Short communication

First record of the ant *Pheidole megatron* Fischer and Fisher, 2013 (Hymenoptera: Formicidae) from Rwanda

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We present the first record of the ant *Pheidole megatron* Fischer and Fisher, 2013 in Rwanda, a species thought to be endemic to the Malagasy region. Specimens were collected in July 2019, in one of the houses located at Kivumu, in the center of Rwanda. They were first morphologically identified as *Pheidole megatron* using a recent identification key. Molecular identification through mitochondrial cytochrome oxidase I (COI) confirmed the morphological identification. The Rwanda sample clustered with samples from the Comoros, and it belongs to the group *megacephala*. Future studies may focus on the biology and ecology of this ant species in Africa. Moreover, we suggest the screening of other African populations of *Pheidole megacephala* using finer genetic markers with higher mutation rates to clarify the identification and spread of the species.

Keywords: ant species, barcoding, biodiversity

Recent studies indicated that ants (Hymenoptera, Formicidae) respond quickly to changing environmental conditions (Nsengimana et al. 2018), and carry out important ecological functions such as predation on pest insects (Eguchi et al. 2011). Like earthworms and termites, ants are ecosystem engineers that improve soil properties and soil function (Lavelle et al. 2006) through leaf litter decomposition, and creation of soil pores that facilitate soil aeration, and soil water infiltration (Fatima et al. 2008). Further, ants facilitate nutrient transport at different soil horizons (Bagyaraj et al. 2016). Furthermore, ants were documented to influence food webs in the soil ecosystem through the introduction of organic matter in the soil (Cammeraat and Risch 2008).

Regarding biodiversity, ants form an important and abundant arthropod group, particularly in tropical ecosystems (Fisher and Bolton 2016). They have both high species richness and complex trophic levels (Graham et al. 2009), and they form the most divergent group among all social insects (Majalakshimi and Channaveerappa 2016). In the Afrotropical and Malagasy regions, Formicidae includes 20 subfamilies classified into 198 genera (AntWeb 2020).

Within Africa, the subfamily Myrmicinae is the largest (AntWeb 2020; Fisher and Bolton 2016), while *Pheidole* Westwood, 1839 is the largest Myrmicinae ant genus, with more than 1000 species worldwide (Salata and Fisher 2020). *Pheidole* species are generally characterised by conspicuous dimorphism between major and minor workers (Casadei-Ferreira et al. 2019). They are a widely distributed and highly diverse ant genus (Shukla et al. 2013). Despite the diverse diet of *Pheidole* species (Pirk et al. 2009), this ant genus is mainly a predator of pest insects (Agarwal and Rastogi 2008; Shukla et al. 2013), particularly in tropical perennial agroecosystems (Rastogi 2004).

In the East Africa region, research conducted in Kakamega forest, Kenya, indicated that the genus *Pheidole* is among the five most species-rich genera after *Tetramorium* Mayr,1855; *Camponotus* Mayr,1861; *Monomorium* Mayr, 1855; and *Crematogaster* Lund, 1831 (Hita Garcia et al. 2009). To date, three species and one subspecies of *Pheidole* are known from Rwanda (AntWeb 2020; Nsengimana and Dekoninck 2020): *P. megacephala* (Fabricius, 1793), *P. melancholica angulata* Stitz, 1911; *P. punctulata* Mayr, 1866; and *P. punctulata angulata* Mayr, 1866. However, there are likely to be more *Pheidole* species present, with other research conducted in southern Rwanda alone recently recording three undescribed species (Nsengimana et al. 2018).

The identification of *Pheidole* to species level is done either by morphospecies taxonomy (Sreedevi et al. 2015)

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or DNA-based taxonomy (Blaxter 2004). Because of uncertainties associated with each type, a number of studies (Hillis 1987; Wortley and Scotland 2006; Heethoff et al. 2011) recommend using a combination of both methods for definitive identification. In some cases, this helps to overcome challenges related to the weaknesses of each identification method (Sreedevi et al. 2015; Borowiec 2016). For these reasons, we investigated both morphological and molecular characteristics to identify ant specimens collected in Rwanda. The specific objective was to confirm the presence of a population that was morphologically identified as *P. megatron* using DNA-barcoding (Hebert et al. 2003).

