Rapid Communication

The establishment of a trap-jaw ant, *Odontomachus ruginodis*, in Hawai‘i

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Abstract

Here we report the establishment in Hawai‘i of a trap-jaw ant, *Odontomachus ruginodis*, a species well known for its potent sting. In June 2017, a farmer on the island of Kaua‘i reported “bites” from large ants and supplied a specimen to the U.S. Fish and Wildlife Service for identification. Additional specimens were found in 2019 and 2020 in multiple locations up to 5 kilometres away from the initial detection. Multiple morphological identifications were made and combined with CO1 genetic assessment confirmed the species to be *Odontomachus ruginodis*, which had not been recorded prior from Hawai‘i. We argue that greater biosecurity protocols are needed in Hawai‘i to prevent further incursions, especially the introduction of the red imported fire ant, *Solenopsis invicta*.

Key words: biosecurity, exotic, hymenoptera, incursion, invasion, sting

Introduction

In June 2017, a farmer on the Hawaiian island of Kaua‘i reported “bites” from large ants in his wetland taro field near the town of Hanalei. The farmer and his farmhands had encountered this ant approximately seven to eight times over the course of a year while tending and harvesting the taro, most commonly after flood events. As part of the report, the farmer collected a single reproductive ant (WGS84 22.210333°N; 159.4738°W) and submitted it to U.S. Fish and Wildlife biologists for identification. The specimen was preliminarily identified as being a species of *Odontomachus*, and notably no species of *Odontomachus* were known to be present in Hawai‘i (Krushelnycky et al. 2005). Workers were subsequently collected at the same location over the next several months. Additional specimens were collected at three nearby locales: ~ 2 km north in Princeville (22.228092°N; 159.486403°W) in March 2019, ~ 2 km west in Hanalei (22.200581°N; 159.499294°W) in June 2020, and ~ 5 km southeast in Kalihiwai Bay Shoreline (22.220117°N; 159.425061°W) in August 2020. Clearly, the species had established a viable population on the island. Here, we formally report the establishment and identification of the new *Odontomachus* in Hawai‘i.

Materials and methods

The identification of the species was conducted multiple times morphologically and using molecular diagnostics. In August and December 2017, a queen and some workers were sent to Fred Larabee (Postdoctoral Fellow, Smithsonian Institution) for species-level morphological identification. In July 2019, another specimen was morphologically assessed by BH by comparing it with specimens within the CSIRO Darwin ant collection, coupled with the online identification sites AntWeb (www.antweb.org) and AntWiki (www.antwiki.org). In 2021, another specimen was photographed and then analyzed genetically. The imaging was conducted using a Leica DMC5400 camera mounted on a Leica M205C dissecting microscope. Montages were compiled using the Leica Application suite v. 4.13 and stacked in Zerene stacker.

DNA extraction (from foreleg) was carried out through the Barcode of Life Data (BOLD) System following a modified Proteinase K membrane-based method (for extraction details, see http://ccdb.ca/resources). Briefly, extracted DNA was eluted in 30–60 µL of prewarm water and between 1–5 µL of eluted DNA template used for PCR amplification using the primers LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) targeting approximately 700 bp from the mitochondrial DNA cytochrome oxidase subunit I (mtCOI) 5’ end partial gene region (Folmer et al. 1994). PCR amplicon of the specimen was assigned a unique identification code that combines the batch within which it was processed, its number within the batch and the year of sequencing (e.g. OZBOL7325-22 for the *O. ruginodis* Smith, 1937 specimen) prior to being Sanger sequenced.

DNA sequence was provided by BOLD system as a fasta format text file and the quality of base-calling via the BOLD system automated artificial processing pipeline therefore could not be verified. The sequence was checked for premature stop codons, intra- and inter-specific amino acid conservation patterns, and unexpected nucleotide insertions/deletions (INDELs) in the partial gene sequence. Due to the unavailability of the .ABI trace file, we therefore processed the fasta format text file through nucleotide alignment with published sequences from GenBank to identify INDEL mutations, and potential miscalling of nucleotide bases from the BOLD system artificial processing pipeline through protein gene assessment using the invertebrate mitochondrial genetic code.

