

# Investigating polyploidy in *Isoetes graniticus* using megaspore morphology and DNA analysis

Katherine Y. Wu,<sup>A,B</sup> Peter W. Schafran,<sup>A,C</sup> Gabriel P. Johnson,<sup>A</sup> W. Carl Taylor,<sup>A</sup> and Elizabeth A. Zimmer<sup>A</sup>

## Introduction

Allopolyploidy, or interspecific hybridization combined with chromosome doubling, is a common method of speciation among plants. In *Isoetes* (quillworts), around 50% of North American species are polyploids. Combined with the large number of putative hybrids, these suggest that for *Isoetes*, allopolyploidy is a significant speciation process.<sup>1</sup>



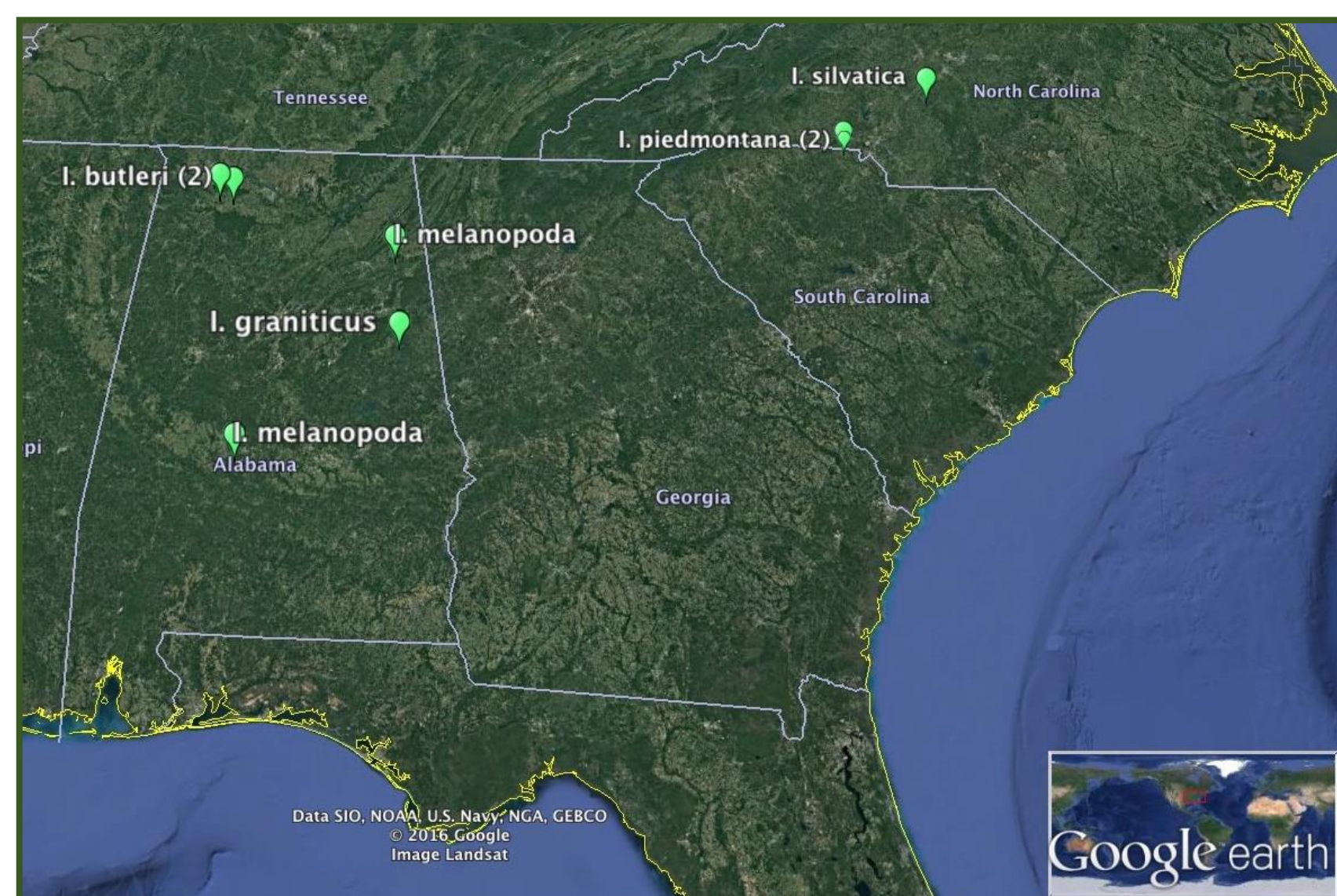
*Isoetes melanospora*

Because of their highly conserved morphology and reticulate evolution, species identification and taxonomic study is often difficult. Morphologically, the megaspore is the richest source of phylogenetically useful variation,<sup>2</sup> but DNA analysis provides a precise way to determine species identification and hybrid origins.<sup>1</sup> This study makes use of both to characterize a specimen of *Isoetes graniticus*, a recently described allotetraploid found on granite outcrops in Alabama.

