First record of the invasive longhorn crazy ant, Paratrechina longicornis (Latreille, 1802) (Hymenoptera: Formicidae) from Mt. Elgon, eastern Uganda

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Research Article

First record of the invasive longhorn crazy ant, *Paratrechina longicornis* (Latreille, 1802) (Hymenoptera: Formicidae) from Mt. Elgon, eastern Uganda

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Abstract

We report the first observation of the invasive longhorn crazy ant (*Paratrechina longicornis*) in the Mount Elgon region of eastern Uganda. About 43 000 ants were sampled in 256 locations throughout the Ugandan foot slopes of Mt. Elgon in the years 2014, 2015 and 2016. We found *P. longicornis* in five locations in and around the town of Budadiri, Sironko district. The visual species identification was confirmed by COI gene-based DNA barcoding. That this species was found in only a small area suggests that it has only been recently introduced. The impact that *P. longicornis* will have on the local agricultural system or the biodiversity within the Mount Elgon National Park remains unclear. The Mt. Elgon region is a unique key biodiversity area where baseline data can be collected now to quantify the effects of *P. longicornis* as it increases its distribution within the region.

Key words: tramp species, barcoding, East Africa, coffee gardens, tourism, biosphere reserve

Introduction

Invasive alien species are animals or plants that have been introduced accidentally or deliberately into a natural environment where they are not normally found (EC 2017). Although ants are small and seem innocuous, some ant species, called "tramp ants", can cause serious damage once introduced to a new habitat. The damage that such invasive ants cause can cost billions of dollars and native species can be pushed to the brink of extinction (Gutrich et al. 2007; O'Dowd et al. 2003; Wylie and Janssen-May 2017). Therefore it's not surprising that five ant species appear in the IUCN's list of world's 100 worst invasive alien species (Lowe et al. 2000).

The longhorn crazy ant, *Paratrechina longicornis* (Latreille, 1802) (Figure 1), might not be the most notorious of all tramp ants, but it is perhaps

Figure 1. Frontal, dorsal and lateral photographs of a *Paratrechina longicornis* specimen collected by Wout Dekoninck in December 2016 at a hostel near the Budadiri Uganda Wildlife Authority headquarter, Budadiri-Bumasola road, 1°10′11.80″N; 34°20′15.75″E. Photos by Camille Locatelli.

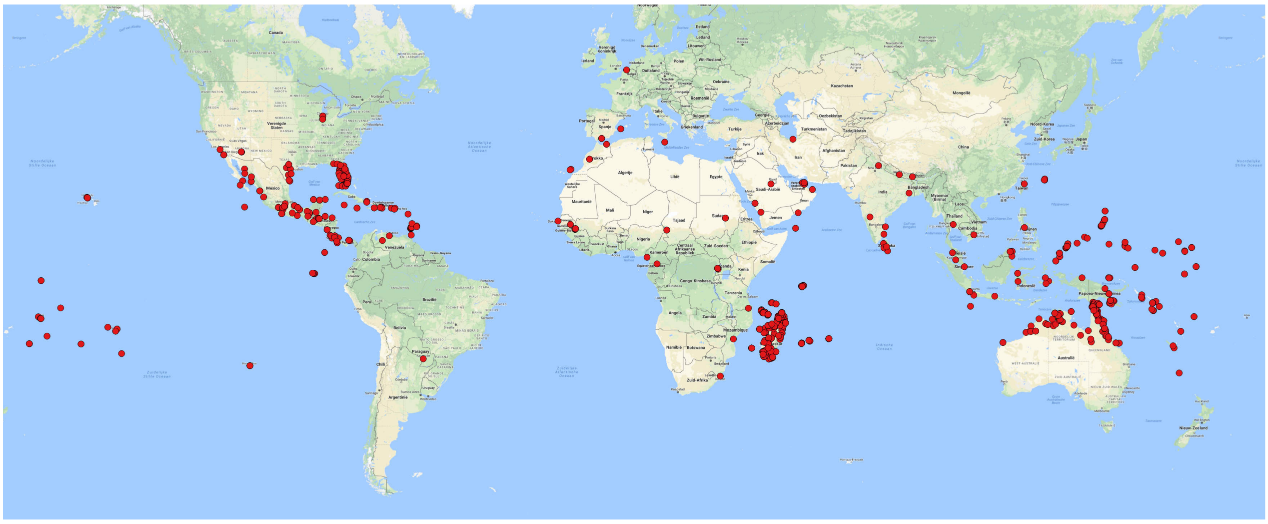

Figure 2. Global distribution of *Paratrechina longicornis*. Data sourced from Antweb (www.antweb.org).

