

Rapid Communication

First morphological and molecular confirmed report of the invasive tropical fire ant, *Solenopsis geminata* (Fabricius, 1804) (Hymenoptera: Formicidae) from Côte d'Ivoire (West Africa)

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Received: 22 November 2016 / Accepted: 24 February 2017 / Published online: 21 March 2017

Handling editor: John Ross Wilson

Abstract

Urban ecosystems house artificial infrastructures such as ports, transport stations and commercial centres which are often introduction foci for exotic species. The connectivity of these structures to other centres specialised in commodity flows located in urban cores, enhances the accidental transport and spreading of exotic species which can become invasive. Despite this fact, targeted investigations to detect establishment of invasive species in tropical urban landscapes are lacking. A survey conducted on ant communities in 15 cities throughout Côte d'Ivoire (West Africa) allowed us to find the highly invasive tropical fire ant (TFA) *Solenopsis geminata* for the first time in the country. DNA analysis of mitochondrial cytochrome *c* oxidase I (COI) of two specimens of *S. geminata* found that they had a close genetic relationship with specimens from Venezuela. From the 15 towns surveyed throughout Côte d'Ivoire, this ant was discovered only in industrial areas of Abidjan near the port zone suggesting an early stage of invasion.

Key words: barcoding, invasive species, urban habitat, port area, Côte d'Ivoire, West Africa

Introduction

Expansion of urban ecosystems coupled with the rise of globalization and worldwide trade drives the spreading and establishment of many organisms beyond their native ranges (Van Ham et al. 2013; Hulme 2009). Nevertheless native and introduced biota in African urban ecosystems to date has received little attention, particularly West African urban habitats where most cities occur in proximity to biodiversity hotspots (Anderson et al. 2013). Consequently, many introduced or potentially invasive

organisms remain undetected, limiting knowledge of the invasive status of these organisms and the identification of regions that may be prone to, or the source of, invaders.

Ants are one of the most important and abundant arthropod groups in most tropical ecosystems (Hölldobler and Wilson 1990), and several ant species are also among the most notorious invasive organisms (GISD 2013; Lach et al. 2010). Many ants have a generalized diet and are able to adapt to urban habitats (Guénard et al. 2015; Angilletta et al. 2007). Introduced ants have generally a tropical or

