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Is the spread of *Wasmannia auropunctata* (Hymenoptera, Formicidae) through the western Palearctic inevitable? First evidence of presence in the Canary Islands

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Abstract

Wasmannia auropunctata, also known as the 'little fire ant' or 'electric ant', is an invasive species that represents a serious threat to biodiversity and public health globally due to its intrinsic characteristics and low detectability. Here, through a combination of morphological and molecular identification, the first record of this species in the oceanic archipelago of the Canary Islands is presented. Molecular data reveal that the population from the Canary Islands is completely different from another population established in Málaga (mainland Spain), which suggests that the western Palearctic has been colonised from at least two independent events. The early detection of this species in a very limited area provides an important temporal window for its eradication and the prevention of its dispersal to other areas.

Keywords

Barcoding, early detection, invasive ant species, little fire ant, oceanic islands

Introduction

Invasive alien species (IAS) are species introduced outside of their natural distribution range that have negative impacts on native communities. IAS are among the greatest global threats to biodiversity, but also to human economy and health (e.g., Reaser et al. 2007; Early et al. 2016; Paini et al. 2016). Social insects, especially ants, are among the worst invaders worldwide (Bertelsmeier 2021; Gruber et al. 2021; Angulo et al. 2022; Tercel et al. 2023). Impacts driven by invasive ants include the reduction of abundance and richness of animal species in undisturbed natural systems (Tercel et al. 2023 and references therein); alteration of ecosystem functions such as scavenging and nutrient cycling (e.g. Angulo et al. 2011; Holway and Cameron 2021; Turner et al. 2021); negative effects on plant-animal mutualisms (e.g., Davis et al. 2010; Fagua and Ackerman 2011; Hansen and Müller 2009; Sinu et al. 2017); effects on human health (Rosselli and Wetterer 2017; Lopez et al. 2024), and high economic costs to mitigate their effects (Gruber et al. 2021; Angulo et al. 2022). A meta-analysis conducted by Angulo et al. (2022), comprising more than 1,300 cases of 12 invasive ant species in 27 countries, since 1930, estimated the economic costs of US$ 51.93 billion to mitigate their impact.

Due to their small size, their generalist nesting habits and frequent association with disturbance environments or habitat, ants are often accidentally transported by humans in goods, mainly together with live plants, wood, soil, litter, etc. (Suarez et al. 2010; Fournier et al. 2019; Suhr et al. 2019; Bertelsmeier 2021). Biological invasions are becoming more frequent due to the recent increase in human trade and tourism, which has resulted in more than 200 species of ants introduced worldwide (Suarez et al. 2010). The early detection and rapid response to the invasive species is considered a first principle of effective and cost-efficient strategies to address invasive species (Rease 2020), followed by subsequent local eradication to prevent range expansion. The faster the response is carried out, the more probabilities for a population to be eradicated (Lodge et al. 2006). In general, the larger the population, area, and coverage, the more limited the possible response options are (Simberloff 2003). Thus, the window of opportunity for potential successful eradication is narrow (Martinez et al. 2020). A key aspect to highlight is that post-invasion management costs greatly exceed those dedicated for pre-invasion management measures, being approximately twenty times higher (Angulo et al. 2022). Early detection and rapid response have allowed local eradications of ant populations worldwide, such as *Solenopsis invicta* in New Zealand and specific populations in Australia (Christian 2009; Wylie et al. 2016), *Pheidole megacephala* (Fabricius, 1793) in Australia (Hoffmann et al. 2010; Hoffmann 2011), *Solenopsis geminata* (Fabricius, 1804) in Australia and Hawaii (Hoffmann et al. 2011), or *Wasmannia auropunctata* (Roger, 1863) in Australia, the island of Marchena (Galápagos) and specific populations in Hawaii (Causton et al. 2005; Vanderwoude et al. 2010). In terms of area, the largest successful ant eradication was that of *S. invicta* that covered 8,300 ha in the port and airport of Brisbane, Australia (Wylie et al. 2016). However, the mean area eradicated is much smaller 68 ha, with more than 60 % of the reported cases being conducted in less than 2 ha (Hoffman et al. 2016).