We assessed the nucleotide identity of the query partial mtCOI gene through a Blast search against the GenBank’s non-redundant DNA database. The top 100 best matched nucleotides from GenBank were downloaded and imported into Geneious Prime (version 2022.2.2; BioMatters Ltd., Auckland, New Zealand) for alignment using MAFFT Alignment (v7.49) with default parameters (i.e., Algorithm: Auto; Scoring matrix: 200PAM / K = 2; Gap Open Penalty: 1.53; Offset value: 0.123). Sequences were trimmed at both

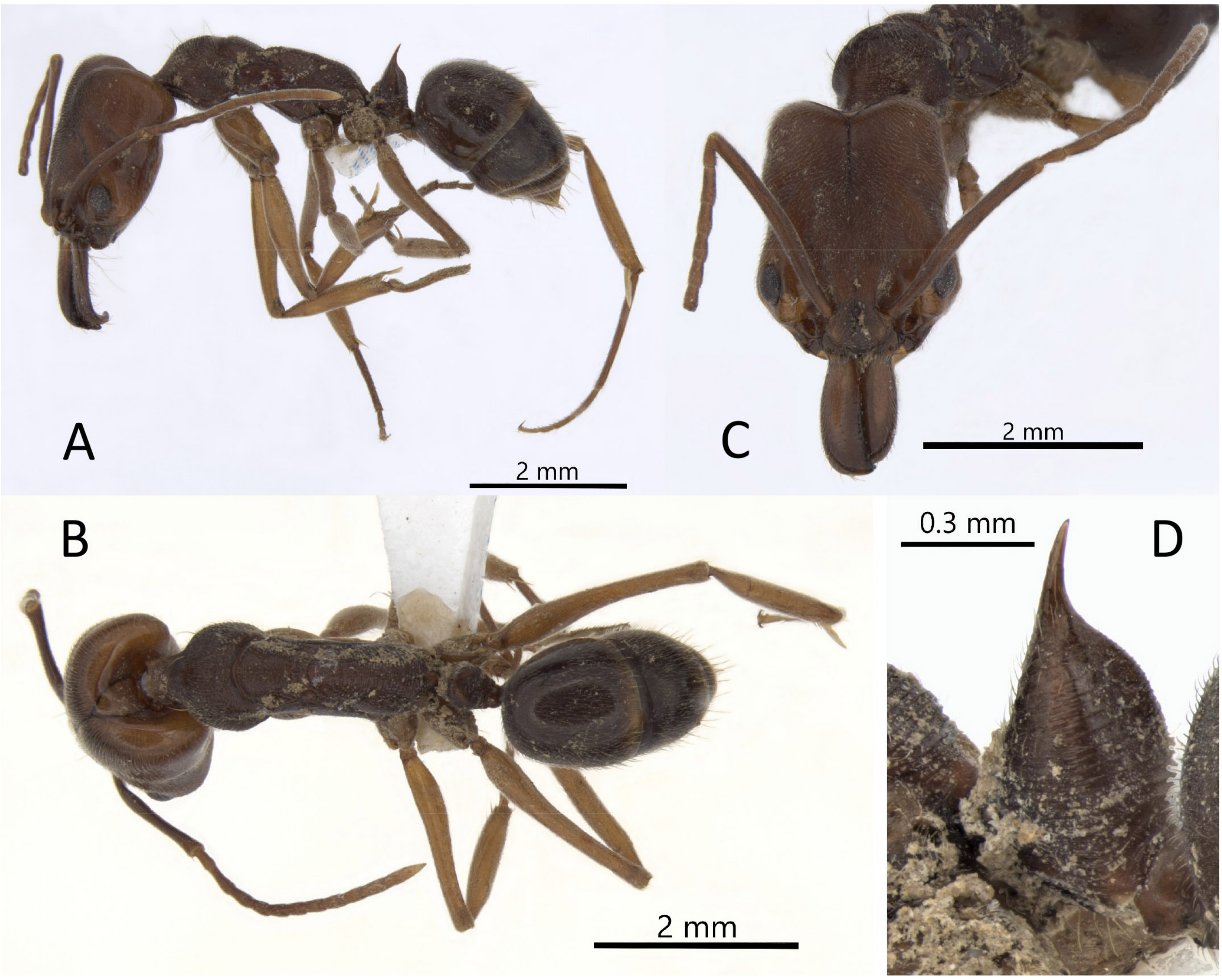
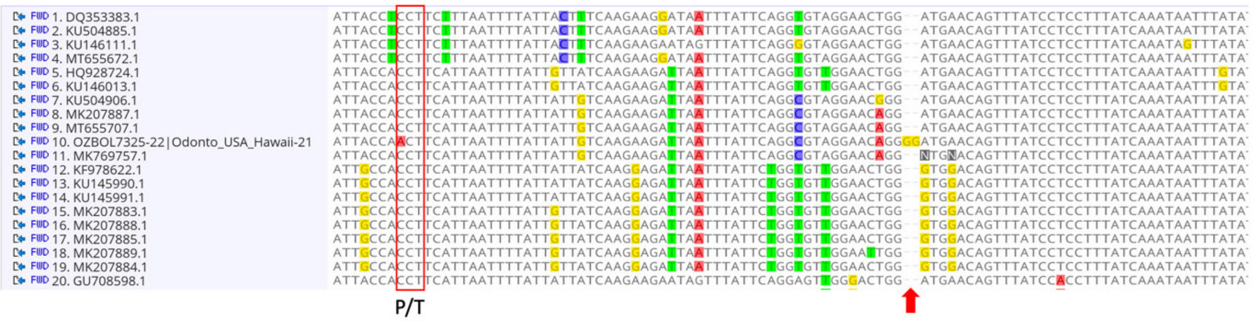


