

Spore sizes reveal ploidy levels in known and new Great Lakes Bog Clubmosses



(*Lycopodiella*, Lycopodiaceae)

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Introduction

- The genus *Lycopodiella* (Lycopodiaceae) currently includes 15 species distributed throughout South America, the Atlantic coastal plain, and the Great Lakes region. Representatives of this group hybridize readily and are commonly found intermingling within the same site.
- Bruce (1975) confirmed three diploid species on the Coastal Plain and found three species in the Great Lakes region: *L. inundata* and undescribed tetraploids “Appressed Inundata” and “Northern Appressa”. Larson and Ballard (2012) documented two more undescribed species, “Robust Inundata” and “Shade Appressed Inundata”.
- Ploidy level serves as a valuable line of evidence for the species delimitation process and can be determined using various methods. Here we take a classical approach by measuring spore sizes to distinguish diploids and polyploids.



Figure 1: The five taxa examined in this study. (A) “Northern Appressa”, (B) “Appressed Inundata”, (C) “Robust Inundata”, (D) “Shade Appressed Inundata”, (E) *L. inundata*.

Methods

- Images of spores were taken with a light microscope at 40x magnification. Measurements were done in ImageJ using the provided scale bar.
- Spores were sampled from multiple individuals of each species, the exception being “Shade Appressed Inundata” where one individual was collected due to its regional rarity.
- Differences in spore sizes across species was analyzed using analysis of variance (ANOVA) in conjunction with the Tukey-Kramer post-hoc test (Figure 3).
- Differences in spore sizes across populations was analyzed using permutational analysis of variance with Bonferonni correction of p-values (Figure 4).