Commonly named as the 'little fire ant' or 'electric ant', *Wasmannia auropunctata* is one of the worst invasive ant species worldwide (Angulo et al. 2022) being included in the IUCN Global Invasive Species Database (GISD) (ISSG 2015), as well as in the list of invasive alien species of concern to the European Union (Commission Implementing Regulation (EU) 2022/1203 of July 12, 2022). Several impacts have been reported associated with *W. auropunctata* such as displacing native ant species, reducing invertebrate populations, harming wild and domestic vertebrates and even humans (Montgomery et al. 2022 and references therein). *Wasmannia auropunctata* is recognised as an extremely serious socioeconomic threat (Gruber et al. 2021), being the second ant species with the highest economic cost reported worldwide (US$ 7.35 billion; Angulo et al. 2022). Several characteristics contribute to its invasive success in new environments: generalist diet; ability to establish in a variety of habitat types; supercolonial social structure; polygyny; clonal reproduction in invasive populations; and, association with human-modified habitats (Wetterer 2013; Montgomery et al. 2022). This species is native to South and Central America, but due to human activity, its distribution has expanded to at least 31 countries and islands worldwide, mainly in tropical and subtropical areas (Wetterer 2013; Montgomery et al. 2022; Hsu et al. 2022; Demetriou et al. 2022; Blight et al. 2023). In the recent years, *W. auropunctata* has spread through the western Palearctic region, being found both indoor in Germany (Geiter et al. 2002; exotic indoor), and the Netherlands (Boer and Vierbergen 2008; several findings during port inspections but without establishment), as well as outdoors Israel (Vonshak et al. 2010), mainland Spain (Espadaler et al. 2018), Cyprus (Demetriou et al. 2022), and France (Blight et al. 2023). The Mediterranean basin is so far the only area where there are outdoor records of the species in temperate climate.

Here, we provide the first record of *Wasmannia auropunctata*, for the Canary Islands, as well as discuss the problematic of the species based on the available information.

Material and methods

The Canary Islands are a volcanic archipelago of seven islands located in the eastern-mid Atlantic Ocean between coordinates 27°37'N and 29°25'N and 13°20'W and 18°10'W, 96 km off the coast of Morocco. Together with four other archipelagos (Azores, Madeira, Savage Islands and Cape Verde), they comprise the biogeographic region of Macaronesia. These archipelagos contribute significantly to the number of unique species in the biodiversity hotspot of the Mediterranean basin (Myers et al. 2000). The Canary archipelago is the most prominent with 40.3% of its fauna and 22.4% of its flora being endemic (Gobierno de Canarias 2024), highlighting the importance of this region in the biogeographical area of the Mediterranean basin. To date, the myrmecofauna of the Canary Islands is represented by 61 ant species and subspecies, of which 19 are endemic, 16 are native, and 26 are exotic to the archipelago (Gobierno de Canarias 2024; Suppl. material 1: table S1). In order to know the biodiversity and distribution of exotic and native ant species, since 2016, more than 1,300 sampling location have been sampled recording of which almost 69% are anthropogenic environments, areas that are more susceptible to the arrival of exotic species (Hernández-Teixidor et al 2020; Pérez-Delgado unpublished data).

In August 2023, in collaboration with a study on the diet (identification of arthropods in stomach contents) of the introduced lizard *Anolis porcatus* Gray, 1840 on a golf course in the south of island of Tenerife, some ant fragments (head and mesosoma) were detected that did not fit with any of the species reported for the Canary Islands (Antweb images http://www.antweb.org; Pérez-Delgado personal collection reference material). Four visits were made (August and September, 2023; August and November, 2024) to the gardens surrounding the golf course and other gardens within the townships. Individuals were collected for morphological identification and subsequent verification by DNA barcoding.

Morphological identification

Individuals were examined under a Zeiss Stemi 2000 stereomicroscope. Identification at species-level was conducted using taxonomic keys (Longino and Fernández 2007) and Antweb images (http://www.antweb.org). Microscopic images of the habitus were taken using a Canon EOS-750D camera and stacked with Zerene Stacker (Zerene Systems LLC. version 1.04).

