoclimate interpretations. Finally, the use of continuous trait variables may have the potential to provide this constraint and evaluate disparity in ecomorphospaces as well as interpret the evolutionary response of plants to geologic perturbations.



**Figure 8.** Stomatal density patterns contradict that found in the literature. *Betula occidentalis*, a mesic facultative species, shows higher stomatal densities and low elevations (a.) and low stomatal densities at high elevations (b.).

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