Short communication

# COMB MORPHOMETRY OF *APIS FLOREA* COLONY DETECTED IN MALTA

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**Abstract**

The red dwarf honey bee *Apis florea* Fabricius 1787, a single open comb constructing honey bee native to Asia, has been expanding its distributional range to Africa and Europe, and also reportedly intercepted in Western Australia. The first fully established colony of *A. florea* in Europe was found in Malta in 2024 near its shipping freeport to the south. The comb was collected, stored, and analysed morphometrically. The following morphometric measurements are reported in this paper: height and width of the comb; surface area of the comb (including crest, worker brood, and drone brood); depth, diameter, and volume of the cells (worker brood, drone brood, and crest); and the angle of the comb against the vertical. The cell measurements were concordant with other cell measurements found in the literature. These data might serve as a reference point for future studies, focusing on comparison of comb morphometry between native and introduced colonies.

Keywords: Apis, *Apis florea*, comb morphometry, honey bees

## INTRODUCTION

The taxonomy of the genus *Apis* Linnaeus, 1758 (Hymenoptera: Apidae) currently has nine recognized species, with three subgenera and an abundance of subspecies. *A. mellifera*, the Western honey bee, is naturally distributed in Europe, Africa, the Middle East, Central Asia and western China (Chen et al., 2016), whereas the other eight species are native to Asia. Based on the worker bees' size, the nine *Apis* species are classified into three subgenera: *Megapis*, the giant honey bees (*A. dorsata* and *A. laboriosa*); *Apis*, the medium-sized honey bees (*A. cerana*, *A. koschevnikovi*, *A. mellifera*, *A. nigrocincta*, *A. nuluensis*); and *Micrapis*, the dwarf honey bees (*A. andreniformis* and *A. florea*). *Apis* are cavity-nesting honey bee species, building nests in dark cavities with multiple combs arranged in parallel (Su et al., 2023), whereas *Megapis* and *Micrapis* are single-comb open-nesting honey bees (Santoso et al., 2018).

The red dwarf honey bee *A. florea* Fabricius, 1787 extends its distribution area across different environments, from tropical and subtropical to arid habitats. Native to Asia, this species is very closely related to and sometimes sympatric with *A. andreniformis* (Hepburn & Radloff, 2011), with which it occupies the basal position of the phylogenetic tree of honey bees. It is considered to be a significant pollinator, especially in dry regions (Ganeshprasad et al., 2022), where it usually outperforms even the cosmopolitan *A. mellifera*. They are small bees (approximately 6 mm long) with a distinctively red abdomen anteriorly and white stripes posteriorly. Although being important ecologically, compared to *A. mellifera* and *A. cerana*, they are not so important economically, as they produce honey in small quantities. Still, due to the limited harvesting capacity, *A. florea* honey is recognised as a high-value delicacy on the market, which reaches a high price (A. Uzunov, personal observation, July, 2023).

*A. florea* is a widespread species, known to occur anywhere between Vietnam to the east and Iran to the west, even extending to the south

of the Himalayas (Uzunov et al., 2025). It has also expanded its reach to the Middle East and Northeast Africa, including such regions as Egypt, Ethiopia, and Jordan (Haddad et al., 2008; Pauly & Zewdu, 2013; Shebl, 2017). Recently, *A. florea* was reported in Australia, where a quarantine area had been declared for the Burrup Peninsula in the Pilbara. In Europe, it was intercepted in harbours in the United Kingdom and Northern Italy, demonstrating the species' exceptional ability for habitat adaptations, with the first ever record of a fully established colony in Europe occurring in the Maltese islands (Uzunov et al., 2024). A fully developed comb, with empty worker and drone brood cells, was covered by approximately 2000 adult bees. The queen was not found, which would indicate that part of the colony absconded with the queen following previous human disturbance (Koeniger & Koeniger, 2024; Uzunov et al., 2025). Since *A. florea* flourishes in warm, arid climates, the Maltese islands offer the perfect opportunity for this honey bee to become a permanent visitor. This might pose challenges to local biodiversity, particularly to *A. mellifera*, including introducing new diseases and competition with native pollinators.

*A. florea* comb is usually constructed encircling a branch in a shady location. They can also be found in bushes, atop tree canopies, and even in rock crevices (Hepburn et al., 2014). Such environmental elements are especially typical for the Maltese islands since the landscape is characterised by traditional rubble walls, which are structures made of limestone rocks without any cementing medium in between. This leaves ample room for *A. florea* to construct their nests.