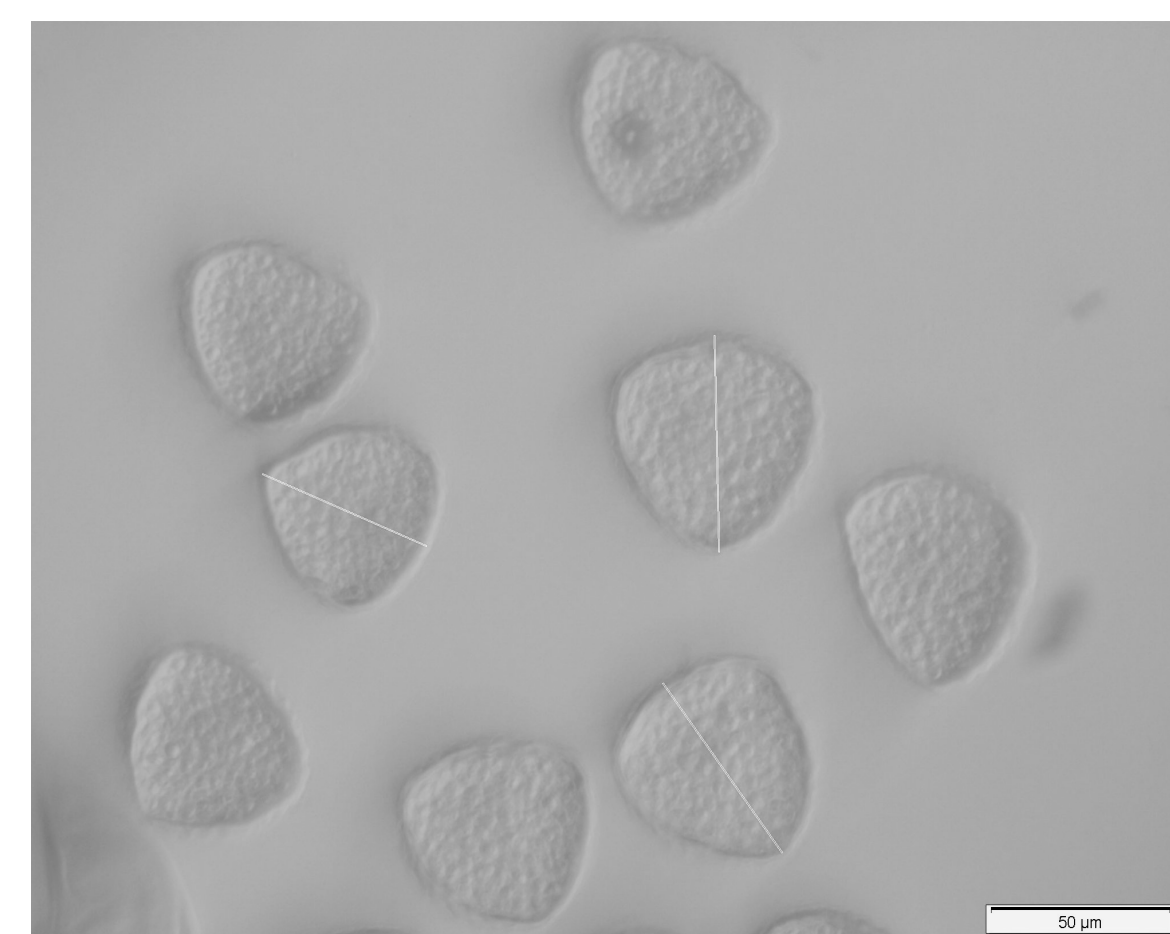


Figure 2: Light microscopy image of *Lycopodiella* spores taken at 40x magnification, with lines showing an example of how the spores were measured in ImageJ.

