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NOTES AND COMMENTS

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Apis florea in Europe: first report of the dwarf honey bee in Malta

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ABSTRACTS

The Red dwarf honey bee (*Apis florea*), is a single-comb open-nesting member of the genus *Apis* with a natural distribution area stretching from the Indomalayan realm on the east to the Persian Gulf on the west. However, it is reportedly colonising new territories mainly due to anthropogenic activities. Nowadays it can be found from Taiwan on the east, to Jordan, the Arabian Peninsula and North-eastern Africa on the west. Here we present the first scientific record of a fully established colony of *A. florea* in Europe. The colony was found on Malta, a crossway of naval routes in the Mediterranean Sea. We documented the incident with photos, collected samples of workers and drones and sequenced the mtDNA COI gene to confirm assignment to *A. florea*. Also, we alert the competent authorities and the beekeeping community to be vigilant and ready to undertake effective eradication measures. In the paper, we discuss the risks and the potential consequences associated with the invasion of *A. florea* in Europe.

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Described by Fabricius (1787), the Red dwarf honey bee (*Apis florea*), is a single-comb open-nesting member of the genus *Apis* (Linnaeus, 1758). The species' adaptability is well documented and reflected in its native distribution area which covers different climatic environments and habitats. Its range stretches from the Indomalayan realm on the east to the Persian Gulf on the west (Ruttner, 1988). *A. florea* is reported colonising new territories (such as the Middle East and eastern Africa) mainly due to anthropogenic activities such as naval commerce and new dispersal corridors resulting from man-made habitats (Silva et al., 2020).

Lord and Nagi (1987) were the first to report *A. florea* in Africa when a population was detected in Khartoum (Sudan). Currently, this honey bee has extended its distribution range both on the east, where it was recorded on the island of Taiwan and the west, having colonised Jordan, the Arabian Peninsula and North-eastern Africa (Haddad et al., 2008; Hepburn & Radloff, 2011; Shebl, 2017; Silva et al., 2020) and it is expected further extend its distribution in the African continent (Abou-Shaara et al., 2021, Dietemann et al., 2009, Silva et al., 2020). Moreover, further colonisation towards the northwest *via* Asia Minor and subsequently into Europe seems possible due to its establishment in Jordan (Haddad et al., 2008) and the absence of major geographical and environmental barriers in the region. Another dispersion event could take place, through the intensive trade across the Mediterranean basin, between North Africa, the Middle East and southern Europe, the latter, characterised by mild winters, a potentially suitable environment for the survival of *A. florea*. In addition, the spread of this honey bee into new territories is further enhanced by its prolific reproductive capacity and absconding behaviour (Hepburn, 2011; Lindauer, 1957; Tirgari, 1971).

The present communication constitutes the first scientific record and description of a fully established colony (Figure 1) of *A. florea* in Europe. This colony was found on Malta, the main island of the Maltese archipelago in the Mediterranean Sea. Following an online report on social media, the location (*Apis florea*, Figure 2) was inspected, and the colony was identified and photographed. The colony found was well developed, with a typical single comb encircling a branch (branch diameter 12 mm), and containing both worker and drone cells. The comb was connected to two branches. The length of the comb (perpendicular to the earth's surface) is 220 mm, the height is 150 mm and the width of the honey crest is 90 mm. The queen was not found and the colony seemed to be in an after-swarm state without brood and food stores. However, no new or old-built queen

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Figure 1. Left: *A. florea* colony hanging on a tree branch of *Acacia saligna*, the Blue-leaved Acacia (photo source: Antoine Borġ Bonaċi), central: *A. florea* worker and drone bees (arrows), right: *A. florea* comb (lower right part of the comb was damaged).

Figure 2. Map of Malta, the main island of the Maltese archipelago, with the location where *A. florea* colony was found, the main Harbour and the Freeport.

cells were observed. The colony size was estimated by counting over 2000 adult bees. No other swarms were observed in the near vicinity.

