Qeios

Peer Review

Review of: "A Developmental Table for the Florida Carpenter Ant *Camponotus floridanus*: Establishing Foundations for Mechanistic Studies of Development and Evolution in Ants"

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The authors present a detailed, image-rich developmental staging table for the Florida carpenter ant *Camponotus floridanus*, describing 17 embryonic stages, four larval instars, and daily pupal progression using a mix of fixed-fluorescent and live time-lapse imaging. Strengths include high temporal resolution time-lapses (5-min intervals across 14 days), combined DIC/confocal imaging, careful morphological descriptions (including gut colour staging and mandibular measurements), and practical staging landmarks intended to enable mechanistic work in eco-evo-devo. The manuscript is thorough descriptively and contains many useful operational details (e.g., embryo fixation, imaging pipelines, and rearing conditions). However, the work leans heavily on descriptive morphology from a small number of colonies/individuals in some critical assays and occasionally asserts interspecific "significant" differences without documenting the statistical procedure or sample sizes.

I enjoyed reading this enriched dataset and the very well-written manuscript.

Critical points:

The paper reports interspecific comparisons (e.g., stages "significantly longer" or "13 times slower than *Drosophila*") but does not present the statistical tests, sample sizes per compared stage, confidence intervals, or p-values in the manuscript—there is no statistical analysis protocol in Materials and Methods tied to these claims.

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Several central assays rely on very limited biological replication: SEM used only two larvae per instar (n = 2 per instar), pupal culturing and some pupal timing measures derive from “a single source colony”, and mandibular-length distributions are reported from larvae collected from a single colony in at least some experiments.

Embryo sampling and live imaging are described (queens from three colonies; embryos collected daily for 14 days), but the manuscript does not give stage-wise N (number of embryos scored per stage), nor inter-replicate variability (SD/SE) for stage durations—preventing assessment of within-species variability and reproducibility.

Homology assertions (e.g., identification of germline “energids,” alignment of *C. floridanus* stages to *Drosophila* and *Monomorium*) are made largely on morphological similarity; while the authors sometimes note that marker confirmation is required, several stronger claims are presented without molecular marker data (e.g., germline markers, segmental gene expression) to substantiate homology.

Time-lapse imaging used “higher than optimal exposure” to reveal internal structures, and images were adjusted in Photoshop (brightness/contrast/levels/sharpness). The manuscript lacks a description of how exposure and post-processing were standardized, controlled, and archived (raw images).

Important experimental confounds (effects of workers or queens on development) are mentioned as “data not shown” (e.g., embryos develop faster without workers), yet no quantitative data, methods, or statistical treatment are provided for these claims—these are precisely the biologically important effects the staging aims to enable and thus require explicit presentation.

The manuscript gives mandibular length ranges per instar but does not describe measurement software, calibration procedures, number of measurers, or inter-observer error.

Several experimental procedures use long-term laboratory colonies (queens collected in 2007; colonies maintained for ~7 years). Long-term lab adaptation can alter developmental timing and symbiont dynamics; the paper does not discuss how long-term domestication may bias developmental durations or morphological features relative to wild populations.

Minor points:

Time-lapse imaging parameters are given (5-minute intervals, Z positions), but the manuscript should explicitly report how many independent embryos were imaged per run and how many independent movies were analyzed for each stage.

Raw datasets (time-lapse movies, measurement spreadsheets, full image stacks) are not referenced for deposition—please deposit and provide accession (movies/measurements essential for reuse).

For the mandibular length histograms (Figure 9), report the exact N per bin, the bin width, and whether any clustering tests (e.g., mixture models, gap statistic) were applied to support four modes.

SEM sample size (2 per instar) is small; note on figure legends that SEM images are illustrative and not quantitative, and increase N for any future morphometric claims.

The authors refer to “significant” differences in stage durations vs Drosophila/*Monomorium—please replace “significant” with precise statistical statements or avoid the adjective until tests are shown.

Larval gut-colour staging is useful, but colour standards/photometric calibration or a colour chart would reduce subjectivity when different labs apply the staging rubric.

The Methods report slightly different rearing temperatures (25°C for routine colonies; 28°C for larval-instar duration assays). Please justify temperature differences and discuss their effect on development.

The paper occasionally uses the spelling “Pharoah” instead of the standard “Pharaoh” (e.g., *Monomorium pharaonis*); please correct for consistency.

For claims regarding *Blochmannia* localization and fluorescence, consider adding a symbiont-specific probe or FISH validation (current description relies on autofluorescence/DAPI ranges and descriptive imaging).

Impressions of results and applicability

The descriptive dataset is **valuable, novel**, and **important**: the detailed morphological staging, gut-colour criteria, mandibular length ranges, and the annotated time-lapse sequence form an excellent operational resource for labs that work on C. floridanus and related ants. If the authors address the outlined quantitative and replication shortcomings (clear sample sizes, statistical analyses, expanded multi-colony replication, and marker validation for key homology claims), the staging table will be widely useful as a foundation for mechanistic work (RNAi, gene expression, epigenetics) in ant eco-evo-devo; in its current form it is an impressive descriptive atlas but limited in the strength of interspecific comparison and generalizability.

Recommendation to editor

Revise prior to acceptance. The manuscript contains **substantial**, useful descriptive material but requires clearer reporting of sample sizes and statistical analyses, expanded replication (or explicit caveats where single-colony data are used), preservation/accession of raw data, and tempering or reinforcement of homology and “significance” claims with marker data or explicit statistical tests.

Declarations

Potential competing interests: No potential competing interests to declare.