the most widespread (Figure 2). Longhorn crazy ants can be found in urban centers and gardens all over the tropics as well as in greenhouses or other manmade structures in cool temperate climates (Kouakou et al. 2018; Wetterer 2008). The species can also be a significant agricultural pest, promoting the growth of populations of phloem-feeding hemipterans (Herrera 2016; Wetterer et al. 1999). Notably, *P. longicornis* was distributed so widely over the globe centuries ago that its native range remains unclear. Although Wetterer 2008 found good evidence that *P. longicornis* is native to Southeast Asia and Melanesia, the recent discovery of four other *Paratrechina* species within the Afrotropical and Malagasy regions suggests otherwise (Forel 1916; LaPolla and Fisher 2014).

Polygyny, unicoloniality and reproduction by colony budding are typical characteristics of tramp ant species (Calcaterra et al. 2016), and *P. longicornis* possesses all these traits (Debout et al. 2007) aiding its success as an invasive species. Although *P. longicornis* is not as aggressive as other invasive ant species such as the red imported fire ant (*Solenopsis invicta* Buren), it has the potential to disrupt local ecosystems and lead to the localized extinction of indigenous ant and other invertebrate species (Wetterer et al. 1999). In addition, because it is omnivorous and displays extremely rapid foraging and recruiting behavior, *P. longicornis* is able to flourish in new areas of colonization (Kenne et al. 2005). It also forms symbiotic relationships with honeydew producing hemipterans with the exchange of honeydew for protection against predators (Koch et al. 2011) which has implications for plant health. Surprisingly though for such a widespread and ubiquitous species, although its implications have been documented for human infrastructure (Wetterer et al. 1999) little is known about the implications of its presence within natural ecosystems (Wetterer et al. 1999).

AntWeb records confirm that *P. longicornis* has recently been observed within Uganda, far inland in the west of the country, namely in the Kibale and Semuliki National Parks (AntWeb 2018). Here we document the first record of *P. longicornis* in the east of Uganda, near the Mt. Elgon National Park. The Mt. Elgon ecosystem can be considered particularly vulnerable to biological invasions of species like *P. longicornis*. Altitudinal differences isolate local species populations from the surrounding rift valley creating a so called "sky island" ecosystem with a high rate of endemism (CEPF 2012). This geographic isolation limits the natural immigration of new species, allowing both the established species to have evolved with few strong competitors or predators and the presence of some unfilled ecological niches, which could facilitate the spread of invasive alien species (MacArthur and Wilson 1967).

Materials and methods

Study area

Mount Elgon is a 4,320 m high, extinct volcano on the boundary between Uganda and Kenya, situated just north of the equator (Figure 3). A series of volcanic cliffs and a change in soils and climate separates its foot slopes from the lowland at about 1,200 m altitude. The local landscape mosaic consists of croplands, tree plantations, pastures and coffee gardens where Arabica coffee is intercropped mainly with bananas, yams, maize or beans. The smallholder agriculture system reaches up to about 1,900 m altitude, where the Mount Elgon National Park starts. This transboundary national park, which is also a UNESCO Man and Biosphere Reserve, stretches up to the top of Mt. Elgon. The local climate has two distinct rainy seasons separated by a pronounced dry period from December to February and by a period of dispersed, less intense rains from July to early August. Average annual rainfall is ca. 2,100 mm (Thiery et al. 2015). Average annual air temperature is 23 °C (NEMA 2001).

