# Characterizing Quantitative Variation in the Glossopodia of Three Californian *Isoëtes* Species

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## Introduction

Historically, finding informative morphological characters to use in *Isoëtes* L. has been difficult due to the highly conserved base morphology and high intra-specific variability. For this reason, the main morphocharacter used for years were the megaspores (Pfeiffer 1922), but this has waned to a degree. This study aims to expand on recent work exploring the glossopodium (Fig. 1), a structure that arose before the division between *Isoëtes* and its extant sister genus *Selaginella* (Greirson and Bonamo, 1979; Pigg 2001), as a potential morphological character (Sharma & Singh 1984; Shaw & Hickey 2005).

In this work, glossopodia from three Californian *Isoëtes* (*I. bolanderi* Engelm, *I. howellii* Engelm, and *I. nuttallii* A.Br.) were histologically sectioned and reconstructed using a computer, and analyzed using MANOVA analysis of linear distances measured on the 3D projections, and a combined elliptical Fourier and principle components analysis. My goal is to determine if there are detectable and consistent morphological differences between the different species of *Isoëtes*, and if there are, does this structure have the potential to be informative taxonomically.

## Methods

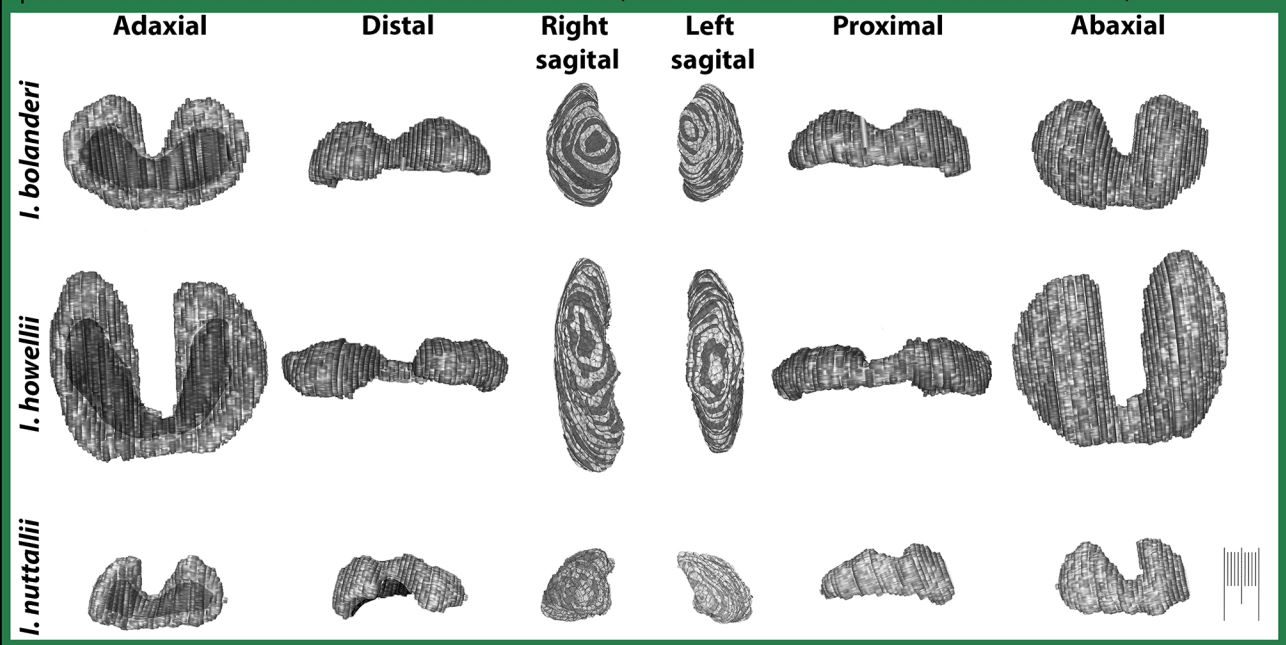
**Preparation of Glossopodia**

- 9 *I. bolanderi*, 6 *I. howellii* and 9 *I. nuttallii* leaves were taken for analysis.
- leaves were embeded in parafin and serial sectioned, stained using the Sharman staining series and imaged with a digital camera.
- glossopodia were reconstructed and examined for qualitative differences between species (Fig. 2).

**Morphometric Analysis**

- linear measurments made using ImageJ.
- measurements analyzed with MANOVA in R-studio.
- silhouettes of reconstructed glossopodia were smoothed using *momocs* and *scales* libraries in R.
- elliptical Fourier analysis (EFA) and principal components analysis (PCA) was run on the smoothed silhouettes to characterize differences in shape between species (Fig. 3).

**Figure 2** - Representative 3D computer reconstructions of the glossopodia of the three sampled species of *Isoëtes*. All reconstructions are to scale (Scale = 0.1mm with 0.01mm increments).




## General conclusions

- Qualitative comparisons among the glossopodia showed differences in the general appearance of each species that was consistent within each (Fig 2).
- The MANOVA analysis found significant differences in the linear distance measurements between all three species in cornua length and absolute width of the sedes (see Freund 2016 for details).
- The EFA/PCA showed the abaxial face and shape of the medimoles base provided the greatest separation of the species (Fig 3), with principal component 1, which describes the length of the cornua lobes, providing the greatest amount of resolution between the three species.
- Both of these results support the glossopodia as being morphologically distinct between the species, with most of the differences being found in the length of the cornua.
- Future work will expand upon the sampling done by increasing the number of samples in the analysis and better quantifying the intra-specific variance. Additionally, 3D computerized tomography is being considered to produce finer detailed images for analysis.

