

Dear Prof. Culley,

It is my pleasure to submit the manuscript titled '3D segmentation of plant bud CT scans support the bud packing hypothesis' for review to the Journal of Applications in Plant Sciences as an Application Article on behalf of my co-authors. My name is Dr. Andras Zsom and I am a Lead Data Scientist at Brown University in the Center for Computation and Visualization (CCV). My team at CCV are not biologists but data scientists and we help faculty members at Brown with their data-intensive research projects.

This project started roughly two years ago when Prof. Erika Edwards (then at Brown University currently at Yale) approached me because her team was struggling to segment microCT scans of plant buds into bud scale and leaf with open source or commercial image segmentation softwares (like ITK-SNAP). The main reason why those tools failed was because the scale and the leaf tissues have a similar intensity.

I was intrigued by the problem and I was happy to collaborate. Initially I tried to develop supervised machine learning models (neural networks) to automatically segment the buds based on tissue structural patterns. Ultimately these approaches were deemed inaccurate. After a couple of failed attempts, we found that the buds can be accurately segmented by a custom image processing pipeline that requires minimal user input. This approach and the preliminary results are described in the manuscript.

After successful segmentation, we calculate various bud metrics some of which are novel in the field to the best of my knowledge (e.g., fractal dimension and compactness). We then use phylogenetically independent contrasts to evaluate correlations between extracted metrics and species-level characteristics. Our results and findings lend preliminary support to the bud packing hypothesis, indicating that the packing of leaves within buds correlates with mature leaf form and suggesting new avenues of research.

I believe the manuscript is a good fit for the journal because we study the structure and development of leaves in the *Viburnum* genus. Our code is publicly available in a GitHub repository and we hope the repository will make this a tool researchers can actually use. The manuscript describes original research, no part of this manuscript is published elsewhere, and we have no conflicts to disclose.

Thanks you for your consideration!

Best wishes,
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