Figure 1. Photographs of the lateral (A), dorsal (B) and frontal (C) views, as well as the posterior-lateral view of the petiole (D) of the *Odontomachus* specimen used for genetic assessment. Photos by Magen Pettit

5’ and 3’ ends to 646 bp, and short sequences, sequences with ambiguous base pairs, and potential INDELs due to significant post-alignment profiles were removed.

Results

Fred Larabee tentatively identified the first specimen collected as *O. ruginodis* Smith, 1937 but did not provide details of the morphological features that were definitive for the species identification, so they are not further elaborated here. The subsequent specimen identified at CSIRO was also identified as *O. ruginodis* based on the following morphological traits including and extending those detailed in Deyrup et al. (1985) (Figure 1): Mandibles with three denticles; Frontal area with fine longitudinal striae, sides of head smooth and shiny; Scape length barely reaching the occipital border; Pronotum with numerous erect setae; Striae over the entire mesosoma except the kapisternum; Head and mesosoma reddish brown to piceous; Petiole conspicuously transversely striate laterally and posteriorly; All surfaces of all gastric tergites with numerous erect setae and all dorsal tergites with semi-



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FWD 1. DQ353383.1                           ATTACCTCCTTCTTTAATTTTATTACTTTCAAGAAGGATAATTTATTCAGGTGTAGGAACTGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 2. KU504885.1                           ATTACCTCCTTCTTTAATTTTATTACTTTCAAGAAGGATAATTTATTCAGGTGTAGGAACTGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 3. KU146111.1                           ATTACCTCCTTCTTTAATTTTATTACTTTCAAGAAGAATAGTTTATTCAGGGGTAGGAACTGG--ATGAACAGTTTATCCTCCTTTATCAAATAGTTTATA
FWD 4. MT655672.1                           ATTACCTCCTTCTTTAATTTTATTACTTTCAAGAAGGATAATTTATTCAGGTGTAGGAACTGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 5. HQ928724.1                           ATTACCACCTTCATTAATTTTATTGTTATCAAGAAGATTAATTTATTCAGGTGTTGGAACTGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTGTA
FWD 6. KU146013.1                           ATTACCACCTTCATTAATTTTATTGTTATCAAGAAGATTAATTTATTCAGGTGTTGGAACTGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTGTA
FWD 7. KU504906.1                           ATTACCACCTTCATTAATTTTATTATTGTCAAGAAGATTAATTTATTCAGGCGTAGGAACGGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 8. MK207887.1                           ATTACCACCTTCATTAATTTTATTATTGTCAAGAAGATTAATTTATTCAGGCGTAGGAACAGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 9. MT655707.1                           ATTACCACCTTCATTAATTTTATTATTGTCAAGAAGATTAATTTATTCAGGCGTAGGAACAGG--ATGAACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 10. OZBOL7325-22|Odonto_USA_Hawaii-21   ATTACCAACTTCATTAATTTTATTATTGTCAAGAAGATTAATTTATTCAGGCGTAGGAACAGGGGATGAACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 11. MK769757.1                          ATTACCACCTTCATTAATTTTATTATTGTCAAGAAGATTAATTTATTCAGGCGTAGGAACAGG--NTGNACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 12. KF978622.1                          ATTGCCACCTTCATTAATTTTATTATTATCAAGGAGATTAATTTATTCTGGTGTTGGAACTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 13. KU145990.1                          ATTGCCACCTTCATTAATTTTATTATTATCAAGGAGATTAATTTATTCTGGTGTTGGAACTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 14. KU145991.1                          ATTGCCACCTTCATTAATTTTATTATTATCAAGGAGATTAATTTATTCTGGTGTTGGAACTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 15. MK207883.1                          ATTGCCACCTTCATTAATTTTATTGTTATCAAGGAGATTAATTTATTCTGGTGTTGGAACTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 16. MK207888.1                          ATTGCCACCTTCATTAATTTTATTGTTATCAAGGAGATTAATTTATTCTGGTGTTGGAACTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 17. MK207885.1                          ATTGCCACCTTCATTAATTTTATTGTTATCAAGGAGATTAATTTATTCTGGTGTTGGAACTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 18. MK207889.1                          ATTGCCACCTTCATTAATTTTATTGTTATCAAGGAGATTAATTTATTCTGGTGTTGGAATTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 19. MK207884.1                          ATTGCCACCTTCATTAATTTTATTGTTATCAAGGAGATTAATTTATTCTGGTGTTGGAACTGG--GTGGACAGTTTATCCTCCTTTATCAAATAATTTATA
FWD 20. GU708598.1                          ATTACCACCTTCATTAATTTTATTATTATCAAGAAGAATAGTTTATTCAGGAGTTGGGACTGG--ATGAACAGTTTATCCACCTTTATCAAATAATTTATA
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Figure 2. Sequence alignment using MAFF Align showing our specimen's partial mtCOI (sequence 10) against a 20 of the 100 GenBank downloaded sequences. Nucleotide shaded in colours are SNPs detected between sequences. Gaps inserted by the MAFF Align program are shown as "--". The red box shows the codon encoding the Proline (P) amino acid (detected in all 73 aligned sequences) and the suspected base pair miscalling in the Hawaiian sample that resulted in a Threonine (T) due to the unexpected substitution from "C" to "A". Note also that sequence MK769757 contains two unknown bases (N) in this initial alignment and was removed from the final dataset. For cleaning of the Hawaiian sequence we removed the two "G" nucleotide insertions (red arrow), and we hypothesised the "A" substitution was a mis-call and should have been a "C" instead (i.e., such that it is translated into Proline instead of Threonine).

erect pubescence. Note that no single feature is definitive for this species, but in combination these features, as well as size, separate this species from other *Odontomachus*.