Sampling

During a recent study of the impacts of private sustainability standards on smallholder coffee production by Vanderhaegen et al. 2018, litter and shrub dwelling ants were collected as indicators of environmental health. Seventy-six coffee gardens were inventoried from July to September 2014

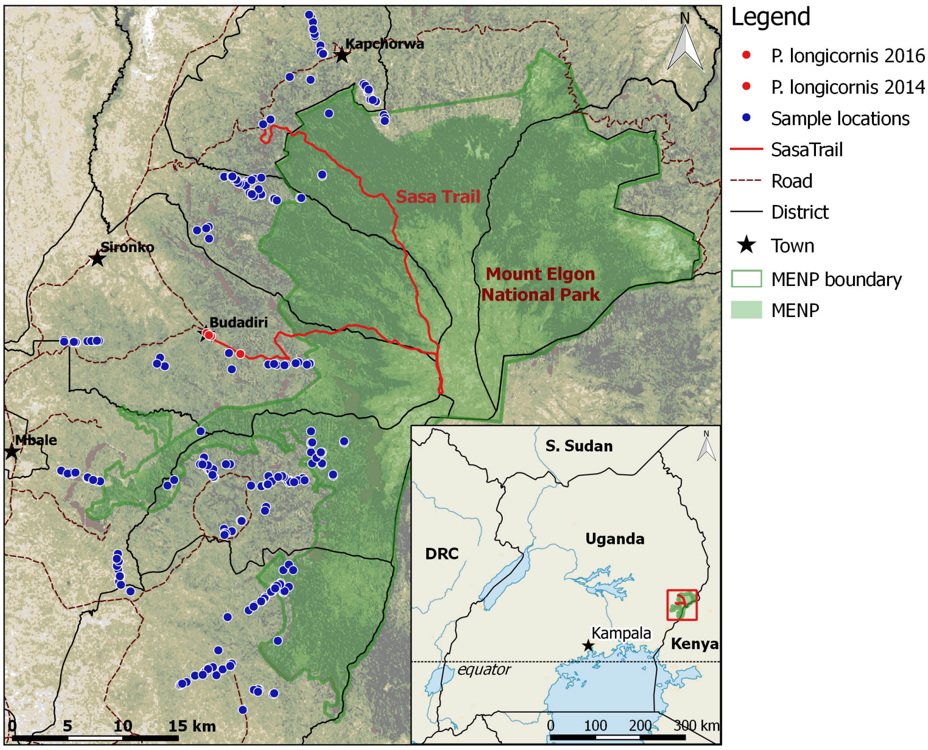

Figure 3. Map of the sampled locations (blue dots) in the Mt. Elgon region and the locations where *Paratrechina longicornis* was collected (red dots). Note that multiple locations where *P. longicornis* was collected are so close to each other that they are not visibly distinct at this scale. For details see Supplementary material Table S1.