Molecular identification

In order to confirm the identification, DNA barcoding was carried out at the Genomic Diversity Service of the Instituto de Productos Naturales y Agrobiología (Spanish National Research Council). DNA was extracted from two specimens with the Mag-Bind Blood & Tissue DNA HDQ 96 kit (Omega Bio-Tek GA, USA) using a KingFisher Flex (Thermofisher), and DNA concentrations were measured using Tecan Infinite 200 Pro (Configuration: Infinite M Nano+). The 5' region (658 bp) of the mtDNA COI gene was amplified using FoldF and FoldR primers (Yu et al. 2012). PCR reaction conditions were as follows: initial denaturation at 95 °C for 4 min, followed by 40 cycles of 94 °C for 45 s, 47 °C for 45 s, and 72 °C for 90 s, and a final extension of 72 °C for 10 min. A volume of 2 μL of diluted DNA extract was amplified with 18 μL of PCR mix (for a total volume of 20 μL), comprised of 12.7 μL of water, 2 μL of 10× NH_4 buffer, 1.2 μL of 50 mM $MgCl_2$, 0.4 μL of 10 mM dNTPs, 0.4 μL of BSA (20 mg/ml), 0.6 μL of each primer (10 μM), and 0.1 μL of Taq polymerase (BIOTAQ). PCR products were sequenced using the Sanger DNA sequencing service of Macrogen (www.macrogen.com). Sequences were then edited in Geneious 2021.1.1 (www.geneious.com). Molecular identification was assessed using the Barcode of Life Data Systems (BOLD Systems) identification engine. A dataset was assembled including our sequenced barcode and a set of barcode sequences including all available haplotypes and countries. A Maximum Likelihood phylogenetic tree was built using RAxML 8.2.11 (Stamatakis 2014).

Results

The first specimens of *W. auropunctata* were detected in the stomach contents of *Anolis porcatus* from the interior of a golf course and in the surrounding gardens (28°3.750'N, 16°43.235'W). Subsequent visits have been made to the surrounding gardens, where areas with a high abundance of workers but only two nests were found. Although it should be noted that it was not possible to sample inside the golf course. Five other ant species were detected during these sampling (Suppl. material 1: table S1), but the only one near the *W. auropunctata* nests was *Brachymyrmex cordemoyi* Forel, 1895.

Morphological identification

Individuals were identified as *Wasmannia auropunctata* unequivocally, which is distinguished by several distinctive features of workers: small ant (1–2 mm total length), monomorphic, pronounced frontal carinae that extend to the back of the head and form the prominent antennal scrobes, the antennae have 11 segmented and a 2-segment apical club; large propodeal spines; and the convex mesosoma which lacks a mesonotal suture (Fig. 1).