The honey storage area, called the crest, consists of deep honey cells that lack a midrib (opposite to *A. andreniformis*) and encircle the supporting branch. Immediately below the crest are the cells that store pollen, and below that is the brood area, where cells from the opposite side meet at the midrib. The brood area is the central portion of the comb, mainly comprising smaller worker brood cells, with the larger drone brood cells at the bottom of the comb. At the tip of the lower edge is where queen cells are constructed if under swarming conditions. Emergency queen cells are found adjacent to the brood area. Hepburn et al. (2014) provide insight into the construction and measurements for *A. florea* comb, including the height and width of the entire comb, as well as the depth and diameter of worker cells and queen cells. Overall, the worker cells of *A. florea* were 30-40% smaller than the worker cells of *A. mellifera*.

This study aims to provide a detailed morphometric analysis of the constructed comb of *A. florea* found in Malta. Understanding this ecological aspect of *A. florea* will serve as a reference in case other combs are identified in the non-native areas of this species, and as a comparison with those from its native habitat.

## MATERIALS AND METHODS

The subject of this study was the comb of the fully established *A. florea* colony (Fig. 1) identified by Uzunov et al. (2024) in the region of Birżebbuġa, close to the main freeport. The exterior features (N=6), as well as other (N=3) morphometrical attributes for a detailed description of the comb structure, were studied by total of nine comb parameters (Tab. 1).


Fig. 1. The comb of *A. florea* found in Malta.
Photo credit: one of the authors (M.C.).

To determine the weight, the entire comb together with its supporting branch was weighed with the use of a weighing balance. A branch

Table 1.

List of the comb morphology parameters, measurement methods applied and units used

| | Parameter | Method | Unit | Comment |
|---|---|---|---|---|
| External comb morphometry | Length | Ruler | cm | |
| | Width | Ruler | cm | |
| | Surface area | Ruler | $cm^2$ | |
| | Number of cells | ImageJ software (real count) | Count | |
| | Weight | Weighing scales | g | See below for more details |
| | Angle of crest | Protractor | Degrees | |
| Internal comb morphometry | Cell diameter | Leica (DVM6) software | mm | See below for more details |
| | Cell depth | Needle method (see below) | mm | |
| | Cell volume | Multiplying cell depth by cell cross-sectional area (CSA) | $mm^3$ | CSA was found using the online calculator linked in the Appendices. |

of *Acacia saligna* with a similar thickness and structure was weighed using the same balance, and its weight was subtracted from the combined weight of the comb and branch to obtain an approximate value for the weight of the comb alone. For the measurement of the angle, a vertical line was digitally superimposed onto a photograph of the crest area, and the angle between this reference line and the crest was measured with the use of a protractor. To measure the cell diameter, a Leica DVM6 microscope with its corresponding software was used to obtain accurate readings. The diameter was defined as the distance between two parallel sides of a cell. For the measurement of cell depth, an entomological needle (no. 2) was inserted headfirst into individual cells until it reached the base, and the upper limit of the cell was marked using a fine permanent marker. The length of the portion of the needle inside the cell was then measured with the use of the same software employed for the cell diameter measurements.

## RESULTS AND DISCUSSION

The entire comb, measured from a single side (Fig. 1), had a width of 14.8 cm and a height of 22.5 cm, resulting in a surface area of 185 $cm^2$. Of this, the worker brood surface area measured 157 $cm^2$ (85%), while the drone brood surface area measured 28 $cm^2$ (15%). The worker brood area contained approximately 3,100 cells, whereas the drone brood area contained approximately 190 cells. The crest area contained an estimated 1,500 cells. The comb had a weight of 60 g, and the angle of the comb relative to the vertical, measured from the crest, was 115°.

The mean diameter, depth, and volume were measured for each cell type. Worker cells had a diameter of 3.13±0.12 mm, a depth of 9.16±0.38 mm, and a volume of 77.73±7.13 $mm^3$, while drone cells had a diameter of 4.30±0.08 mm, a depth of 9.60±0.93 mm, and a volume of 153.83±15.58 $mm^3$. Crest cells had a diameter of 3.40±0.36 mm and a depth of 24.13±7.12 mm, with cell volume not applicable in this case (since the crest cells are more irregular and cannot be taken as cylinders).