Results

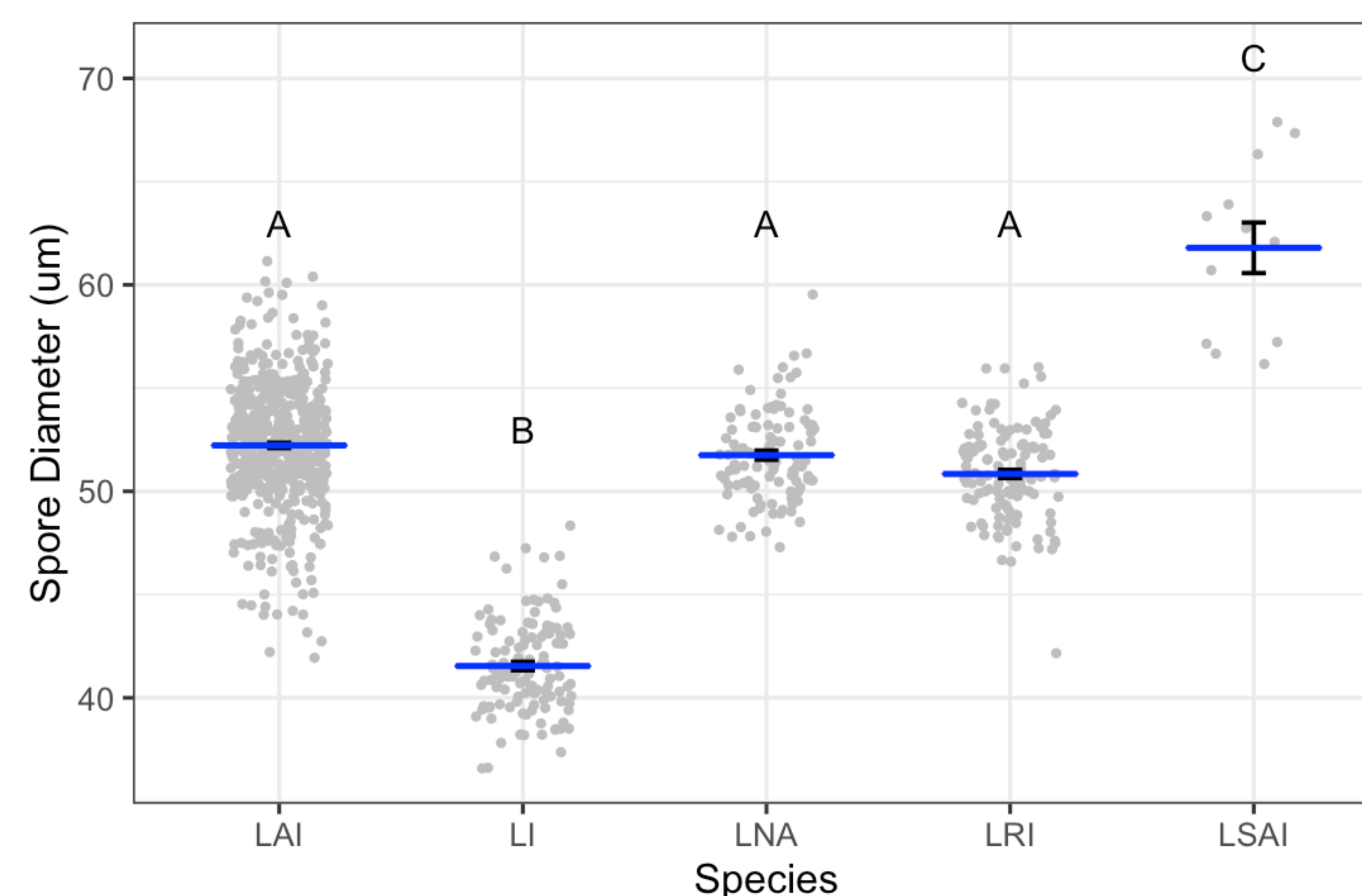


Figure 3: Spore sizes measured in micrometers are graphed as a function of species using R software. ANOVA and Tukey-Kramer post-hoc test were used to determine significant variation between species. We conclude that at least one of the means is different from the other ($F_{4,886} = 435.4$, $p < 0.001$), and our post-hoc test indicates that *L. inundata* (2x) and Shade Appressed Inundata (6-8x) are significantly different from the other species, while Appressed Inundata(4x), Northern Appressa(4x), and Robust Inundata(4x) show no significant variation among them.

