

Peer Review Report

1. BASIC REPORTING

- The manuscript is written in clear and professional English. Minor edits could improve clarity, particularly in lines such as 23, 45, and 63.
- The literature review is comprehensive and current, including recent studies (2024–2025). Comparative studies on ecological/geographic distribution of *Apis cerana japonica* could further strengthen the context.
- Figures and tables are of high quality and appropriately labeled. Figures 1 and 2 are especially effective.
- Raw data have been shared via DRA and FigShare, with sufficient metadata provided.

2. EXPERIMENTAL DESIGN

- The research question is clearly defined and relevant, focusing on microbiota changes in overwintering *Apis cerana japonica*.
- Sample collection from four colonies across three time points (BO, OW, AO) ensures systematic representation.
- High-throughput sequencing using MiSeq and analysis via DADA2, SILVA DB, and GLMM are robust and properly described.
- The study design is methodologically sound and ethically appropriate.

3. VALIDITY OF THE FINDINGS

- The dataset is strong in sample size ($n=360$) and depth (coverage $>99\%$).
- Statistical analyses (NMDS, PERMANOVA, GLMM) are well-executed and support conclusions.
- Functional implications of bacterial shifts are plausible but speculative, as no SCFA or gene expression data are presented.
- Conclusions are generally supported by data, with proper limitations discussed.

4. GENERAL COMMENTS

Strengths:

- Novel investigation on *Apis cerana japonica* with ecological and phylogenetic relevance.
- Identification of potential novel species is a significant contribution.

Areas for Improvement:

1. Add functional validation or explicitly state as a limitation.
2. Discuss inter-colony variability more thoroughly.
3. Deepen discussion on ecological factors (e.g., pollen/honey quality) with caution regarding speculative claims.

5. CONFIDENTIAL NOTES TO THE EDITOR

This manuscript presents original and robust data with an appropriate methodological approach. Minor revisions, particularly in the discussion and minor language edits, would further improve the clarity and impact. The work is suitable for publication after minor revision.

Recommendation

 Minor Revision