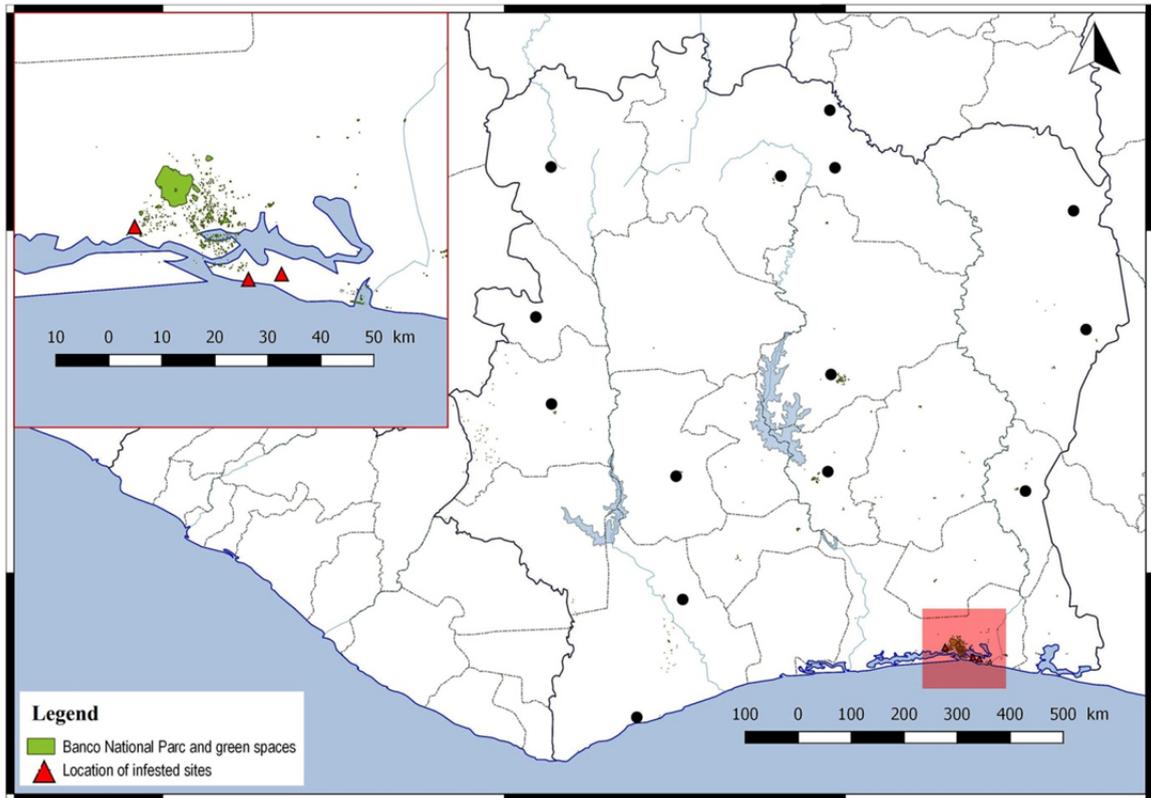


Figure 1. Location of the fifteen sites screened for invasive ant species in Côte d'Ivoire (dark dots). Red triangles are locations in the city of Abidjan where *Solenopsis geminata* was collected, which are, from West to East, Yopougon, Treichville, and Koumassi.

subtropical origin, and many of them share similar ecological traits such as polygyny, unicoloniality, omnivory, nest type and tramp behaviour. All these traits facilitate their success in the new colonised environment (Morrow et al. 2015; Holway et al. 2002). Moreover their small size simplifies accidental transport by humans via tourism and commercial exchange to locations outside their geographical ranges where they often become a serious threat for native ant species and other organisms (McGlynn 1999). As such, ants are a useful model group to track the invasion process (Bertelsmeier et al. 2015a).

One of the world's most notorious and invasive pest ants is the tropical fire ant (TFA), *Solenopsis geminata* (Fabricius, 1804). This species, native to tropical Central and South America (Suarez et al. 2010), has spread through commerce to many parts of the world (Wetterer 2010). In its native range, TFA is both an important pioneer species (Perfecto 1991) and generalist keystone predator (Risch and Carroll 1982). *Solenopsis geminata* occupies both natural habitats and disturbed ecosystems like urban areas and agro-ecosystems where it may be a serious

pest (Holway et al. 2002). The damage this ant causes on other invertebrates and vertebrates is largely documented (Wauters et al. 2014; Plentovich et al. 2009). Because of its numerical and ecological dominant behaviour *S. geminata* is often considered as an extirpator species and described as an important biocontrol agent of invertebrate pest species (Yusa 2001). TFA also has direct socio-economic impacts, in particular due to its painful sting which can seriously affect humans and domestic animals (Wauters et al. 2014). With global climate change, this ant species is recently predicted to have a 6.3% increase in its suitable habitat on all six continents by 2080 (Bertelsmeier et al. 2015b).

In Western Africa, although already reported from Liberia, Cameroon, Nigeria, Senegal and Guinea, relevant molecular information seems lacking. Additionally, all reports of TFA in this region are based on information dating back to 1958 (EPPO 2014; Wetterer 2010). Due to the lack of scientific knowledge of TFA in Africa, African populations of *S. geminata* were not considered in the recently published global invasion history reconstruction of

Table 1. Location of sites in Abidjan where *Solenopsis geminata* was observed and their distances from the port zone.