The different measurements for worker cells, drone cells, and crest cells are shown and compared with the use of appropriate figures. Box plots were drawn to show the variation present between the three types of cells.

As seen in Fig. 2 (A), crest cells had the highest degree of variation in terms of the diameters of the cells, with a higher median and lower and upper quartile than the worker cells. The variation of crest cell measurements makes sense due to the oval shape of the crest, and its orientation around the support branch. Worker cells and drones exhibited relatively low variation, and drone cells clearly have the largest diameter.

Cells of the crest consistently had a much higher depth than those of workers and drones, and

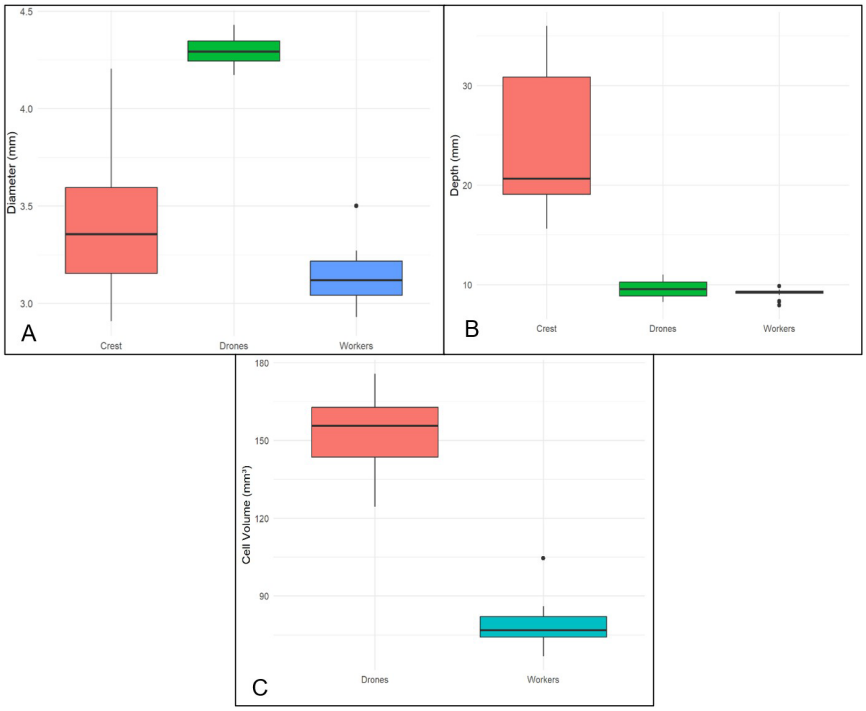


Fig. 2. Boxplots showing the variations of different internal parameters measured: A - for diameters of worker, drone, and honey cells (crest); B - for depths of worker, drone and honey cells (crest); C - for volume for worker and drone cells.

Table 2.

A comparison of measurements between this study and those by Hepburn et al. (2014)

| Parameter | Present study | Hepburn et al. (2014) | Comment |
|---|---|---|---|
| Comb length (cm) | 14.8 | 16.2 | Single measurement |
| Comb height (cm) | 22.5 | 16.9 | |
| Worker cell diameter (mm) | 3.13 ± 0.12 | 2.90 ± 1.50 | Mean value from 30 measured cells (present study) |
| Worker cell depth (mm) | 9.16 ± 0.38 | 9.30 ± 0.70 | Mean value from 30 measured cells (present study) |
| Queen cells depth (cm) | Not available* | 1.41 ± 0.15 | |
| Queen cells diameter (cm) | Not available* | 0.47 ± 0.09 cm | |

*No queen cells were found in the Maltese comb, which is why no comparisons are made above.

they also showed much higher variation (as seen in Fig. 2, B). This is shown by the large interquartile range, from just below 20 mm to just above 30 mm. Drones have a slightly more extensive variation than worker cells, but nowhere close to that exhibited by the crest.

As clearly seen in Fig. 2 (C), drone cells had considerably higher volumes than worker cells, with a higher degree of variation. The ratio of drone cell volume to worker cell volume is found to be approximately 2:1.