Samples of worker and drone bees were collected and stored in sample tubes containing 99% ethanol for genetic analyses. Consequently, the colony, alongside the section of the branch was removed and sealed in a box with all adult bees being culled using 99% ethanol. The comb was appropriately stored, so that it would be protected from wax moths. As soon as this colony was discovered and positively identified as *A. florea*, it was immediately removed and the competent authorities were informed. The immediate removal of the colony was a must due to the risk of swarming or absconding. The empty comb was visually inspected and photographed. Thereafter, the comb with dead specimens of *A. florea* was donated to the Biology Department at the University of Malta.

The sampled worker and drone bees were used for DNA barcoding, amplifying the *cytochrome c oxidase subunit I* (COI) region of *mt*DNA (Folmer et al., 1994). Briefly, thoraxes were dissected and pooled from 3 workers and 3 drones. DNA from the two pools were extracted using a commercial kit Quick DNA Microprep Plus Kit (Zymo Research, Irvine, CA, USA) as reported in Tiritelli et al. (2024). Amplification of COI region was performed using HotStarTaq Polymerase (Qiagen, Hilden, Germany) and the PCR assay was performed on Applied Biosystems®2720 Thermal Cycler (ThermoFisher Scientific) (see Selis et al., 2024). The obtained amplicons were visualised on a 1.5% agarose gel, purified using ExoSAP-IT Express (ThermoFisher Scientific, Waltham, MA, USA) and sequenced with Sanger methodology through SeqStu-dio™ (ThermoFisher Scientific) (Cilia et al., 2022).

The obtained sequences (631 bp) confirmed that the samples belong to *A. florea*, and BLAST analysis showed high similarity (97.62%) to *A. florea* (GenBank Accession Numbers: *A. florea* workers PP905460, *A. florea* drones PP905461).

The survival of *A. florea* colonies under the climatic and environmental conditions present in the Mediterranean basin is a probable scenario. Therefore, if the identified colony has already swarmed, or if there are other unidentified colonies, it is likely that *A. florea* will become established in the Maltese Islands. The lack of food and brood and the relatively small population (ca. 2000 workers and drones) on a well-developed comb having both worker and drone cells indicate the probability of an after-swarm status. Nevertheless, queen cells were not observed. For comparison, Seeley et al. (1982) reported more than 6000 workers as an average colony size for *A. florea* in Thailand.

The establishment of *A. florea* in Malta would represent a serious risk to natural biodiversity due to competition for resources and habitats, in a territory that harbours numerous endemic species and where anthropogenic pressures are already high. This is also the case for other invasive species (*Megachile sculpturalis, M. disjuntiformis, Xylocopa pubescens* and *X. aestunas*) reaching the Mediterranean countries (Bortolotti et al., 2018; Catania, 2023; Flaminio et al., 2023; Ruzzier et al., 2020). Furthermore, it should be noted that *A. florea* is a host of many harmful pathogens including Black Queen Cell Virus, possibly Thai Sacbrood Virus, the parasitic mites *Euvarroa sinhai* and *Tropilealaps clareae*, the common non-parasitic facultative kleptophage *Neocypholaelaps indica* and an unknown microsporidian (Delfinado-Baker et al., 1989; Fries, 2011; Needham et al., 2001; Warrit & Lekprayoon, 2011). As some of these pathogens are known to switch hosts, the introduction of *A. florea* into new areas can thus pose a real threat to the health of Western honey bees (*Apis mellifera*) and wild bees. Such invasion incident, combined with climate change and diminishing forage grounds, is expected to increase the constraints on the endemic *A. m. ruttneri* (Sheppard et al., 1997, Zammit-Mangion et al., 2017) population in Malta, which according to Uzunov et al. (2023) represents only a small percentage of the current *A. mellifera* colonies present in Malta.