Location	Coordinates	Description of microhabitats	Distance from Port zone	Record	Reference
Koumassi	05°17'28.4"N 003°57'04.9"W	Bare soil with short grass	4.3 km	12 /05/2009	Yeo et al. (2016)
Treichville	05°17'08.2"N 004°00'30.0"W	Soil with lawn	0 km	16/05/2009	Yeo et al. (2016)
Yopougon	05°22'12.9"N 004°05'29.9"W	Soil with short grass	10.9 km	25/11/2014	This study

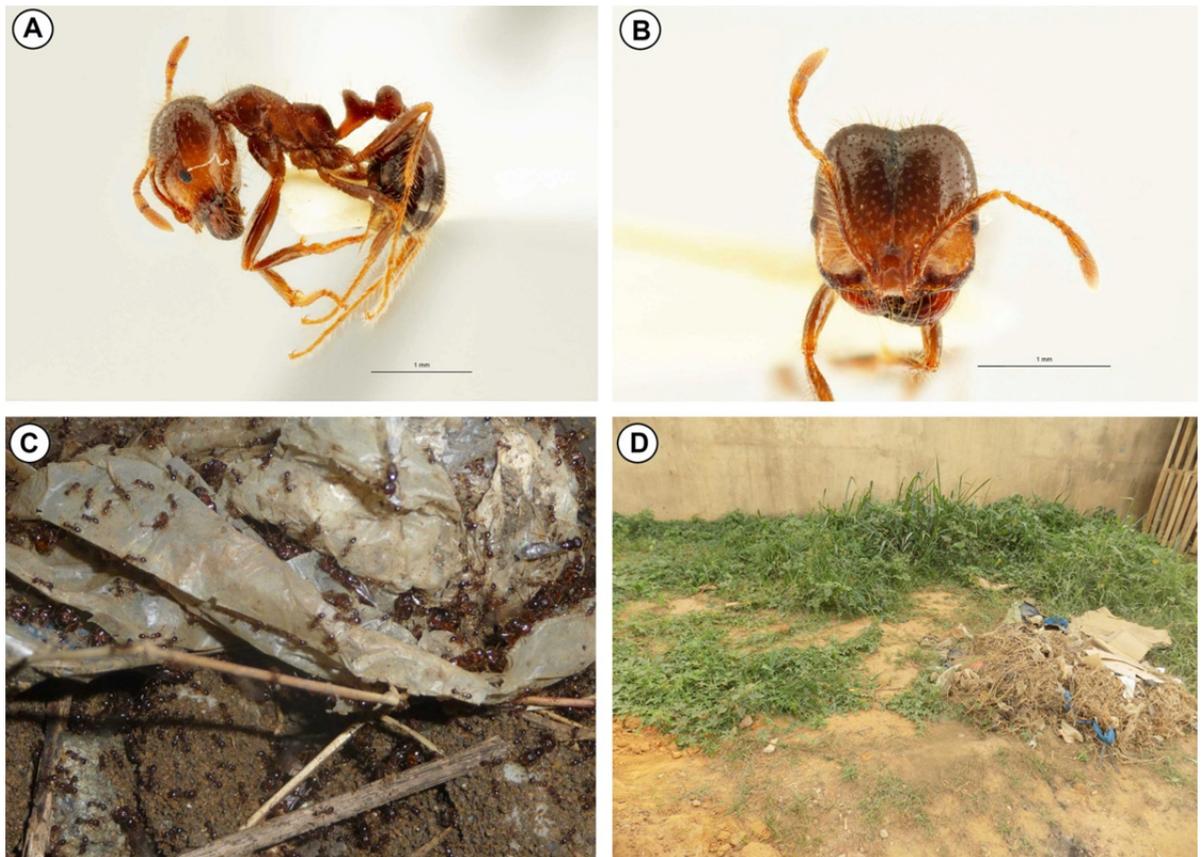


Figure 2. (A) Lateral and (B) Frontal views of major worker of *Solenopsis geminata*, (C) *S. geminata* nesting below a plastic bag (different worker castes and gynes are visible), (D) Infested microhabitat where *S. geminata* was discovered in the industrial zone of Yopougon (photographs by C. Locatelli and L.M.M. Kouakou).

this species (Gotzek et al. 2015). Here we report the first official record of TFA in Côte d'Ivoire, West Africa, identified initially morphologically and confirmed using mitochondrial cytochrome *c* oxidase subunit I (COI) DNA analysis.