Not many data are available in the literature of measurements of a comb of *A. florea* in this level of detail. Therefore, any comparisons made with past studies will not be complete ones. For example, Hepburn et al. (2014) provided a number of measurements for combs of *A. florea*. Comparisons between the measurements given by Hepburn et al. (2014) and those reported here are given in Table 2.

The dimensions of the comb can vary, and probably the comb described by Hepburn et al. (2014) was a more mature one. The cells have more or less the same dimensions between the two studies. No data were found in the literature which provide adequate comparison with the other measurements taken during the present study. That being said, it is good to

keep in mind that although there may be some differences in comb structure or morphometry between the Maltese comb and other native combs, such differences might arise from the different capabilities of that particular colony, and might not necessarily imply the colony's adaptability to a new environment.

## CONCLUSION

This study provides new information about *A. florea* comb morphometry, based on the comb of an established colony found in Malta. These results will be used for further research on comparisons of combs constructed by different colonies, especially comparing combs from native versus introduced colonies. This might give insight about the degree of adaptability of *A. florea* when it comes to comb construction under different climatic and environmental conditions.

## REFERENCES

Chen, C., Liu, Z., Pan, Q., Chen, X., Wang, H., Guo, H., Shi, W. (2016). Genomic analyses reveal demographic history and temperate adaptation of the newly discovered honey bee subspecies *Apis mellifera sinisxinyuan* n. ssp. *Molecular Biology and Evolution, 33*(5), 1337–1348. https://doi.org/10.1093/MOLBEV/MSW017

Ganeshprasad, D. N., Lone, J. K., Jani, K., Shouche, Y. S., Khan, K. A., Sayed, S., Sneharani, A. H. (2022). Gut bacterial flora of open nested Honey bee, *Apis florea*. *Frontiers in Ecology and Evolution, 10*, 837381. https://doi.org/10.3389/FEVO.2022.837381/BIBTEX

Haddad, N., De Miranda, J. R., Bataena, A. (2008). Discovery of *Apis florea* in Aqaba. *Journal of Apicultural Research, 47*(2), 172-173. https://doi.org/10.1080/00218839.2008.11101448

Hepburn, H. R., Pirk, C. W. W., Duangphakdee, O. (2014). Honey bee nests composition, structure, function. *Springer*. https://doi.org/10.1007/978-3-642-54328-9

Hepburn, H. R., & Radloff, S. E. (2011). Biogeography of the dwarf honey bees, *Apis andreniformis* and *Apis florea. Apidologie, 42*(3), 293-300. https://doi.org/10.1007/S13592-011-0024-X/FIGURES/1

Koeniger, N., & Koeniger, G. (2024). *Apis florea* erstmals in Europa - was ist zu tun? *Deutscher Landwirtschaftlicher Verlag, Bienen und Natur, 12*, 36-41.

Pauly, A., & Zewdu, A. H. (2013). Apini and Meliponini from Ethiopia (Hymenoptera: Apoidea: Apidae: Apinae). *Belgian Journal of Entomology, 16,* 1-36.

Santoso, M. A. D., Juliandi, B., Raffiudin, R. (2018). Honey bees species differentiation using geometric morphometric on wing venations. *IOP Conference Series: Earth and Environmental Science*. https://doi.org/10.1088/1755-1315/197/1/012015

Shebl, M. A. (2017). Discovery of *Apis florea* colonies in Northeastern Egypt. *African Entomology, 25*(1), 248-249. https://doi.org/10.4001/003.025.0248

Su, Y. C., Chiu, Y. F., Warrit, N., Otis, G. W., Smith, D. R. (2023). Phylogeography and species delimitation of the Asian cavity-nesting honey bees. *Insect Systematics and Diversity, 7*(4). https://doi.org/10.1093/ISD/IXAD015

Uzunov, A., Galea, T., Chen, C., Cilia, G., Costa, C., Mifsud, D. (2024). *Apis florea* in Europe: first report of the dwarf honey bee in Malta. *Journal of Apicultural Research, 63*(5), 1122-1125. https://doi.org/10.1080/00218839.2024.2386888

Uzunov, A., Koeniger, G., Scheiner, R., Galea, T., Koeniger, N. (2025). *Apis florea* in Malta! A short visit or a permanent stay? *Bee World*, 1-6. https://doi.org/10.1080/0005772X.2025.2499756

**Appendices**

Link to online calculator used to calculate cell cross-sectional area:
https://www.gigacalculator.com/calculators/hexagon-calculator.php