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Peer Review

Review of: "Programmed Cell Death and the Origin of Wing Polyphenism in Ants: Implications for Major Evolutionary Transitions in Individuality"

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Ants are eusocial and exhibit wing polyphenism between winged queens and wingless workers. In the manuscript, the authors examine whether this wing polyphenism is caused by programmed cell death (PCD) via autophagy and use ancestral state estimation to test if PCD was likely present in the ancestor of this clade.

In general, I found the manuscript clear and well-written. The authors examined wing disks of an impressive array of ant species, and the immunocytochemistry methods seem appropriate.

However, I have a few issues with the ancestral reconstruction analysis. Importantly, in the methods section for ancestral state estimation, the authors did not include the method used to estimate ancestral state, making it difficult to evaluate these results. In particular, I am unsure how ASE was used to predict PCD at the node between Leptanillinae and Martinlinae if data was unavailable for these groups. Please include (and cite) the method used and clarify the choice to include Leptanillinae and Martinlinae.

In Figure 5, the cladogram requires further explanation. In the methods section, the authors mention that the Romiguier et al. (2022) ant phylogeny was used for their ASE analysis; however, the figure legend cites three alternative sources of phylogenetic relationships within the Formicidae. Additionally, the use of Polistes (paper wasps) as an outgroup seems misleading here, as Polistes are not a sister group to the ants and eusociality originated independently in the social wasps. I suggest redoing this figure, restricting it to only the species analyzed and presenting the trimmed Romiguier phylogeny used in the ASE.

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Declarations

Potential competing interests: No potential competing interests to declare.