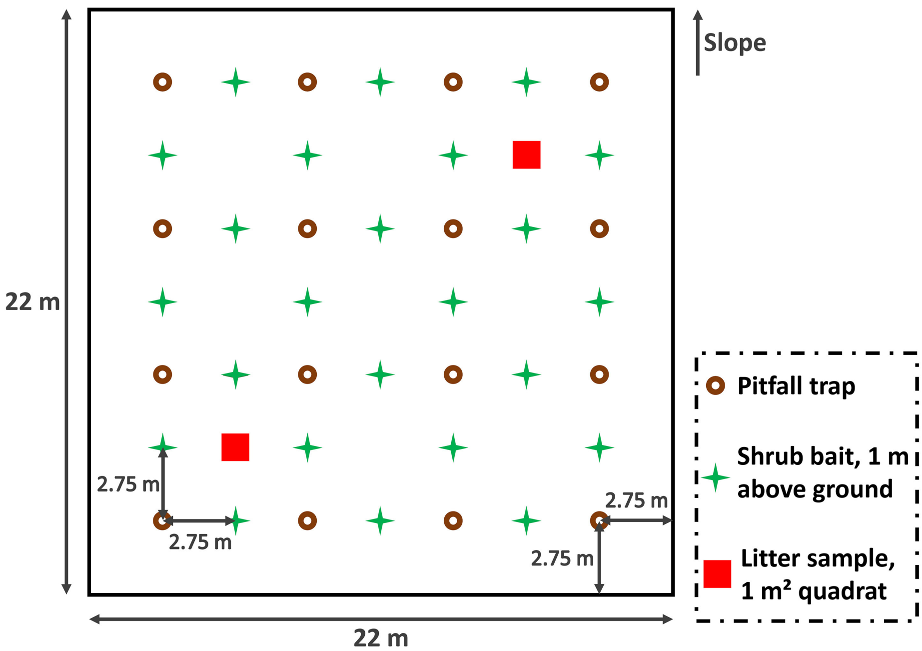

Figure 4. Survey design at each site.

(Figure 3, Supplementary material Table S1.). Three sampling methods were applied, based on the Ants of the Leaf Litter standard protocol (Agosti and Alonso 2000) (Figure 4). First, sixteen pitfall traps were set in a 4 × 4 array with 5.5 m spacing and operated for 24 hours. Second, 24 tuna baits were placed 1 m above ground in the shrub layer among the area of the pitfall traps and attending ants were hand collected after 30 minutes. Third, litter was collected in two haphazardly positioned 1 m^2 quadrats, with visual ant collection for 7 minutes in the field and further extraction using Winkler bags over the following 72 hours.

A second inventory of the ant fauna was conducted from July to September 2015 using 173 plots positioned along 10 transects radiating in different directions along the Ugandan slopes of Mt. Elgon (Figure 3). Plots were placed in all main land use types. During this second study ants were collected using a simplified methodology compared to the 2014 sampling, only the third step was applied: 7 minutes visual collections *in situ* and the Winkler extraction from litter collected in two 1 m^2 quadrants per plot. In December 2016 a final small survey was conducted to reconfirm the presence of *P. longicornis* at 7 locations within the urban center of Budadiri and along the Budadiri-Bumasola road. Ants appearing to be *P. longicornis* were collected during 30 minutes of visual collection at each site. Notably, no sampling was conducted in the larger urban centers near to Mt. Elgon as the research was focused on Mount Elgon National Park and its surrounding agricultural landscape.

Ant processing and morphological identification

The samples were sorted at the Royal Belgian Institute for Natural Sciences (RBINS), Belgium. We used various keys to identify ant genera and species (Fisher and Bolton 2016; LaPolla and Fisher 2014; LaPolla et al. 2013). Additionally, voucher specimens were photographed using a Canon-Cognisys automated focus stacking setup (Brecko et al. 2014). Images were compared with online databases and shared with international experts to confirm their species identification (AntWeb 2018). Voucher specimens of this study are deposited at RBINS under the accession number RBINS IG 32.905.

COI gene-based DNA barcoding

DNA extraction, amplification and sequencing

To further confirm the morphological identification of *P. longicornis* we conducted COI analyses on two specimens. Individual total genomic DNA was extracted using the Nucleospin Mini-kit (Machery-Nagel). DNA was digested overnight on a thermoshaker and was eluted in 2 × 50 µl buffer (70 °C). PCR was performed in a final volume of 9.06 µl PCR-mix with 1 µl template DNA. The PCR-mix contained 1 µl 2 mM dNTP (Qiagen), 1 µl PCR buffer 10x (Qiagen), 1 µl 2 µM mtDNA COI Primer LCO1490F (5’-GGTCAACAAATCATAAAGATATTG-3’) (Folmer et al. 1994), 1 µl 2 µM mtDNA COI Primer HCO2198 (5’- TAAACTTCAGGGTGACCAAAAAA TCA-3’) (Folmer et al. 1994), 0.3 µl Mg Cl_2, 0.8 µl 0.25 mg/ml BSA, 0.06 µl 5 Units/µl Taq platinum (Qiagen) and 3.9 µl H_2O (for 1 µl DNA, 2.9 µl H2O for 2 µl DNA). Bands were visualized in a 1.2% Midori green stained agarose gel.

PCR amplification was performed under the following conditions: Initial denaturation at 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 45 s. These steps were repeated for 40 cycles, followed by a final extension step at 72 °C for 7 min. The PCR products were stored at 25 °C.

