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FishCuTv2: An Extensible Software for microCT-Based Whole-Body Skeletal Phenomics in Zebrafish

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Introduction

- Zebrafish are a powerful model for rapid-throughput skeletal biology [1].
- Previously, we developed software for microCT-based phenomics in zebrafish, FishCuT(v1) [1].
- FishCuT enables rapid (<5min/fish) profiling of 600 measures within 24 vertebrae of adult zebrafish (Fig 1,2).
- We showed that analyzing vertebral patterns confers higher sensitivity, with similar specificity, in discriminating mutant populations compared to analyzing a single vertebra [1].
- One limitation of FishCuTv1, due to its architecture, is difficulty in extending analysis to other skeletal structures and traits.

Objectives

- Within FishCuT, incorporate an extensible architecture that facilitates open-source development of new phenotypic algorithms in pursuit of whole-body phenomic analysis.

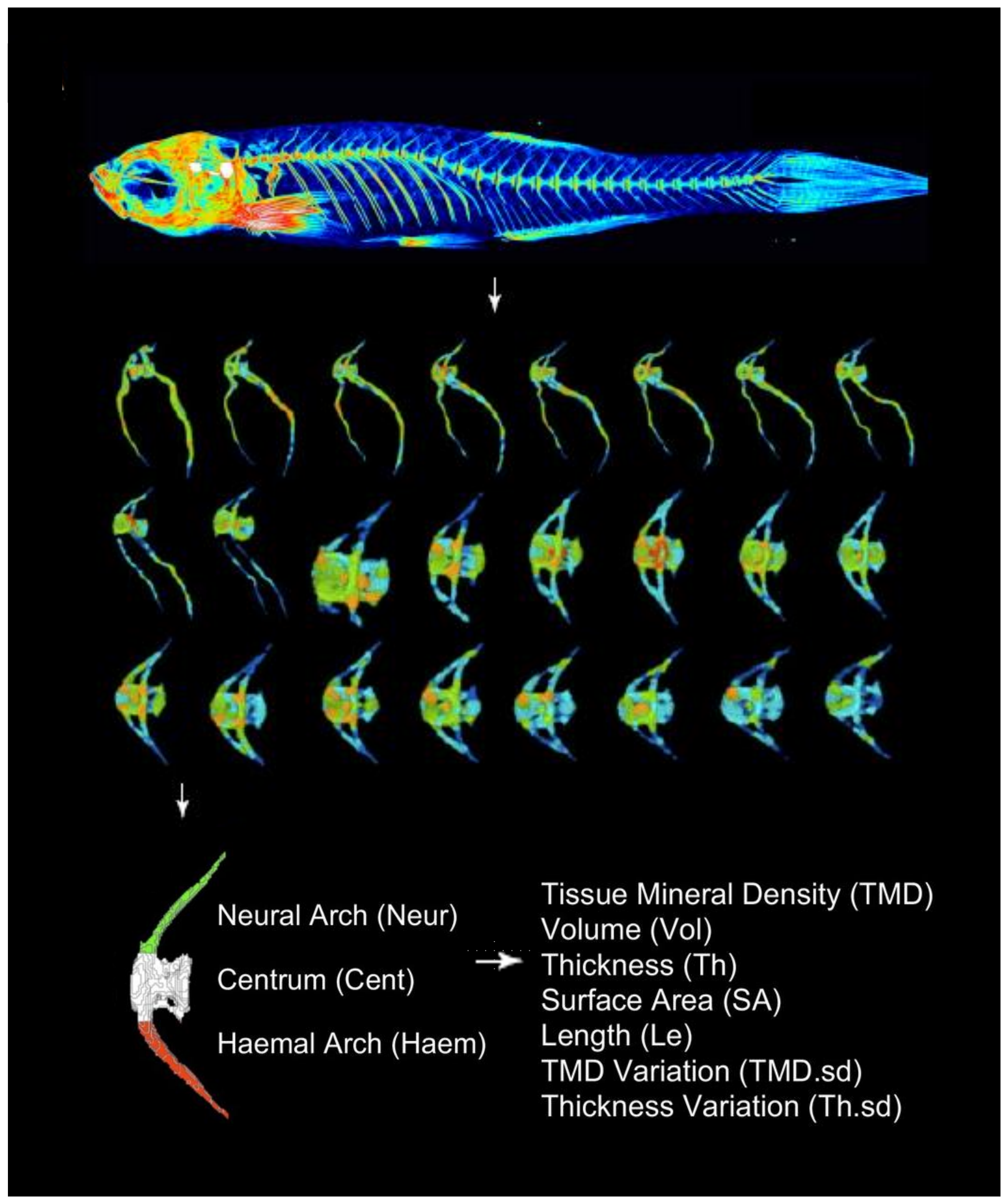

Fig 1: FishCuT isolates individual vertebrae (shown: 24 vertebrae from a single fish), segments each vertebra into three elements (neural arch, centrum, and haemal arch), and computes measures for each element.