Material and methods

Sampling and identification

From September 2014 to December 2015, fifteen cities in Côte d'Ivoire were screened for invasive ant species (Figure 1). Ants were collected using tuna

fish lures (Yeo et al. 2016; Bestelmeyer et al. 2000) on 200m long linear transects, geo-referenced with a Garmin GPSMAP 64S. A brief description of the microhabitats present along each transect was noted. Lures (n = 20 per transect) were operational for one hour and ants were collected at 15; 30; 45 and 60 minutes. In total, 45 transects were sampled giving 900 lures. Samples were stored in individual Eppendorf minitube of 2 ml filled with ethanol (96%) and labelled. After identification of *Solenopsis geminata* in the laboratory, we came back a few months later to the place where this species was collected to look for and

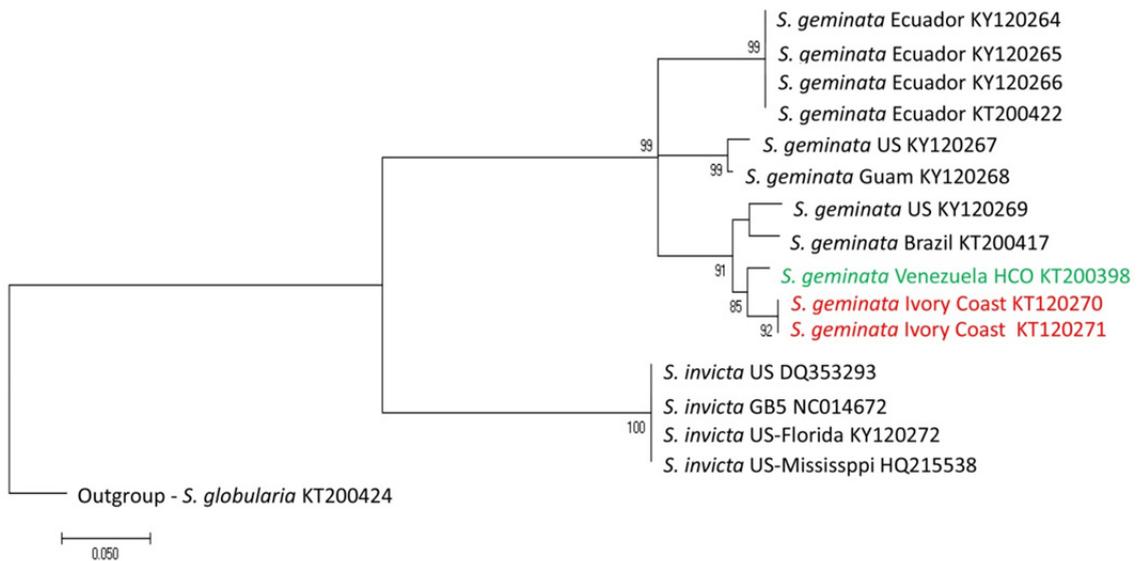


Figure 3. Maximum likelihood (ML) trees of *Solenopsis geminata* and *Solenopsis invicta* Buren, 1972 (HKY+I+G model estimated by JmodelTest) in MEGA v7. *Solenopsis globularia* (Smith, 1858) (KT200424) was used as an outgroup (GB=GenBank).

to attempt the excavation of its whole nest(s), with the objective to estimate reproductive traits (e.g. polygyny/monogyny, worker number and nesting behaviour). We again used tuna lures along a 200 m long linear transect to estimate limits of foraging activities in the infested area.

Because ants are often highly polymorphic, all collected workers and species were identified, though note the identification of *Solenopsis geminata* could only be achieved with certainty using the major workers as minor workers are highly simplified (Trager 1991). We first used Bolton (1994) to identify workers to genus, and then we compared our specimens with the reference collection of Yeo et al. (2016) located at Lamto Ecological Research Station for identification to morphospecies. Our specimens were then photographed using the digitization equipment at the Royal Belgian Institute of Natural Sciences (RBINS) (Brecko et al. 2014), and the images were then compared with image banks (Taylor 2007; AntWeb 2002) and finally with specimens from the RBINS entomological collection for definitive species identifications. Voucher specimens of our study were deposited at RBINS, Belgium under the accession number RBINS IG 32.422 and in Lamto Ecological Research Station, Côte d'Ivoire.