## Methods

1. Samples of the following species were collected across the Southeastern United States:

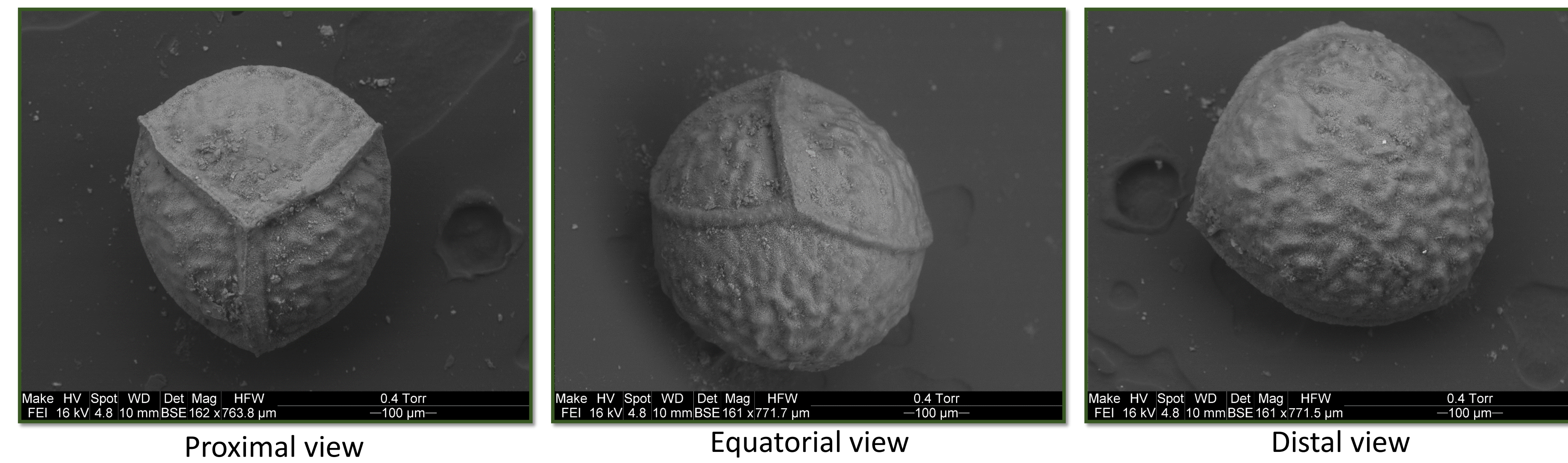
- I. graniticus*
- I. piedmontana*
- I. silvatica*
- I. melanopoda*
- I. butleri*



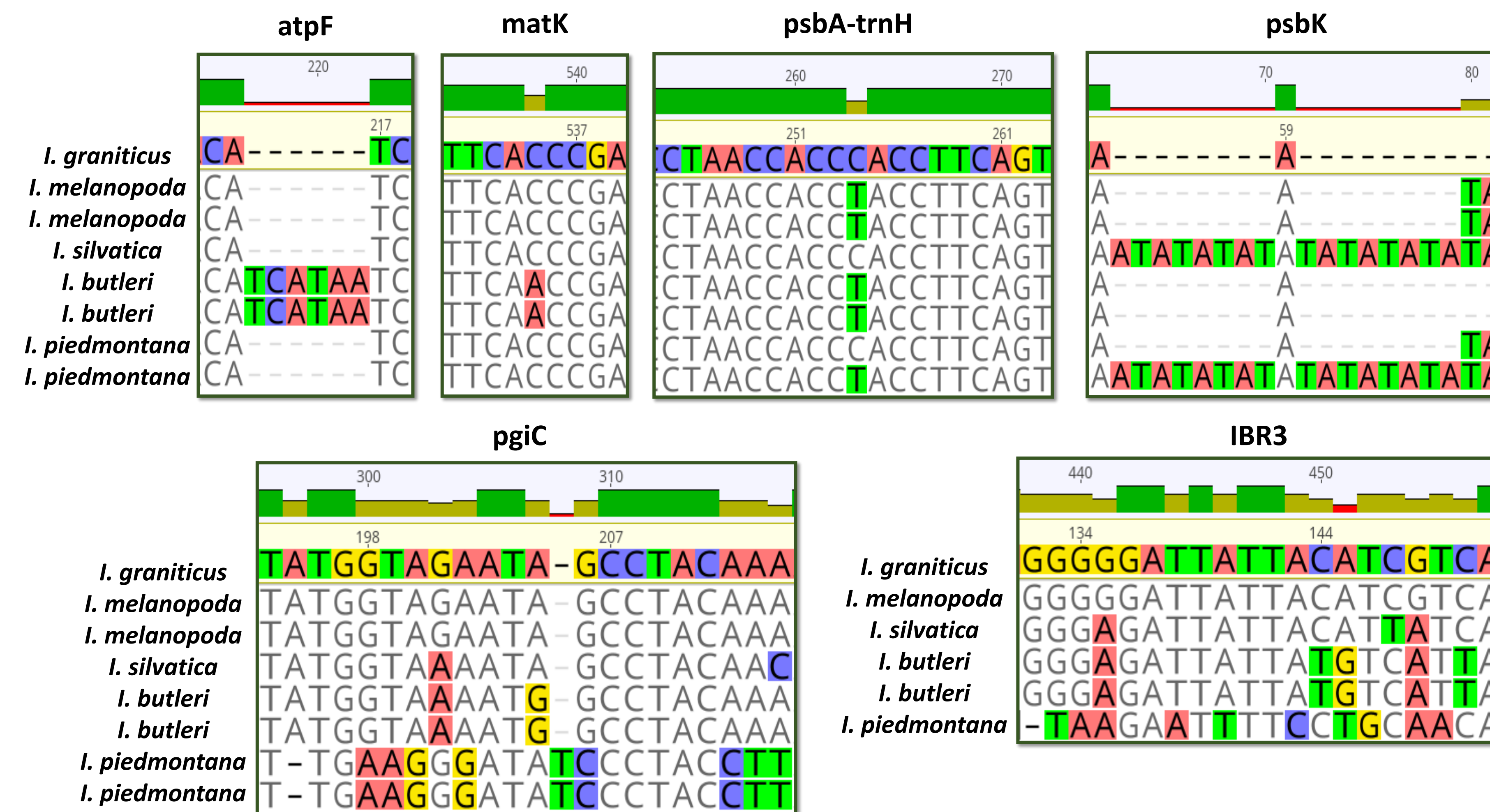
2. Spores of *I. graniticus* were photographed under the scanning electron microscope (SEM) and compared with the spores of other sampled species described in the literature.