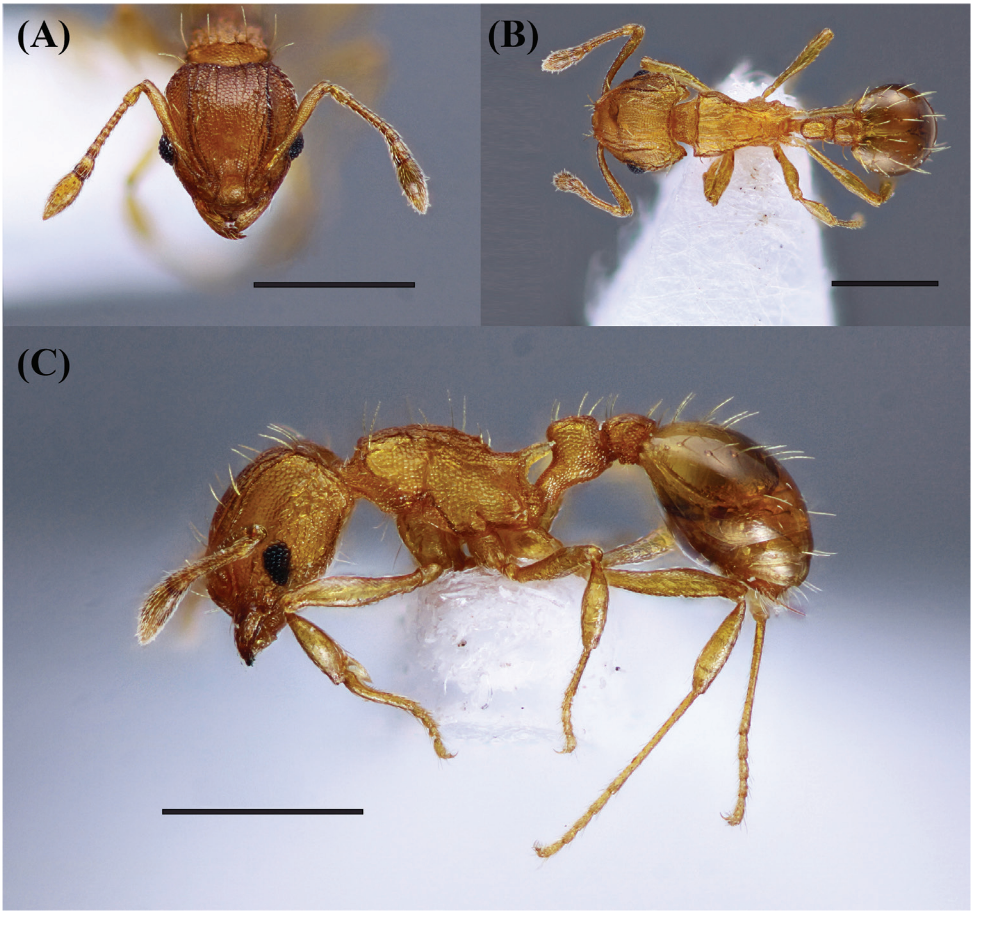

Figure 1. *Wasmannia auropunctata* worker, head (**A**), dorsal (**B**), and lateral (**C**) views. Scale bars: 0.5 mm.

Molecular identification

Two 619-bp sequences with no ambiguities were obtained (GenBank accession numbers PQ629112–PQ629113). Sequences were 99.5% identical to sequences identified as *W. auropunctata* from Ecuador (PREEC1745-22 and FROGB105-16). Sequences were clustered into a group comprising sequences from Ecuador and Peru. There was just an available sequence from the western Palearctic, being this sequence generated from a sample from Málaga (Spain; ANTEU4632-22). This sequence was 100 % identical to a sequence from Argentina (ANTI521-16) and was placed within a group comprising sequences from Argentina and Gabon. Mainland Spain and Canary Islands sequences displayed a similarity of 91.3% (Fig. 2).

Discussion

Genetic and morphological analyses have confirmed the presence of *Wasmannia auropunctata* in Tenerife, thus expanding its known distribution in the western Palaearctic. Globalisation and the massive movement of goods have meant that goods are not always subject to effective customs inspections (Perrings et al. 2010). This has allowed the rapid range expansion through the transport of ant-infested agricultural, horticultural and construction materials (Suarez et al. 2010; Suhr et al. 2019; Bertelsmeier 2021). This species has experienced an increase in its distribution worldwide, with 53.8 % of records at countries and islands levels occurring in the last 25 years (Wetterer 2013; Demetriou et al. 2022; Hsu et al. 2022; Montgomery et al. 2022; Blight et al. 2023). The first outdoor report for this species in a temperate climate area was in 2009 (Vonshak et al. 2010), as previous records in this area had occurred in greenhouses. Genetic results show that, at least, two independent colonisations have occurred in the western Palearctic. This is supported by the high genetic divergences between Tenerife and Málaga populations (8.7 %), and the fact that they were not placed within the same phylogenetic group (Fig. 2). This high divergence substantially exceeds what is typically considered to be found within species (Ratnasingham and Hebert 2013), suggestive of either cryptic or undetected species within what has been described as *W. auropunctata*. We also highlight the lack of genetic information of this species from introduced areas, especially in the western Palaearctic, as of the six already known established populations, there is only available barcode data for one population (Málaga, mainland Spain). This genetic information would be crucial to manage the expansion of *W. auropunctata* as it would allow knowing the origin of each of the populations and focus efforts on the potential entry routes of the species.

Although all regions of the planet are suffering impacts derived from biological invasions, these impacts in insular ecosystems can be exacerbated due to the vulnerability of island ecosystems to invasive species (Reaser et al. 2007; Bellard et al. 2017). About 43 % of the ant species reported for the Canary Islands are considered exotic (Schifani et al. 2018; Staab 2019; Hernández-Teixidor et al. 2020), and seven of them are recorded in the IUCN list of invasive species (ISSG 2015; Suppl. material 1: table S1). Those seven