FishCuTv2

- We have modularized the architecture of FishCuT to facilitate development of new extensions to measure additional skeletal traits.
- New modules can be implemented retroactively to analyze existing microCT data.

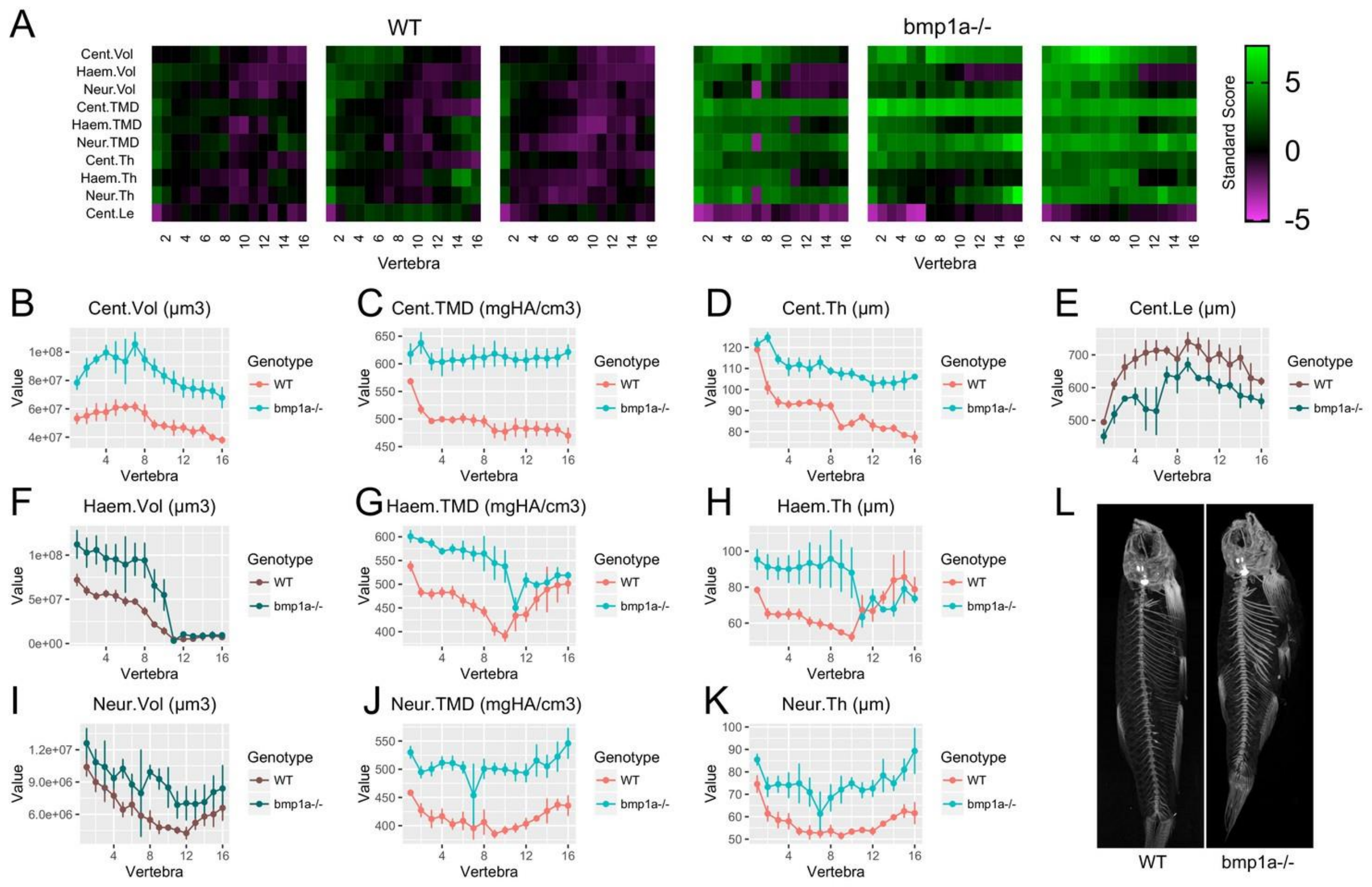

Fig 2: (A) Skeletal barcodes for WT and *bmp1a-/-* fish (n = 3/group), each representing a single fish. (B–K) Phenotypic features plotted as a function of vertebra. Plots associated with significant differences have a lighter coloring scheme. (L) Maximum intensity projection of microCT scans.

