



Updating the saga of the small hive beetle (*Aethina tumida*): molecular inference of the origin of the South American invasion

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In the last three decades, the small hive beetle *Aethina tumida* Murray 1867 (Coleoptera: Nitidulidae) has drawn attention worldwide after a series of successful invasions into contrastingly different habitats (Lounsberry et al. 2010). The rapid pace at which *A. tumida* is expanding its range has mobilized intensive efforts to understand the source of the invasions, the impact on the local fauna, and how to mitigate this beetle's damage to wild and managed honey bees (Mutinelli et al. 2014). Information about the genetic makeup of an invasive founding population is critical to understand its chances to succeed in a new environment (Lawson Handley et al. 2011). Determining the magnitude and the number of introductions is an important step in assessing the risk to the local fauna as well as the likelihood of admixture of different genetic pools from successive invasions (Andersen et al. 2004). Collection of this type of data is also important in determining gaps in the regulatory control of cross-border movement of goods and products.

Aethina tumida is native to Africa (Neumann and Elzen 2004), where it is considered a minor pest, causing severe damage mainly to weak and stressed bee colonies (Neumann and Ellis 2008). Even though adults can be found in large numbers in honey bee colonies, they have little direct impact. The larvae, on the other

hand, may cause serious problems during their development, with heavy damage to the combs (Neumann and Elzen 2004). These beetles can exploit alternative food sources such as fruits (Ellis et al. 2002), which can be an additional concern in tropical regions in terms of management or eradication efforts.

The first introduction of *A. tumida* was reported in the USA in 1998; the putative route of invasion was through South Carolina and subsequently spreading to Georgia and Florida (Hood 2000). Shortly after this introduction, *A. tumida* had been reported in 29 states by 2003 (Neumann and Elzen 2004). DNA analysis of mitochondrial COI gene fragments indicated the presence in the USA of two haplotypes, which closely resembled haplotypes in South African populations (Evans et al. 2000, 2003). Subsequently, invasions were reported in Australia (Gillespie et al. 2003), southern Italy (Granato et al. 2017), Mexico and Central America (Neumann et al. 2016), and South America (Al Toufailia et al. 2017). Here, we report the molecular identification of the putative origin of the South American invasion by the small hive beetle. Molecular genetics analyses can help us to understand the timing and magnitude of the original introduction of *A. tumida* into Brazil, as well as serving as a basis for studies on how this alien species adapts to a novel environment, the conditions of its founding, and the chances of the parasite succeeding in following generations.

Specimens of *A. tumida* were collected at the first reported site of infestation in Brazil (22° 42' 50.14" S, 47° 37' 33.80" W; Al Toufailia et al. 2017; WAHID interface OIE: <http://www.oie.int/wahis/public.ph>), from hives of Africanized honey bees in 2018. The fresh samples were

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first identified morphologically upon collection, transferred to tubes containing 95% EtOH and stored at -20°C . Fourteen beetles were used for DNA extraction. The total genomic DNA was extracted using a modified CTAB method (Doyle and Doyle 1987). The PCRs were performed using specific primers for cytochrome oxidase I (COI) gene fragments of *A. tumida* forward primer 5'-GGTGGATCTTCAGTTGATTAGC-3' and reverse primer 5'-TCAGCTGGGGGATAAAATTG-3' (Evans et al. 2000) (Supplementary online material S1). Sanger sequencing was conducted in the Animal Biotechnology Laboratory at the University of São Paulo.

In addition to the sequences from beetles sampled in Brazil, 77 *A. tumida* COI sequences were retrieved from NCBI GenBank database and incorporated in the analysis (Supplementary online material S2). Sequences

were edited to 721 bp and aligned with sequences from GenBank database using the ClustalW algorithm in MEGA X (Kumar et al. 2018). The sequences obtained in Brazil were deposited in NCBI GenBank database with the accession numbers (MK286579–MK286592). The software DnaSP 5.10 (Librado and Rozas 2009) was used to define the number of haplotypes that were used in subsequent analyses.

