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1 Background

- ❑ **The Small Hive Beetle** *Aethina tumida* a parasite of the *Apis mellifera* causes major damage to honeybee colonies, particularly when weakened
- ❑ the last few decades, *tumida* spread to many countries around the world and, where it has been observed, eradication is often difficult, if not impossible.
- ❑ Infestation with *A. tumida* listed by the World Organisation for Animal Health (WOAH) and regulated in the European Union (EU)



2 Context

- ❑ **July 2022** the presence of the Small Hive Beetle confirmed for the first time in a French department located in the Indian Ocean, **Reunion Island**
- ❑ this area of the Indian Ocean, species is known to be endemic in Madagascar and has been reported Mauritius since
- ❑ **Molecular characterisation** represents key approach for identifying the origin of the introduction of invasive species.

3 Which method?

- ❑ **Sequencing** mitochondrial cytochrome oxidase subunit I gene is a widely used tool for identifying insects. The sequences obtained are compared with those available in reference databases (e.g. BOLD genebank)
- ❑ **Genomic DNA** from an adult and a larva collected in two separate outbreaks was extracted using the QIAamp® DNA Mini

- ❑ To amplify the **COI gene**, PCR assays were performed eight different oligo primers: SHB1, SHB2, SHB3, SHB4 (Evans 2000), AT3014 and A1859 (Howland and Hewitt, 1995) and LCO1490, HCO2198 (Folmer, 1994)
- ❑ Amplicons were sequenced with the same primers, in both directions, using a SeqStudio Analyser

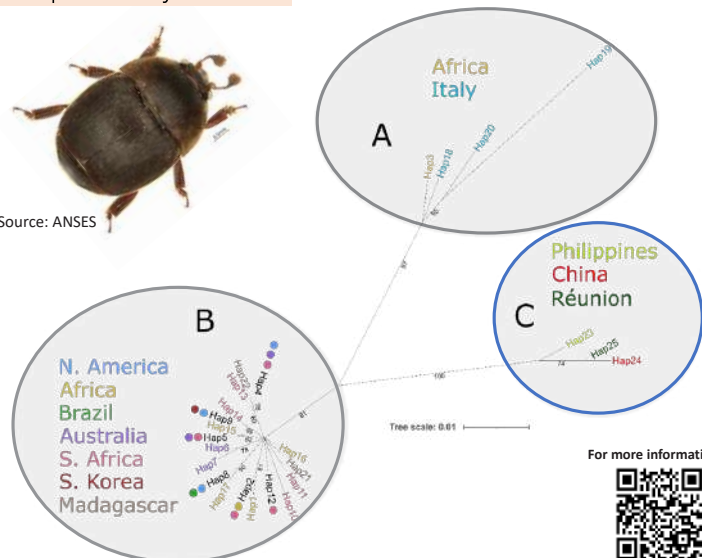
- ❑ sequences were aligned with MUSCLE and the alignment was trimmed with trimAl resulting in 1,003 sites.
- ❑ maximum likelihood phylogenetic analysis was performed with RAXML v0.9.0 using the HKY+I substitution model which was selected by Modeltest v0.1.6.
- ❑ Node support was calculated from 600 bootstrap

4 Results

- ❑ The two sequences (758 bp) were deposited in GenBank databases under accessions OQ547271 OQ547272
- ❑ COI sequences from the two specimens belonged to a same new haplotype: **Hap25 defined in this study**.
- ❑ Phylogenetic analysis revealed that this haplotype is closely related to *tumida* specimens previously found in **Philippines (Hap23) and China (Hap24)**
- ❑ **new haplotype Hap25** is assigned to the **clade C**, one of the 3 clades previously described (Del Cont et al., 2021).



Source: ANSES



5 To conclude

- ❑ epidemiological investigations provided substantial evidence
- ❑ Several hypotheses remain equally introduction exchanges
- ❑ presence *tumida* Mauritius continent, coming directly linked African recent
- ❑ sequencing currently available region, analysing specimens Mauritius essential

finding highlights the complexity of *tumida* global spread and the importance of further genetic studies to support biosecurity strategies and prevent future invasions.

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In July 2022, an outbreak of Small Hive Beetle (*Aethina tumida*) was reported for the first time in a French department in the Indian Ocean, Réunion Island. *Aethina tumida* is a parasite of the *Apis mellifera* that causes significant damage to honeybee colonies, particularly when weakened. Over the past few decades, it has spread to many countries worldwide, and eradication is often difficult, if not impossible, once introduced. In this area of the Indian Ocean, it is endemic to Madagascar and has been reported in Mauritius since 2016, posing a serious threat to neighbouring territories, including Réunion Island. Tracing the origin and pathway of introduction remains challenging. Alongside epidemiological investigations, molecular characterisation of specimens is a valuable tool for identifying the source of the invasion. Sequencing the cytochrome oxidase I (COI) gene is a widely used method for identifying insects. The sequences are compared with those available in the databases (BOLD, GenBank, etc.). Phylogenetic analyses of *A. tumida* specimens from various countries have identified three distinct clades, with 24 different haplotypes observed to date. In this study, beetles collected from two separate outbreaks were initially identified morphologically. Molecular analyses were then performed on two specimens (an adult and a larva) from these outbreaks. Genomic DNA was extracted using QIAamp® DNA Mini Kit, and PCR amplification was performed using eight oligonucleotide primers. The amplified fragments were sequenced using a SeqStudio Analyser, followed by phylogenetic analysis. The phylogenetic results revealed that both specimens belonged to a newly defined haplotype, H25, which is part of clade C. This suggests an introduction from either an unidentified African source or the Asian continent, since this haplotype closely resembles those previously identified in China and the Philippines. Due to the lack of genetic data on *A. tumida* in Mauritius, an introduction from this territory cannot be excluded. This finding highlights the complexity of *A. tumida*'s global spread and the need for further genetic studies. Understanding invasion pathways is crucial for improving biosecurity measures and preventing future introductions of this parasite, which is listed as a notifiable disease by the World Organisation for Animal Health (WOAH).