Module to Measure Neural Arch

- We developed a module to quantify neural arch area, height, and width to demonstrate FishCuTv2's extensibility.
- Neural arch measurements exhibited high correlation ($R^2 = 0.86$) with manual measurements.

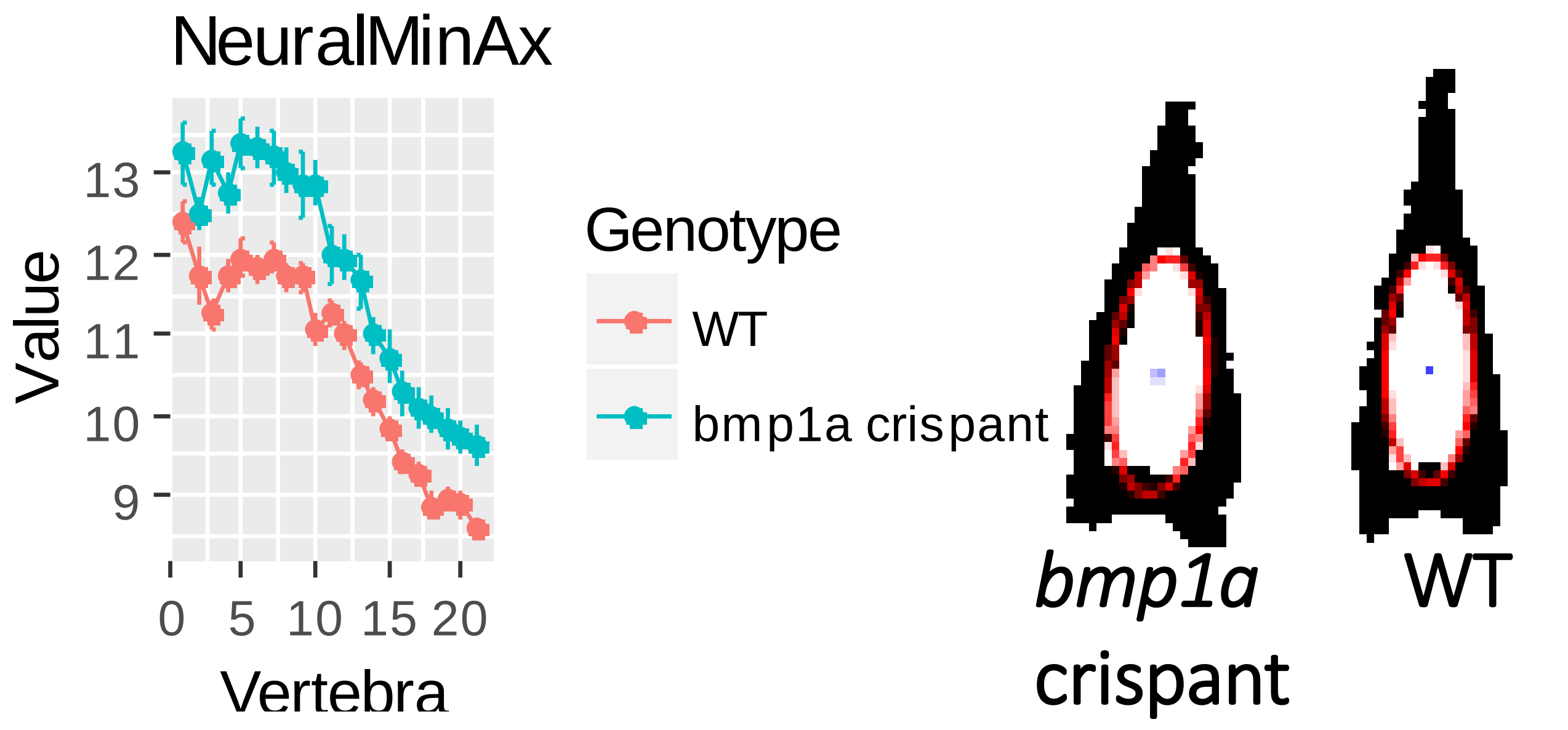

Fig 3: Quantitative and qualitative analysis of neural arch area and effective minor axis (neural arch width) for *bmp1a* crispants.

- In *bmp1a* crispants, we detected a statistically significant ($p < 0.05$) increase in neural arch width (NeuralMinAx) (Fig 3). This was consistent with previous studies showing increased neural arch area in *bmp1a* germline mutants [2].

Atlas-Based Craniofacial Analysis

- We investigated methods for analyzing the craniofacial skeleton.
- We created a synthetic template for the craniofacial skeleton by deforming and registering microCT scans of 50 wildtype animals, creating an "average skull."
- We used the atlas to identify regions of craniofacial shape change in *bmp1a* crispants (Fig 4) [3].