Through sequence alignment against the GenBank sequence database, our specimen's partial mtCOI gene sequence was found to have a 2 bp "GG" insertion (i.e., at nucleotide positions 325 and 326 when aligned against MT655707.1), resulting in 4 "G"s' being called (Figure 2). Furthermore, a nucleotide substitution was also detected in the fasta format text file that was provided by the BOLD System that resulted in an unexpected amino acid change from Proline (P; present in all 100 sequences downloaded from GenBank; representing different *Odontomachus* species groups; Fernandes et al. 2021) to Threonine (T) (Figure 2). We edited the Hawaiian *Odontomachus* sequence by removing the two extra "G" bases, and by hypothesising that the Threonine amino acid (from the codon ACT) should be a Proline (encoded by the codons CCT/CCC/CCA/CCG). Correction of the "ACT" codon (from Threonine) to one that encoded a Proline amino acid would lead to a 100% nucleotide identity with the Honduras *Odontomachus ruginodis* specimen (MT655707.1) reported by Fernandes et al. (2021) assuming it was the codon "CCT". Alternatively, our specimen's sequence would share 99.7–99.8% nucleotide identity with MT655707.1 if the hypothesised Proline amino acid involved either the codons CCC, CCA, or CCG. Both alternatives indicate that the species in Hawai‘i is *Odontomachus ruginodis*.

Discussion

Our morphological and genetic assessments identify the *Odontomachus* now present in Hawai‘i to be *O. ruginodis*. But even with this determination, we recognise that there has been much past confusion between *O. ruginodis* and *O. brunneus* (Patton, 1894) (reviewed in Wetterer 2020), and additionally it is quite possible that one or both species might represent additional cryptic species. Regardless, this is the first *Odontomachus* species to establish

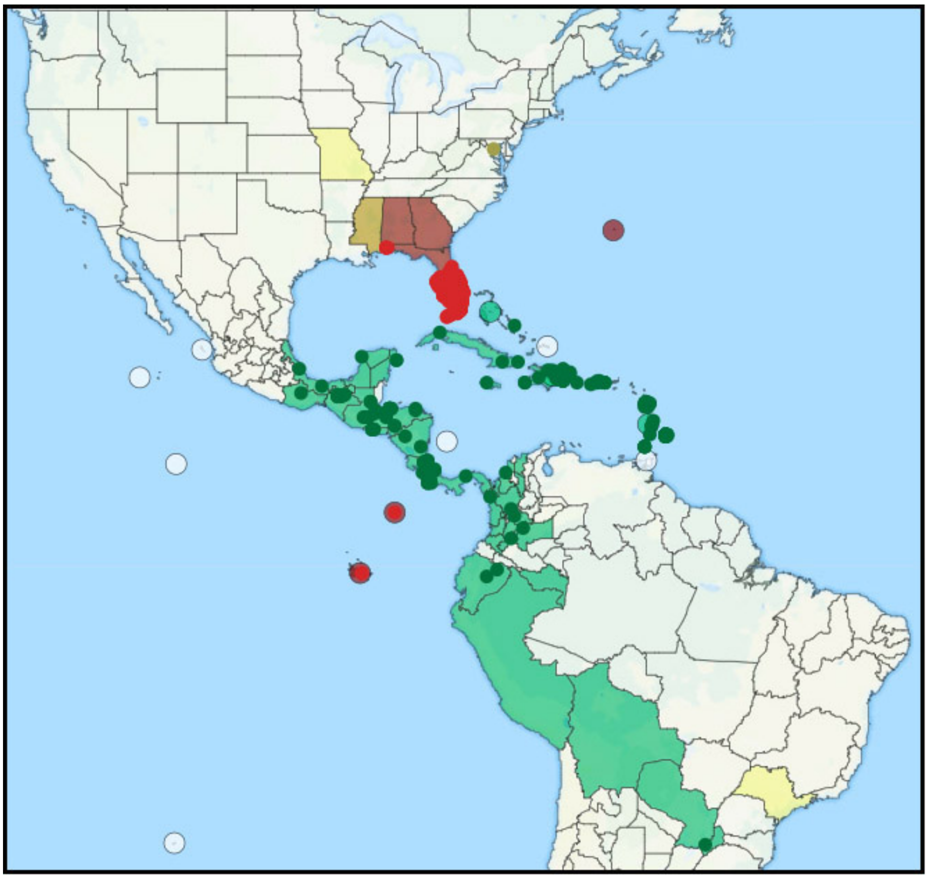

Figure 3. Native (green) and exotic (all other colours) ranges of *O. ruginodis*. Sourced with permission from AntMaps www.antmaps.org