The location where the colony of *A. florea* was found is about 2.5 km in a direct line from the Birżebbuġa Freeport (Figure 2) where most cargo is handled. Interestingly, in the commercial port of Genova, Italy, in September 2022, a swarm of *A. florea* was intercepted (and immediately destroyed) on board of a cargo ship (https://agronotizie.imagelinenetwork.com/agricoltura-economia-politica/2022/09/16/apis-florea-chi-e-l-ape-asiatica-trovata-a-genova/77048). Past *A. florea* introductions in the Middle East and Africa together with the insularity of the Maltese islands indicate that most likely the swarm entered through naval traffic. This type of transportation represents a possible route for the introduction of alien invasive species, such as the Dwarf honey bee into Europe. The present communication aims to alert the competent authorities and the beekeeping community to be vigilant and further monitor the potential presence of *A. florea* colonies in the Maltese Islands and Mediterranean coastal areas and to be prepared with effective eradication measures.

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Disclosure statement

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Data availability statement

Sequences were submitted to Genbank under the accession numbers PP905460 (*A. florea* workers) and PP905461 (*A. florea* drones).

References

Abou-Shaara, H. F., Mahfouz, H. M., & Owayss, A. A. (2021). Species distribution modeling of potential invasion of dwarf honey bee, *Apis florea* Fab., to Africa and Europe after occurrence in Egypt in view of climatic changes. *Journal of Plant Protection and Pathology*, *12*(9), 609–614. https://doi.org/10.21608/jppp.2021.206735

Bortolotti, L., Luthi, F., Flaminio, S., Bogo, G., & Sgolastra, F. (2018). First record of the Asiatic bee *Megachile disjunctiformis* in Europe. *Bull. Insect*, *71*, 143–149.

Catania, R. (2023). The recent spread of the carpenter bee *Xylocopa pubescens* (Hymenoptera, Apidae) in Europe, and first record for the aegean archipelago. *Sociobiology*, *70*(2), e8678. https://doi.org/10.13102/sociobiology.v70i2.8678

Cilia, G., Flaminio, S., & Quaranta, M. (2022). A novel and non-invasive method for DNA extraction from dry bee specimens. *Scientific Reports*, *12*(1), 11679. https://doi.org/10.1038/s41598-022-15595-8

Delfinado-Baker, M., Baker, E. W., & Phoon, A. C. G. (1989). Mites (Acari) associated with bees (Apidae) in Asia, with description of a new species. *American Bee Journal*, *129*, 609–613.

Dietemann, V., Pirk, C. W. W., & Crewe, R. M. (2009). Is there a need for conservation of honeybees in Africa? *Apidologie*, *40*(3), 285–295. https://doi.org/10.1051/apido/2009013

Fabricius, J. C. (1787). Mantissa insectorum. Hafniae, Proft [in Latin, cited from Maa 1953].

Flaminio, S., Bortolotti, L., & Cilia, G. (2023). First report of *Xylocopa aestuans* in Italy: A new species for Europe? *Bulletin of Insectology*, *76*, 161–166.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*(5), 294–299. PMID: 7881515

Fries, I. (2011). Diseases of Asian honeybees. In H.R. Hepburn, & S.E. Radloff (Eds.), *Honeybees of Asia* (pp. 69–93). Springer Verlag.

Haddad, N., de Miranda, J. R., & Bataehna, A. (2008). Discovery of *Apis florea* in Aqaba Jordan. *Journal of Apicultural Research*, *47*, 173–174.

Hepburn, H. R. (2011). Absconding, migration and swarming. In H.R. Hepburn, S.E. Radloff (Eds.). *Honeybees of Asia* (pp. 69–93). Springer Verlag.

Hepburn, H. R., & Radloff, S. E. (2011). Biogeography. In H.R. Hepburn, & S.E. Radloff (Eds.), *Honeybees of Asia* (pp. 69–93). Springer Verlag.

Lindauer, M. (1957). Communication among the honeybees and stingless bees in India. *Bee World*, *38*(1), 3–14. 34-71 https://doi.org/10.1080/0005772X.1957.11094970

Lord, W. G., & Nagi, S. K. (1987). Apis florea discovered in Africa. *Bee World*, *68*(1), 39–40. https://doi.org/10.1080/0005772X.1987.11098907

Needham, G. R., Gerson, U., & Sammataro, D. (2001). Mite biology. In T.C. Webster, & K.S. Delaplane (Eds.), *Mites of the honey bee* (pp 1–16). Dadant.