Previous work has demonstrated the presence of two divergent lineages in *A. tumida* populations (Granato et al. 2017). We performed a phylogenetic analysis including haplotypes of both lineages and an outgroup, *Triacanus* sp. (NCBI No. KC966655). Bayesian analyses were conducted in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), using the GTR+G+I mutation model. The analyses were performed in three runs for

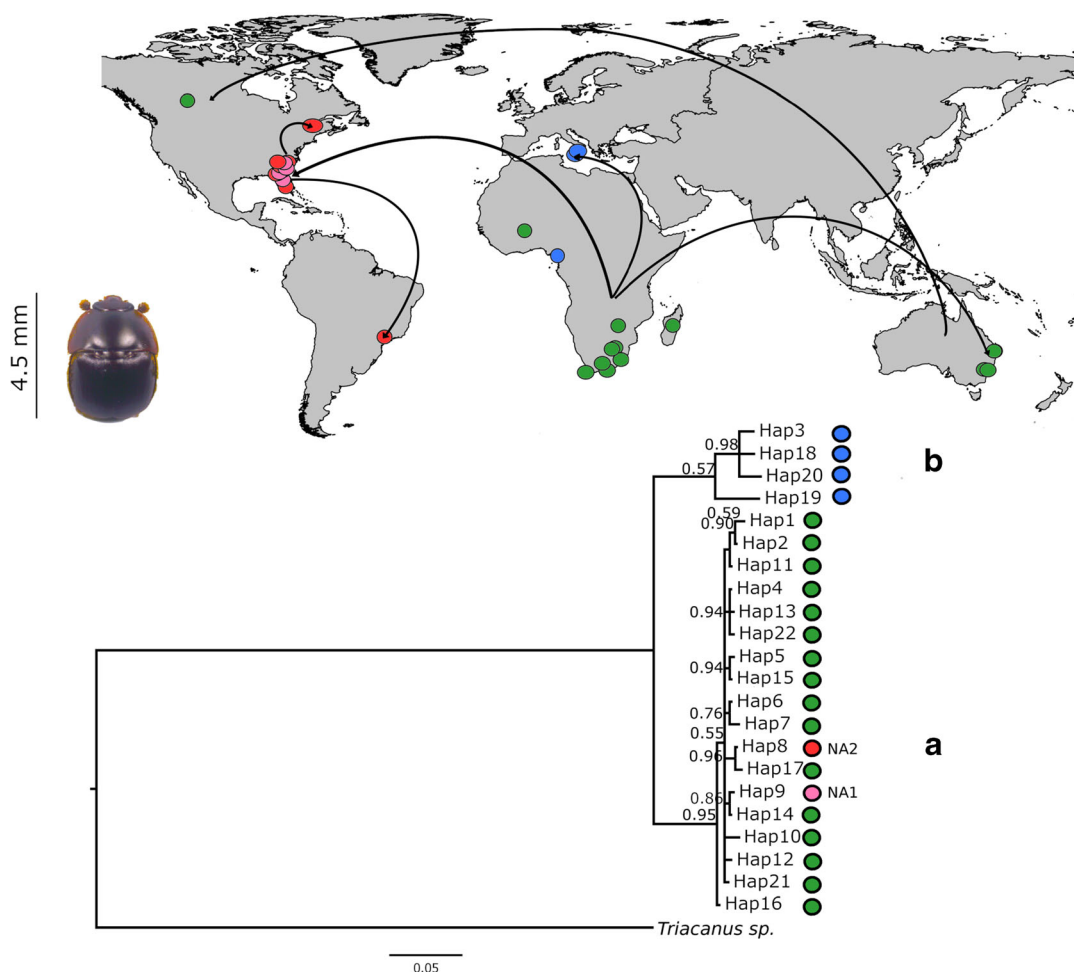


Figure 1. Global geographic distribution and Bayesian phylogenetic relation among COI haplotypes of *Aethina tumida* (Coleoptera: Nitidulidae).