DNA extraction, amplification and sequencing

Two worker specimens of *Solenopsis geminata* (from two lures placed 80 m apart at Yopougon)

were selected for DNA molecular analysis. Genomic DNA extraction was conducted at RBINS. DNA of the specimens was extracted with the Standard commercial mini-kit (Nucleospin Mini-kit) following the manufacturer's protocol. PCR was performed with a volume of 9.06 µl and mixed with 1 µl template DNA. PCR mixed product was amplified with Primer LCO1490F [5'-GGTCAACAAATCAT AAAGATATTG-3' (Folmer et al. 1994)] and Primer HCO2183 [5'-CCAAAAAATCAAATARATGY-3' (Folmer et al. 1994)]. PCR reactions were performed in a final volume of 10.06 µl with 1 µl 2 µM Primer, 1 µl 2 mM dNTP (Qiagen), 1 µl 10x PCR-buffer (Invitrogen), 0.3 µl 50 mM MgCl₂ (Invitrogen); 0.8 µl 0.25 mg/ml BSA (Biolabs); 3.9 µl sterile H₂O and 0.06 µl 0.5 U/µl Taq platinum (Invitrogen). The cycling parameters were set under the following conditions: initial denaturing at 95 °C for 3 minutes followed by 40 cycles at 95 °C by 30 s; 48 °C by 30 s and 72 °C by 45 s.

The final extension step was done at 72 °C for 7 minutes and the program was paused at 25 °C. The results of the PCR reaction were checked in 1.2% Midori green-stained agarose gel electrophoresis. The purified PCR products are sequenced in both directions on an ABI 3130xl capillary DNA sequencer using the BigDye Terminator v3.1 chemistry (both Life Technologies). The obtained trace-files (Forward and Reverse) were processed using CodonCode Aligner (CodonCode Corporation, <http://www.codoncode.com>).

The sequenced fragment of the COI-gene (± 657 bp) is considered to be the standard barcoding region for animals (Hebert et al. 2003).

Phylogenetic analysis

Sequences data were subjected to blastn searches in Genbank (<http://www.ncbi.nlm.nih.gov/genbank>). Related species of the genus *Solenopsis* were retrieved and aligned with the obtained sequences using the Clustal-W algorithm (Thompson et al. 1994) in MEGA v7.0 (Kumar et al. 2016). A Maximum Likelihood analysis in MEGA v7.0 was used to reconstruct the phylogenetic relationships among the sequences with a HKY+I+G substitution model, selected by JmodelTest (Darriba et al. 2012). *Solenopsis globularia* (Genbank accession number KT200424), was selected as outgroup. Bootstrap support values were based on 500 bootstrap replicates and only bootstrap values of more than 70% were considered to be supportive (Hillis and Bull 1993). Our nucleic acid sequences were then submitted to the GenBank database under the accession codes KY120270 and KY120271.

Results and discussion

The morphological identification of *Solenopsis geminata* (TFA) was based on: (1) its polymorphic caste; (2) the major worker head (Figure 2B), which is almost square and endowed with a median groove down the middle of cephalic dorsum in full-face view; (3) 10-segmented antennae with a 2-segmented club; and (4) the surface of the body which is smooth and shiny with numerous standing hairs.

We found TFA in the industrial zone of Yopougon at 10.9 km from the port zone of Treichville. *Solenopsis geminata* was not recorded from any of the other 14 Ivorian cities prospected for invasive ant species, suggesting that the species is still in an early stage of invasion. However, a morphological similar ant species was previously collected in 2009 (but identified to the morpho-species level only as *Solenopsis* sp. 3 by Yeo et al. 2016) in the port zone of Treichville and in the industrial zone of Koumassi at 4.3 km from Treichville in the city of Abidjan (Table 1). It seems most probable that *S. geminata* had been introduced to the port zone of Treichville by sea freight, and that it spread from the port zone to the freight yard of the industrial zone during the transport of materials and goods or through natural spread (winged queens can fly kilometres from their natal nests (Holway et al. 2002)). However, despite careful search carried out in 2015, we did not recollect TFA from Treichville and Koumassi. As

such the only area known to be currently invaded by TFA in Côte d'Ivoire is the site of Yopougon in the city of Abidjan.