Purified PCR products were sequenced in both directions on an ABI 3130xl capillary DNA sequencer using the BigDye Terminator v3.1 kit (Life Technologies). Trace-files were checked for mistakes and the contigs were aligned and merged in Codon Code Aligner v6.02 (Codon Code Corporation, www.codoncode.com). The obtained COI sequences were aligned with GenBank database sequences using the Basic Local Alignment Search Tool (nBLAST).

Phylogenetic analysis

The sequenced COI fragment (± 657 bp) is the standard barcoding region for animals (Hebert et al. 2003). The extracted sequences were aligned using ClustalW in MEGA v7.0 (Kumar et al. 2016) and run through a maximum likelihood (RAxML-HPC BlackBox v.8) (Stamatakis 2014) analysis run in the online CIPRES platform (Miller et al. 2010). *Lepisiota* species samples collected during the same inventory and sequenced using the same methodology were used to root the tree. 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). A Bayesian analysis was run using Mr Bayes v.3.2.0 (Ronquist and Huelsenbeck 2003). The specific nucleotide substitution models were determined by Partition Finder v.1.1.1. (Lanfear et al. 2012).

Results

Among the 33,000 ants collected in the 76 coffee gardens in five districts covering the Ugandan slopes of Mt. Elgon, two *P. longicornis* specimens were found. Both specimens were collected in the same coffee garden in the year 2014 but by different collection methods. One specimen was collected using tuna baits on coffee shrubs and the other one was collected with a pitfall trap. The sampled coffee garden was located 3.5 km from the regional town of Budadiri at 1305 m altitude and along the road from Budadiri to the start of the Sasa trail (Figure 3). Of the 10,000 ants collected in an additional 173 sampling sites located in all types of land uses in 2015, none were *P. longicornis*.

Four of the seven locations surveyed in the third targeted survey in 2016 contained *P. longicornis*. Two locations were guesthouses frequented by tourists, at the local Ugandan Wildlife Authority compound, where tourists have to report before entering the National Park and the other two were at the market of Budadiri.

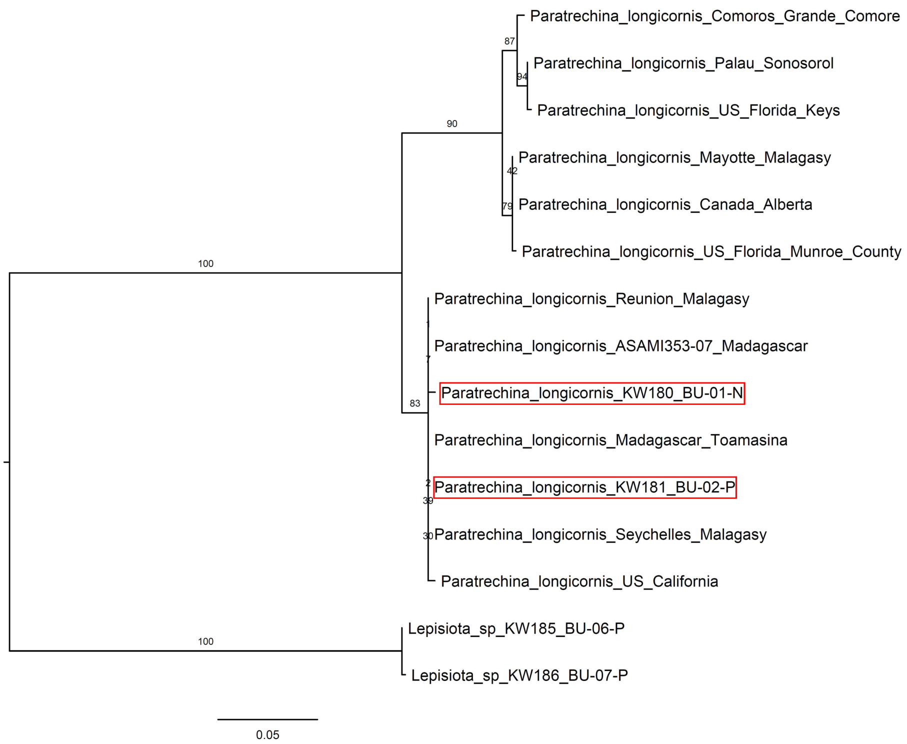

Figure 5. Maximum likelihood tree of *Paratrechina longicornis* specimens from different locations worldwide. The two specimens collected at Mt. Elgon are indicated by the red frames.