in Hawaiʻi, and as such any potential future identification changes would not change Hawaiʻi's incursion statistics.

The native range of *O. ruginodis* appears to be the Caribbean and central America to NW South America (Figure 3). Populations in North America are most likely exotic (Wetterer 2020), as are populations in distant islands including Bermuda and the Galapagos. Another record of an alate male from New Caledonia (AntWeb 2022), if correct, is the only other known record outside of the immediate American region. Therefore, this incursion into Hawaiʻi is a significant dispersal event for this species.

The potential impacts of *O. ruginodis* within Hawaiʻi are unclear. No impacts have ever been quantified for this species in any exotic location. Instead, any such influences, such as interference with native ant species, have just been speculated (e.g. Deyrup and Carrel 2012). Indeed, we are unaware of any impacts being reported for any *Odontomachus* species in an exotic location. However, given that *Odontomachus* species are renowned for having a powerful sting, it is likely that humans and other animals will be subject to stinging events by *O. ruginodis*. Already there have been at least two reports of people being stung within buildings, one being a school. Notably, the taro fields are incorporated into the Hanalei National Wildlife Refuge because of the endangered native waterbirds that forage and nest in and around the fields, and so it is anticipated that this stinging ant species will negatively affect these ground-nesting birds and their chicks, as occurs when other invasive ant species interact with ground active or nesting birds (Drees 1994; Plentovich et al. 2009, 2018).

Our genetic analysis also serves to highlight issues for species identification that may also affect this work and the work of others. Molecular diagnostics based on the partial mtCOI gene (Hebert et al. 2003) has provided greater confidence in species confirmation for over two decades. This technique has also demonstrated the presence of genetically distinct species groups, including in *Odontomachus* (Fernandes et al. 2021). Our specimen, based on the edited partial mtCOI gene, appears most likely to belong to *O. ruginodis*, and potentially matching a sequence from a Honduras sample (Fernandes et al. 2021). But that species determination is also dependent upon the specimens in BOLD having a correct species designation, and not being conflicted with the *O. ruginodis* vs *O. brunneus* misidentification issue. A similar mis-identification issue can occur from the use of partial vs whole mitochondrial genome. Additional and informative markers can be missed in partial mtCOI gene analyses which can greatly impact interpreting genetic information in the context of biological invasions, including species identification (Tay et al. 2022).

Our study also highlighted a need to analyse the original .ABI trace sequence file to confirm nucleotide identity and to avoid the over-reliance on an artificial sequence processing pipeline, especially for Sanger sequencing out files. There is a need to avoid accepting nucleotide identity at face value without confirmation of integrity. Pseudogenes and NUMTs-associated sequences are widespread in public databases (Vyskočilová et al. 2018; Kunz et al. 2019) and can complicate molecular diagnostics (Tay et al. 2016), especially for identifying the geographic origins of an exotic species. Further molecular analyses of the Hawaiian *Odontomachus* population will be needed to ascertain population origins if there is an attempt to trace the incursion pathway.

Despite the clear scientific knowledge of the significant economic, social, and environmental issues of invasive ant species, as well as the ability of ants to be unintentionally transported within imported goods, new ant species continue to arrive and establish within Hawai‘i. We argue that greater biosecurity protocols are needed to prevent further incursions, especially to prevent the introduction of red imported fire ant, *Solenopsis invicta* Buren, 1972, and other highly invasive ant species.

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Authors’ contribution

All authors contributed data and knowledge and drafted the manuscript.

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