We found TFA in an open area characterised by the presence of short grass or lawn. When discovered at Treichville and Koumassi, TFA was found in microhabitats dominated by pavements (Yeo et al. 2016; Table 1). It is possible that neighbouring areas also harboured the species and dedicated search should be carried out to estimate the area invaded by TFA in the port zone of Abidjan with more precision. Although this ant is polymorphic (Wetterer 2010), only minor and intermediate workers were attracted to our lures in 2014, probably because the detected population was still in the early stage of establishment and the size of colony was too low to produce major workers (Tschinkel 1988) or because our experiment did not last long enough to attract major workers. The major workers were collected by nest excavations (Figure 2A, 2B).

Solenopsis geminata co-occurred with high abundance of woodlice below garbage of rotting grass (Figure 2D) whereas on lures TFA was the single species exploiting the lure, generally in very high numbers (in total 1358 workers captured). The numerical and behavioural dominances are characteristic of invasive ant species, and the fact that no other ant species co-occurred with TFA confirmed that it may seriously disturb native ant communities. Another exploration conducted in July 2016 in industrial zone of Yopougon allowed us to collect one nest in which we counted 35 major workers, 106 gynes (winged queens) and 93 dealate queens in addition to numerous minors workers (Figure 2D). This last investigation confirmed the establishment of *S. geminata* and the existence of polygynous colonies, at least on the site of Yopougon where large number of flying sexual highlights the potential for dispersal to other areas, and subsequent invasion.

In addition, our study is the first molecular confirmed report of the highly invasive *Solenopsis geminata* in Côte d'Ivoire. Comparing Ivorian *Solenopsis geminata* sequences with online sequences from GenBank, COI barcoding results (Figure 3) confirmed that both our specimens of *Solenopsis* match with sequences available on GenBank for the species *Solenopsis geminata*. The BLAST results for both Ivorian *Solenopsis* sequences also indicate a match with *Solenopsis geminata*. The highest similarity (Query Coverage 100%, Identity 98%) was found with a *Solenopsis geminata* sequence from Venezuela (KT200398, GB3 in tree).

The detection of *S. geminata* at Yopougon, which is only at 1.5 km from the Banco National Park, is giving serious cause of concern, especially because

this natural park is already under pressure due to increasing urban development around its boundary, and the spread of other invasive ants along its access roads (Yeo et al. 2016). Otherwise, considering the challenge of conducting eradication and its low success rate for invasive ants (Hoffmann et al. 2016), we recommend a rapid eradication of Ivorian populations of *Solenopsis geminata* while we still are in the early phase of invasion. Also, an extensive monitoring of industrial zones in Ivorian port cities should be established as they appear to be one of the main potential entrance zones for invasive ants.

Acknowledgements

This paper is a result of the project with financial support from the Belgian Directorate-General for Development Cooperation (DGD), part in Global Taxonomy Initiative, within the framework of the CEBioS programme (CBD/GTI-02/MLS/2015.53). This research was partly supported by International foundation for Science, Stockholm, Sweden, through a grant D/5837-1 to Lombart Mesmer Maurice KOUAKOU. We are grateful to Ben Hoffmann and two other reviewers which the positive suggestions greatly improved the quality of this paper. We are also grateful to Jonathan Brecko (RBINS) for his helpful advice for the digitalization of our specimens and to Gontran Sonet (RBINS) for his help preparing and calibrating the sequencer. The molecular research was funded by the FWO project (G0D2915N) – Poneroid Ants of Ecuador (Formicidae: Agroecomyrmicinae, Amblyoponinae, Ponerinae, Proceratiinae, Paraponerinae).

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