Ribas-Marquès, E., & Díaz-Calafat, J. (2021). The Asian giant resin bee Megachile sculpturalis Smith 1853 (Hymenoptera: Apoidea: Megachilidae), a new exotic species for the bee fauna of Mallorca (Balearic Islands, Spain). *Journal of Apicultural Research*, *60*(3), 506–511. https://doi.org/10.1080/00218839.2021.1874177

Ruttner, F. (1988). *Biogeography and taxonomy of honeybees*. Springer-Verlag.

Ruzzier, E., Menchetti, M., Bortolotti, L., Selis, M., Monterastelli, E., & Forbicioni, L. (2020). Updated distribution of the invasive *Megachile sculpturalis* (Hymenoptera: Megachilidae) in Italy and its first record on a Mediterranean island. *Biodiversity Data Journal*, *8*, e57783. https://doi.org/10.3897/BDJ.8.e57783

Seeley, T. D., Seeley, R. H., & Akratanakul, P. (1982). Colony defense strategies of the honeybees in Thailand. *Ecological Monographs*, *52*(1), 43–63. https://doi.org/10.2307/2937344

Selis, M., Cilia, G., Wood, T. J., & Soon, V. (2024). Taxonomic revision of the *Stenodynerus fastidiosissimus* species-group in Western Europe and North Africa (Hymenoptera: Vespidae: Eumeninae), February 2024. *Zootaxa*, *5418*(1), 34–56. https://doi.org/10.11646/zootaxa.5418.1.2

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

Sheppard, W. S., Arias, M. C., Grech, A., & Meixner, M. D. (1997). *Apis mellifera ruttneri*, a new honey bee subspecies from Malta. *Apidologie*, *28*(5), 287–293. https://doi.org/10.1051/apido:19970505

Silva, D. P., Castro, A. C. F., Vilela, B., Ong, X. R., Thomas, J. C., Alqarni, A. S., Engel, M. S., & Ascher, J. S. (2020). Colonizing the east and the west: Distribution and niche properties of a dwarf Asian honey bee invading Africa, the Middle East, the Malay Peninsula, and Taiwan. *Apidologie*, *51*(1), 75–87. https://doi.org/10.1007/s13592-019-00711-x

Tirgari, S. (1971). On the biology and manipulation of Apis (Micrapis) florea F. in Iran. Proceedings of 23rd international beekeepers Congress, pp. 330–332.

Tiritelli, R., Flaminio, S., Zavatta, L., Ranalli, R., Giovanetti, M., Grasso, D. A., Leonardi, S., Bonforte, M., Boni, C. B., Cargnus, E., Catania, R., Coppola, F., Di Santo, M., Pusceddu, M., Quaranta, M., Bortolotti, L., Nanetti, A., & Cilia, G. (2024). Ecological and social factors influence interspecific pathogens occurrence among bees. *Scientific Reports*, *14*(1), 5136. https://doi.org/10.1038/s41598-024-55718-x

Uzunov, A., Mifsud, D., Galea, T., Cutajar, S., Zammit-Mangion, M., & Meixner, M. D. (2023). Development, behaviour, productivity, and health status of the native honey bee Apis mellifera ruttneri vs. the introduced A. m. ligustica in Malta. *Apidologie*, *54*(4), 34. https://doi.org/10.1007/s13592-023-01008-w

Warrit, N., & Lekprayoon, C. (2011). Asian honeybee mites. In H.R. Hepburn, & S.E. Radloff (Edrs.), *Honeybees of Asia* (p. 69–93). Springer Verlag.

Zammit-Mangion, M., Meixner, M., Mifsud, D., Sammut, S., & Camilleri, L. (2017). Thorough morphological and genetic evidence confirm the existence of the endemic honey bee of the Maltese Islands *Apis mellifera ruttneri*: Recommendations for conservation. *Journal of Apicultural Research*, *56*(5), 514–522. https://doi.org/10.1080/00218839.2017.1371522