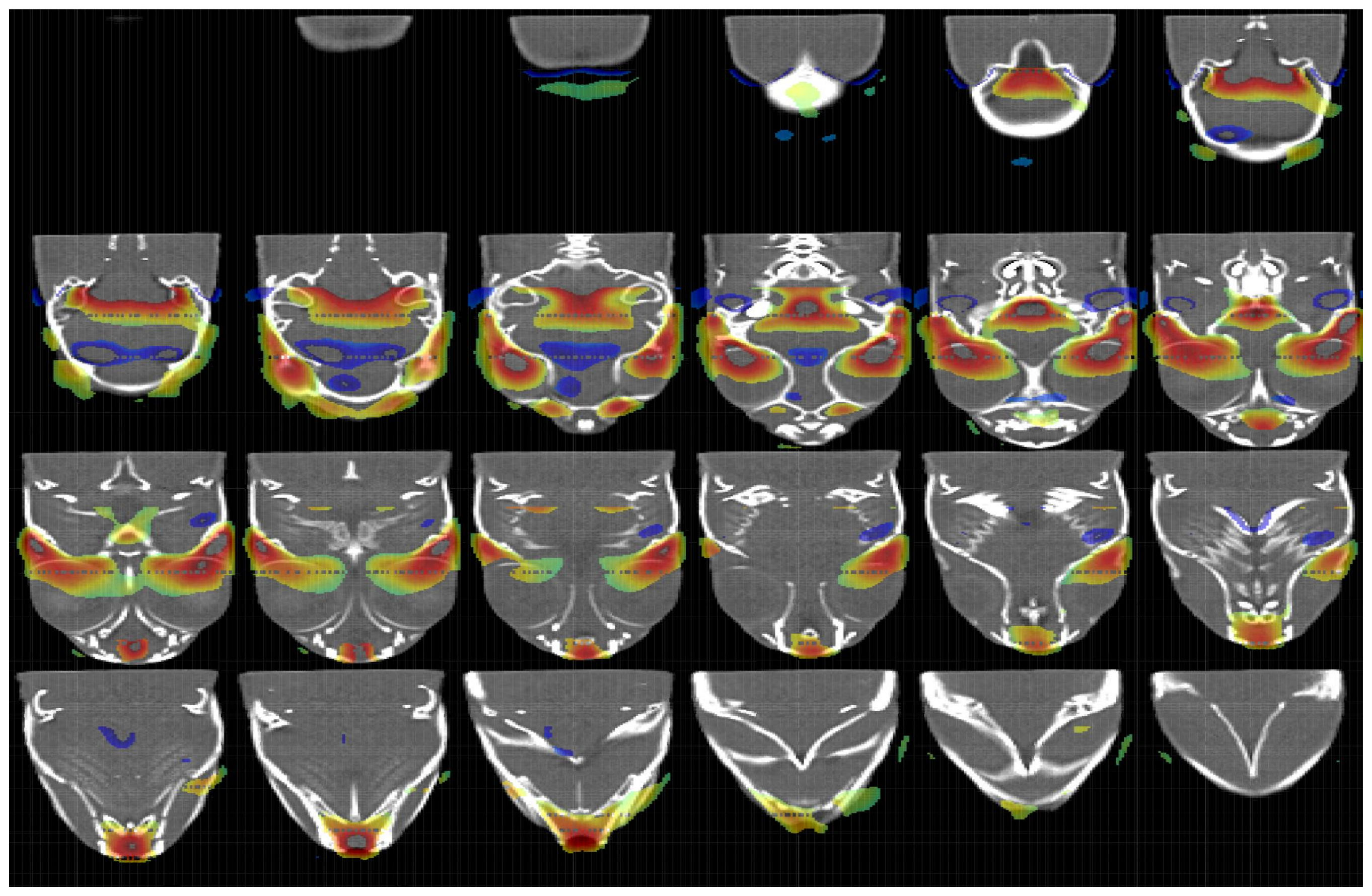

Fig 4: Visualization of statistical shape analysis in craniofacial skeleton of *bmp1a* crispants compared to atlas skull. Shown are slices of the synthetic template (dorsal to ventral direction). Warmer colors indicate contraction; cooler colors indicate expansion.

Discussion

- We improved FishCuT's architecture and prototyped new phenotypic modules for higher content descriptions of whole-body microCT datasets.
- FishCuTv2 facilitates collaborative development and retroactive screening of microCT data to identify new gene-to-phenotype relationships.

References

[1] Hur et.al., eLife 2017, [2] Charles et.al., Bone 2017. [3] Maga et.al, Journal of Anatomy 2017.

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