2×10^6 generations, with five chains each and three replications. Trees were sampled every 1000 generations with 25% burn-in. We have also inferred the evolutionary relation by a maximum likelihood method on MEGA X (Kumar et al. 2018) using the general time-reversible model (GTR+G+I) allowing it to run for 10,000 replications.

Characterization of the COI fragment confirmed the presence of *A. tumida* in Brazil via DNA barcoding diagnosis. Sequences from the 14 individuals indicated the presence of a single COI haplotype in Brazil. Haplotype H8 is identical to one of the two haplotypes present in the USA (NA2) (Evans et al. 2000). At present, 22 different haplotypes have been described worldwide (Figure 1 and Supplementary online material S2), of which two have been found on more than one continent (Figure 1). Both Bayesian and maximum likelihood trees converged to the same tree topology even though branch size and node support differed (Figure 1 and Supplementary online material S3). The most widespread is H4, which has been reported in Australia, South Africa, and Canada (Alberta), followed by H8 which is present in the USA and Canada (Québec) and now in Brazil. Haplotypes H4 and H8 are very similar to haplotypes present in southern Africa, separated only by a few mutational steps. Phylogenetic analysis confirmed the presence of two lineages (A and B) as previously reported by Granato et al. (2017) (Figure 1). The haplotype found in Brazil belongs to group A, which contains the most widely spread haplotypes and has been found on two continents besides Africa.

Specimens of *A. tumida* found in Brazil have the same COI haplotype (H8 = NA2) as the beetles that are present in North America (Evans et al. 2003). Even though it is not conclusive that the source of the introduction was the USA or Canada, the wide genetic diversity and high gene flow observed in southern Africa make it unlikely that Africa was the source of the introduction into Brazil. The presence of two widely dispersed haplotypes in North America (H4 and H8) and the identification of one of these haplotypes in Brazil suggest that a single or a few invasion events of *A. tumida* originating from the same North American region occurred in Brazil. In North America, populations in Georgia and Florida have strong biases toward H8, and may be strong candidates as the source of the invasion into Brazil (Evans et al. 2003).

The low haplotype number of North American populations of *A. tumida* does not allow a definitive determination of the exact region of the pest invasion in Brazil. After the initial report of *A. tumida* in Piracicaba, São Paulo State, subsequent investigations rapidly reported *A. tumida* in honey bee hives in other

cities in São Paulo and Rio de Janeiro, raising the question of how widespread *A. tumida* has become in Brazil. In conclusion, we found strong evidence of a North American origin of the populations of *A. tumida* in Brazil, probably originating from one or a few invasion events. Further investigations are necessary to understand the population dynamics, as well as to guide immediate control strategies and long-term integrated management of *A. tumida* in this country. The possibility that *A. tumida* might parasitize native stingless bee colonies, as reported for *Melipona beecheii* in Cuba by Peña et al. (2014), could have an enormous negative impact on Brazilian biodiversity and must be thoroughly investigated.

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AUTHORS' CONTRIBUTION

ASC, DAA, and EMGC conceived and designed the study. EMGC and PLS collected and analyzed the data. ASC provided reagents and analytical tools. ASC, DAA, and EMGC wrote the manuscript. All authors read, corrected, and approved the manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no conflict of interest.

Actualisation de la saga du petit coléoptère des ruches (*Aethina tumida*): déduction moléculaire sur les origines de l'invasion sud américaine

Les espèces envahissantes / Abeilles africanisées / Brésil / Gène COI / Petit coléoptère de la ruche

Update der Geschichte des kleinen Beutenkäfers (*Aethina tumida*): Molekulare Rückschlüsse auf den Ursprung der südamerikanischen Invasion

Invasive Art / Afrikanisierte Honigbienen / Brasilien / COI Gen / Kleiner Beutenkäfer

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