3. DNA was extracted from leaf tissue for all of the samples, using a Qiagen® DNeasy Plant Mini Kit and amplified for four plastid (atpF, matK, psbA-trnH, psbK) and four nuclear (gapC, IBR3, LFY, pgiC) markers using standard PCR protocol. The products were treated with ExoSAP-IT and Sanger sequenced. All sequences were edited and analyzed in Geneious 9.1 (<http://www.geneious.com>, Kearse et al., 2012).<sup>3</sup>

## Results



**Figure 1:** SEM pictures reveal a megaspore with a mix of short ridges and small tubercles along the surface, which flatten out as they approach the equatorial ridge.



	atpF	matK	psbA-trnH	psbK	pgiC	IBR3
<i>I. melanopoda</i>	N/A	N/A	1 transition	1 indel	N/A	1 transition 9 transversions 2 indels
<i>I. silvatica</i>	N/A	N/A	N/A	2 indels	1 transitions 2 indels	6 transitions 5 transversions 2 indels
<i>I. butleri</i>	1 indel	1 transversion	1 transition	N/A	4 transitions 2 indels	12 transitions 7 transversions 1 indel
<i>I. piedmontana</i>	N/A	N/A	N/A	1 indel	27 transitions 31 transversions 15 indels	51 transitions 110 transversions 49 indels

**Table 1** shows the phylogenetically informative variation among sample sequences when compared to sequences of *I. graniticus*. If two samples of the same species were used, any difference had to be common to both samples. The marker IBR3 showed the most variation across species. A sample of the variation present in each marker is shown in **Figure 2** above.

## Discussion

Hybrids and allopolyploids often have an intermediate spore morphology when compared to their parent species.<sup>1</sup> Comparison of megaspore morphology of *graniticus* to that of sampled species of the Southeastern United States suggests descent from *I. butleri* is unlikely because of the lack of granularity.<sup>4</sup> Future work in this area will involve measuring sample megaspores as a test for polyploidy, since polyploids tend to have larger megaspores. This will be confirmed with a root tip squash.

DNA analysis suggests that *I. melanopoda* and *I. silvatica* are prime candidates for *I. graniticus* parentage, while *I. piedmontana* and *I. butleri*, though they inhabit the same region of the US, are unlikely to have any direct relation to *I. graniticus*. Work is underway to confirm this by sequencing the highly variable LFY region. This will be combined with next-gen chloroplast sequencing to determine the chloroplast donor species.

## References & Acknowledgements

<sup>1</sup>Hoot, Sarah B., Nancy S. Napier, and W. Carl Taylor. (2004). "Revealing unknown or extinct lineages within Isoetes (Isoëtaceae) using DNA sequences from hybrids." *American Journal of Botany* 91(6), 899-904.

<sup>2</sup>Hickey, R. James. (1986). "Isoetes Megaspore Surface Morphology: Nomenclature, Variation, and Systematic Importance." *American Fern Journal* 76(1), 1-16.

<sup>3</sup>Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647-1649.

<sup>4</sup>Brunton, Daniel F. (2015). "Key to the Quillworts (Isoetes: Isoëtaceae) of the Southeastern United States." *American Fern Journal* 105(2), 86-100.

<sup>A</sup>Smithsonian National Museum of Natural History, Department of Botany.

<sup>B</sup>Mount St. Mary's University

<sup>C</sup>Old Dominion University

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