## References

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**Figure 1** - The leaf base of *Isoëtes*, including the structures of the leaf, ligule, and glossopodium. Images are not to scale, and represent the average appearance of *Isoëtes howellii*.

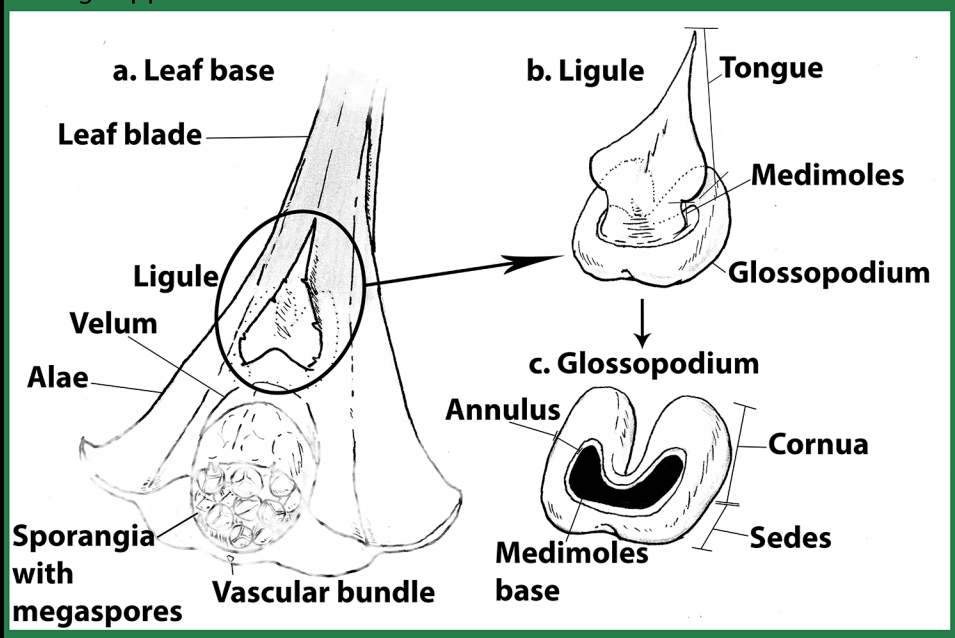
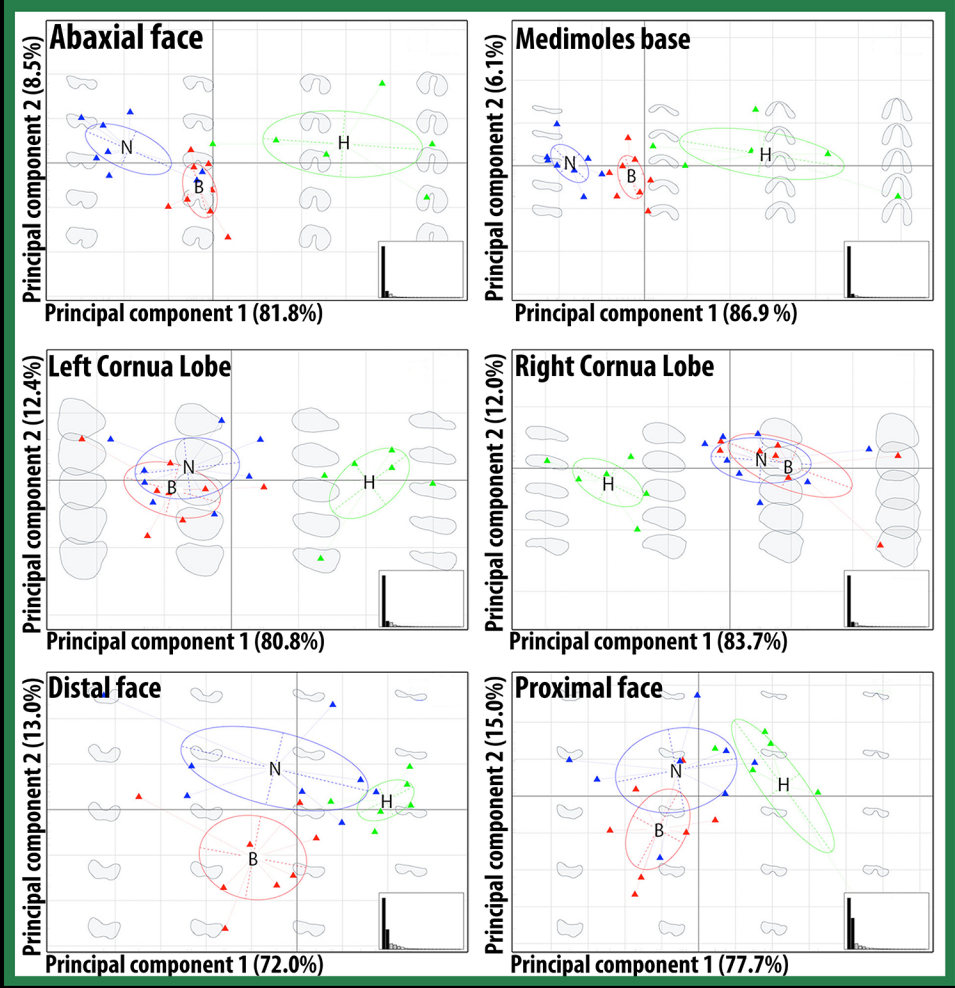



**Figure 3** - PCA of glossopodial EFA, with PC 1 on the x-axis and PC 2 on the y-axis. Triangles represent the individuals sampled in the study. Ellipses represent the 95% confidence limit for the centroid of each species B) *I. bolanderi*, H) *I. howellii*, N) *I. nuttallii*.

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