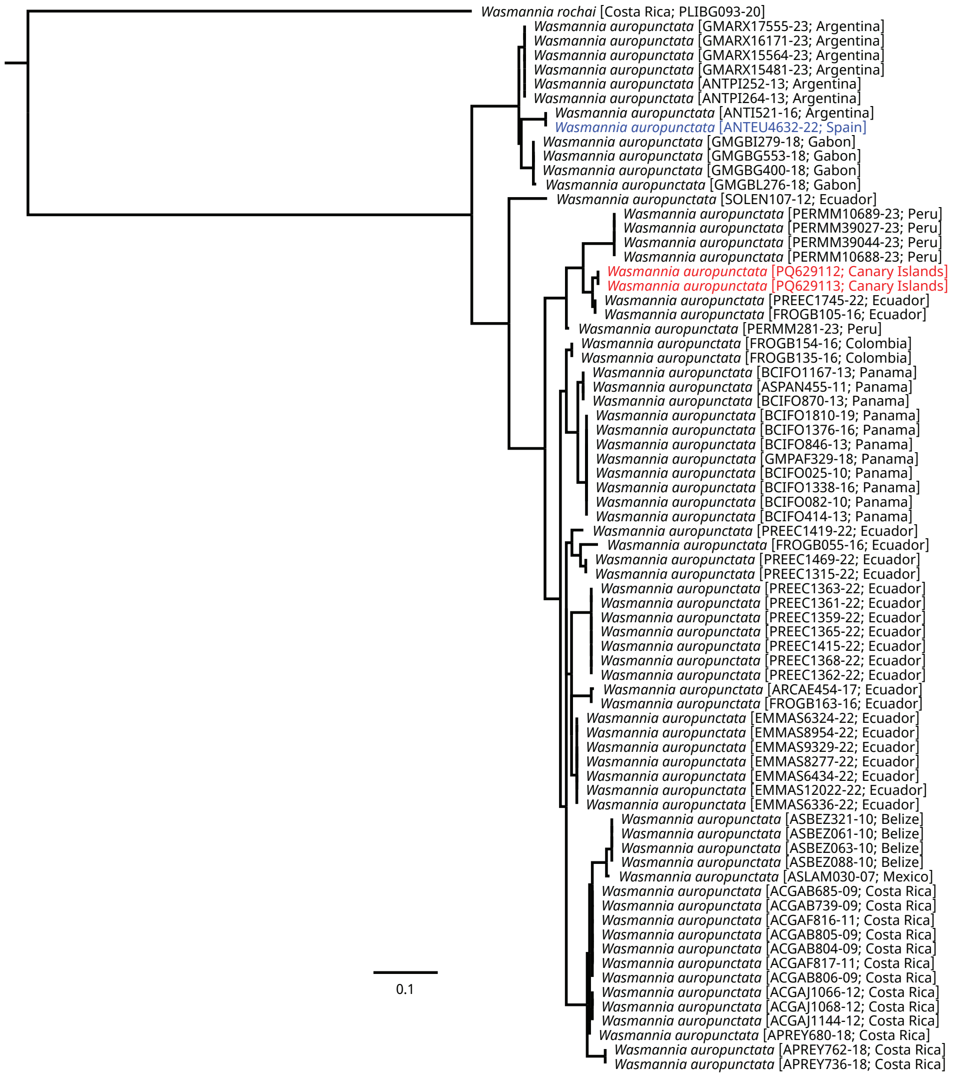

Figure 2. Maximum-likelihood tree showing the phylogenetic placement of *Wasmannia auropunctata* based on the mitochondrial data set (cox1) from a sample of 74 individuals and 619-bp DNA sequences. BOLD accession numbers and countries in brackets. Reference sequence codes correspond to GenBank accession numbers. *Wasmannia rochai* was used as an outgroup in the analysis. Red = specimens sequenced in this study (Canary Island). Blue = sequence from Málaga (mainland Spain).

species are widespread worldwide and produce serious impacts for human goods and notable effects on ecosystems (e.g., Wetterer et al. 1999; Plentovich et al. 2009; Wetterer 2010, 2012; Boase 2014; Zhou et al. 2014; Montgomery et al. 2022; Angulo et al. 2024). *Wasmannia auropunctata* like other tramp species, has characteristics that are key to its invasive success, such as high rates of polygyny, high interspecific aggressiveness, high density of individual, and colony foundation by budding instead of nuptial flights (Passera 1994; Holway et al. 2002; Heinze et al. 2006). However, there are two key aspects of its invasive potential. Firstly, outside its natural range, *W. auropunctata* builds supercolonies that can extend hundreds of kilometres, with multiple interconnected nests that exhibit low intraspecific aggression between workers from distant areas (Breton et al. 2004; Montgomery et al. 2022). This behaviour allows population growth, reaching estimated densities of more than 20,000 workers/m^2 of which 40 will be queens (Souza et al. 2008). This population is larger than the estimate for *Solenopsis invicta* with 2,000–4,000 workers/m^2 (Macom and Porter 1996) and may be one of the largest of all ant species in the world (Montgomery et al. 2022). This high density of individuals is one of the main causes of the environmental and economic problem posed by *W. auropunctata* outside its native range. Secondly, while natural populations in conserved environments show a haplodiploid reproductive system typical of Hymenoptera, the populations in disturbed and introduced areas consist in queens produced by thelytokous parthenogenesis, and males generated by a male clonality system (Fournier et al. 2005; Foucaud et al. 2007). This unusual reproductive strategy seems to be a key factor in the successful establishment of small founder colonies (Mikheyev et al. 2009). In general, the genetic diversity of the founding population is low because few individuals arrive and reproduce (Lawson et al. 2011). This may lead to genetic bottleneck, causing inbreeding and loss of heterozygosity leading to loss of fitness (Dlugosch and Parker 2008). However, the reproductive strategy of *W. auropunctata* allows for the preservation of heterozygosity when genetic diversity is low and avoids the consequences of inbreeding (Foucaud et al. 2007; Rey et al. 2013). It is likely that this clonal reproductive system is responsible for the success of *W. auropunctata* in human-modified habitats (Montgomery et al. 2022) and contributes significantly to its invasive potential.