COI gene-based barcoding confirmed our morphological identifications of *P. longicornis*. A BLAST query resulted in a very high similarity with five of the 11 randomly selected *P. longicornis* sequences from BOLD and GenBank, namely from California (USA) and the Malagasy region, and a high similarity with the six other selected *P. longicornis* specimens from Canada, Florida (USA), the Comoros, Palau and the Malagasy region (Figure 5).

Discussion

Of the 256 locations sampled in all land uses within and around Mt. Elgon National Park, only 5 locations were found to contain *P. longicornis*. This limited distribution shows that *P. longicornis* has not yet invaded large areas of rural land around Mt. Elgon National Park indicating that this species has only recently been introduced to the Elgon region. AntWeb records confirm however that *P. longicornis* has already been observed in western Uganda and although not documented in scientific literature, it is also highly probable that *P. longicornis* is present in many urban habitats and cities in Uganda, e.g. *P. longicornis* was collected in 2016 in Mukono at the NaFORRI center by Naturinda Z (*pers. comm.* 2016).

The specific location of our first observations in 2014 might be an indication of how *P. longicornis* could have arrived at Mt. Elgon. Due to the recent peace and security in the region, tourism is increasing. A major tourist activity is the four-day round-trip to the top of Mt. Elgon, Wagagai peak (UWA 2018). The Sasa trail is the original and busiest route leading to Wagagai peak (Figure 3). Most tourists spend a night in one of the local hotels located along the Budadiri- Bumasola road between Budadiri and the start of the Sasa trail, before ascending Mt. Elgon. Other rural areas on the slopes of Mt. Elgon are rarely visited by foreigners. That *P. longicornis* was found only here, close to the hotels at the start of the Sasa trail indicates that *P. longicornis* could have arrived associated with the tourism industry. This is further strengthened by visual searches in 2016 around tourism facilities close to the area where *P. longicornis* was observed in 2014, with *P. longicornis* being found at two hostels nearby and in the Uganda Wildlife Authority (UWA) local headquarter of Budadiri. These findings are not so surprising because tourism and outdoor recreation are known as major global pathways for the movement of non-native species (Anderson et al. 2015).

If *P. longicornis* spreads further in the region near Mt. Elgon National Park this could impact the local agricultural systems, including the important coffee growing industry, as the species is known to be very efficient at tending homopteran scales (Wetterer et al. 1999). In return for sugary honeydew *P. longicornis* ants protect scales which in turn can multiply rapidly. Large numbers of phloem-feeding scales reduce plant health and crop productivity. Globally the economic losses due to scales on coffee have been estimated to be US$ 5 billion per year (Rutherford and Phiri 2006).

Additionally, *P. longicornis* could have negative impacts on local biodiversity. The Elgon region is noted as having high ant diversity (over 200 species) by Vanderhaegen et al. 2018 with more species likely yet to be found as over the border in Kenya an even higher ant diversity is known to occur (Garcia et al. 2013). Besides ant communities, other local fauna could also be affected such as stingless bee species which have been shown to be outcompeted for honeydew by *P. longicornis* in Madagascar (Koch et al. 2011).

Importantly, the increase in tourism is likely to result in more exotic species being introduced within the Mt. Elgon region. Indeed, within the surveys we also found *Pheidole megacephala* (Fabricius, 1793), *Tapinoma melanocephalum* (Fabricius, 1793) and *Cardiocondyla wroughtonii* (Forel, 1890). The Mt. Elgon region is a unique area where baseline data can be collected now to quantify the effects of *P. longicornis* as it increases its distribution within the region.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Sampling sites, methods and records of *Paratrechina longicornis*.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_Vanderhaegen_etal_Table_S1.xlsx