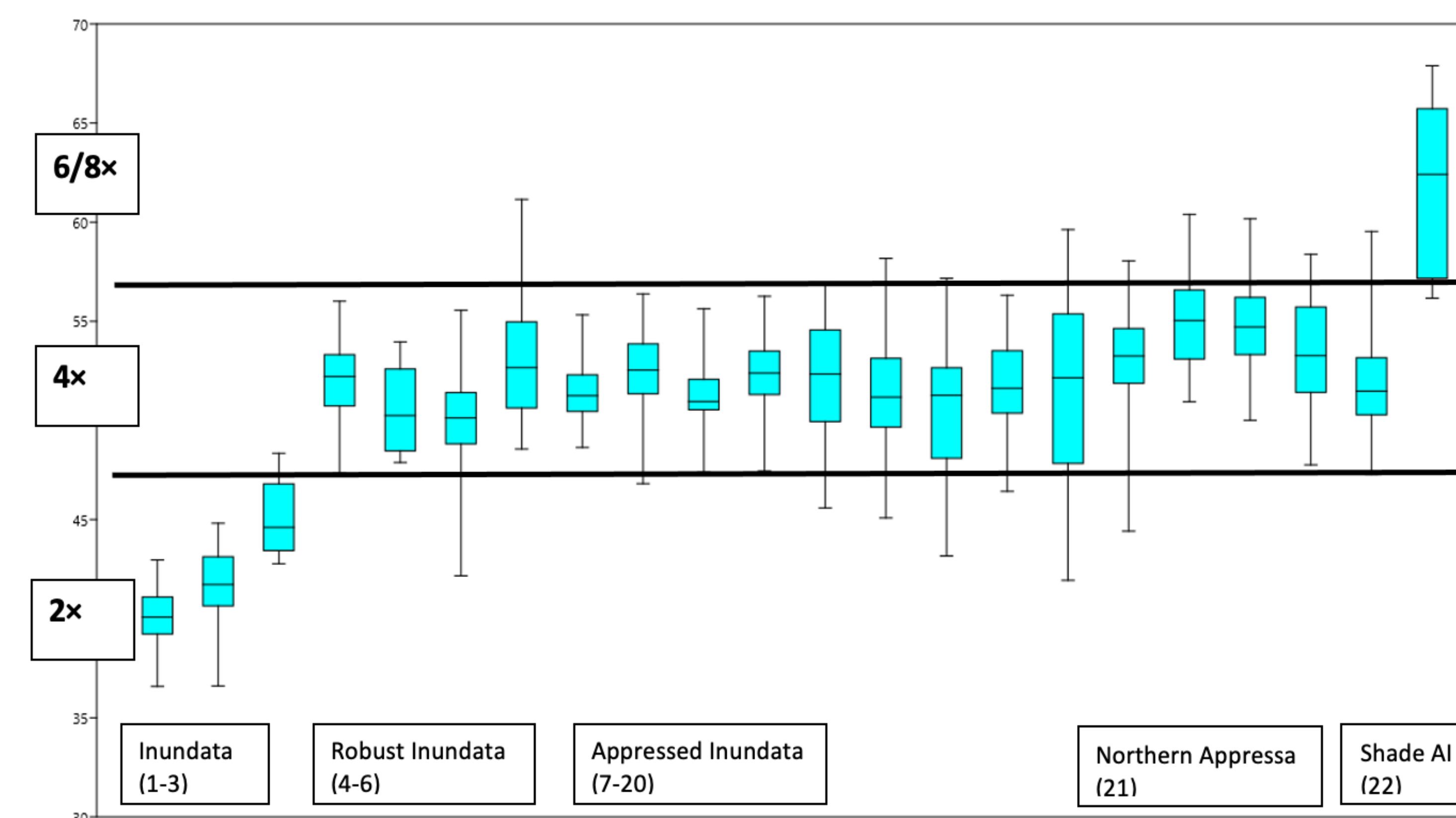


Figure 4: Boxplots created in PAST software show variation in spore sizes by population of *Lycopodiella*. A permanova test with Bonferonni correction of p-values shows that (1) populations 1-3 (all *L. inundata*) are statistically distinct from all others; (2) that populations 6 (Robust Inundata) and 17-20 (Appressed Inundata) are distinct from other presumed tetraploid populations; and that population 22 (shade Appressed Inundata) is distinct from all others.

Conclusions

- Data reveals a diploid size range for *L. inundata*; a tetraploid size range for “Appressed Inundata”, “Northern Appressa” and “Robust Inundata”; and a hexaploid or octoploid size range for “Shade Appressed Inundata”, the latter being a new higher ploidy level for North American *Lycopodiella*.
- These new findings support the recognition of five distinct *Lycopodiella* species within the Great Lakes region, confirming the ploidy level for *L. inundata*, “Appressed Inundata”, and “Northern Appressa”, and revealing the ploidy level in “Robust Inundata” and estimating a new ploidy level in “Shade Appressed Inundata”.

Broader Impacts

- This research contributes valuable evidence to support recognition of species in Great Lakes *Lycopodiella*.
- The broader study provides up to date species specific information for Natural Heritage programs in the Great Lakes region concerning species endangerment and guides conservation efforts for these rare species and the coastal plain marsh community that they inhabit.

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Literature Cited

- Bruce, J. G. 1975. Systematics and morphology of subgenus *Lepidotis* of the genus *Lycopodium*. Ph.D. dissertation, University of Michigan, Ann Arbor, MI.
- Larson, J. and H.E. Ballard, Jr. 2012. Phenetic analyses of Great Lakes *Lycopodiella* (Lycopodiaceae). Botany 2012, Columbus, OH.