Given the ecological and economic problems of this species, eradication programs have been proposed worldwide for controlling early-detected populations (e.g., Espadaler et al. 2018; Blight et al. 2023; Wang et al. 2024). There are, however, some examples of local eradications of *W. auropunctata.* In Marchena island (Galápagos archipelago, Ecuador) the species was eradicated from an area of 21 ha (Causton et al. 2005). In the Hawaiian archipelago there are also several examples of local population detection and eradication. Conant and Hirayama (2000) reported *W. auropunctata* for the first time in 1999 for the islands of Hawaii (Big Island), Maui, Kauai and Oahu. Since then, due to the existence of monitoring plans, new populations have been found, resulting from the involuntary transport from infected areas within the same archipelago (Vanderwoude et al. 2016). The spread of *W. auropunctata* throughout the Hawaiian Islands is likely to have significant economic and ecological repercussions. The projected economic costs for the Big Island alone are likely to exceed U$100 million annually (Vanderwoude et al. 2016). *W. auropunctata* management in the Hawaiian archipelago is estimated to cost US$ 6.1 billion over 35 years if current management efforts are maintained (Lee et al. 2015). However, if current management is not maintained the cost could be increased slightly more than double (US$ 12.9 billion). In contrast, with improved prevention actions, rapid response to newly detected infestations and enhanced management, the cost could be reduced to US$ 51 million over 35 years (Lee et al. 2015).

In our case study, *W. auropunctata* has so far only been found in an urban garden area with an estimated maximum area of no more than 55 ha (i.e., the area of the golf course and surrounding gardens). However, to correctly delimit the extent of this population it is necessary to prospect inside the golf course and to intensify sampling in other garden areas of the township. The golf course is located in one of the most important tourist townships within Tenerife, with a high population density (2,263 inhabitants/km^2) and with abundant garden areas and one of the most important ports of the Canary Islands with a daily traffic of more than 1300 vehicles (https://www.puertosdetenerife.org/memorias-anuales/). Although intensive sampling has been carried out since 2016 within the Canary Islands (Hernández-Teixidor et al. 2020; Pérez-Delgado unpublished data), *W. auropunctata* has not been detected anywhere else. However, due to the characteristics of the species (body size and behaviour) its potential presence in other localities should not be ruled out. As this golf course hadn't been sampled before, we cannot firmly state that this record constitutes a very recent colonisation (i.e., months or a couple of years). However, based on available data (Hernández-Teixidor et al. 2020; Pérez-Delgado unpublished data), its current distribution seems to be restricted to the golf course and its surroundings in Tenerife Island. All these suggest that control and eradication measures should be taken as soon as possible in order to avoid *W. auropunctata* spreading throughout the island or reaching other islands of the archipelago, which could pose a risk to biodiversity and a potentially high socio-economic cost. In addition, effective biosurveillance systems are needed to detect the transport of the species and reduce the gap between arrival and detection of the species.

Author contributions

AJP-D conceived the study. AJP-D, EJ-G, DL conducted fieldwork. AJP-D, DS performed the genetic analysis. All authors contributed to writing and editing the manuscript.

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Supplementary material 1

Canarian checklist of ants

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Data type: docx

Explanation note: The distribution of the species of ants present in the Canary Islands and their corresponding category of origin is indicated, as well as which are included in the list of invasive species of the IUCN and which were found in the area of presence of *Wasmannia auropunctata*.

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