Specimens were collected in a house located at 2°3′15.01″ S, 29°47′53.02″ E, at an elevation of 1 826 m, in Kivumu, central Rwanda. Sampling was done in July 2019 by hand collection. Specimens were conserved in 30 ml of ethanol (96%) and taken to the laboratory of the Royal Belgian Institute of Natural Sciences (RBINS). They were first identified to the subfamily and genus levels based on morphological characteristics using a microscope and dichotomous keys (Fisher and Bolton 2016), and to the species level (Fischer and Fisher 2013). Specimens were then digitised using a Canon 600D Camera equipped with a Canon MP-E 65 mm 1: 2.8–5× Macro Photo Lens mounted on StackShot (Brecko et al. 2014). Digitised images (Figure 1) were compared with image banks from AntWeb (https://www.antweb.org/) for confirmation of the species assignation.

Further, the DNA of one specimen was extracted using the NucleoSpin® tissue extraction kit (Macherey-Nagel). A fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the LCO1490[5′-GGTCAACAAATCATAAAGATATTGG-3′] and HCO2198[5′-TAAACTTCAGGGTGACCAAAAAATCA-3′] universal primers (Folmer et al. 1994). The PCR amplification was done in 11 µl reaction mixture containing 1 µl of DNA template, 5.5 µl Multiplex (Qiagen) mastermix, 1 µl of each primer diluted at 2 µM, and 2.5 µl double distilled H_2O. The PCR products were checked on a 1.5% agarose gel. Furthermore, the positive amplifications were purified using the ExoSAP-ITTM protocol, following the manufacturer's instructions, and sequenced in both directions on an ABI 3230xl capillary DNA sequencer using BigDye Terminator v3.1 chemistry (ThermoFisher Scientific). The quality of the sequencing output was checked with Geneious® R11 (Biomatters Ltd), then strands were trimmed, corrected, translated into amino acids, and assembled using the same software. The generated sequence is available in GenBank with access code MT410993.

The generated sequence was searched against the identification system on GenBank (https://www.ncbi.nlm.nih.gov), and the Barcode of Life Data System (BOLD) (https://www.boldsystems.org). Because of many misidentified sequences present in online databases, specifically for the *P. megacephala* group, we only used recently published sequences based on specimens used as part of a revision of ants from the Malagasy region and which belong to the group *P. megacephala* (Fischer and Fisher 2013; Sarnat et al. 2015; Salata and Fisher 2020). These were aligned with the generated sequence using ClustalW in Geneious® R11, and the final alignment was trimmed to retain only the COI Folmer region

Figure 1: Frontal and lateral views of a minor worker of *Pheidole megatron* collected by Nsengimana Venuste in July 2019 at 2°3′15.01″ S, 29°47′53.02″ E, at an elevation of 1 826 m, in central Rwanda

(658 bp). Duplicates following the definition of Geneious® were discarded, and a Neighbor-Joining (NJ) tree was constructed with a Tamura-Nei distance model and 1 000 bootstrap replicates. The support threshold for the bootstrap values was set to 75%. Three *Pheidole jonas* sequences were used as the outgroup to root the NJ-tree.

The species was identified morphologically as *Pheidole megatron* (Fischer and Fisher, 2013). The species was a minor worker characterised by an ovoid head shape, smooth and shiny face and promesonotum, and had the postpetiole longer than the petiole. This identification was supported by DNA-barcoding. The BOLD and GenBank best matches were CASENT0136865-D01 (GU709802) and CASENT0137386-D01 (GU709800), with 98.15 and 98.1 similarity percentages, respectively. The NJ tree construction showed that the generated sequence clustered with almost maximum branch support with the sequences of *P. megatron* (Figure 2).

Pheidole megatron was described from the Comoros and belongs to the *megacephala* group (Fischer and Fisher 2013). In the Comoros, *P. megatron* was found in dry forests, coastal shrubs, coconut plantations, and beaches, specifically under rotten wood and branches at elevations ranging from 10 to 35 m (Fischer and Fisher 2013). This study is the first indicating the presence of *P. megatron* from Rwanda, and the species is common in the sampled area, particularly in the dry season. The origin of the species is unknown. In the latter case, a DNA screening of
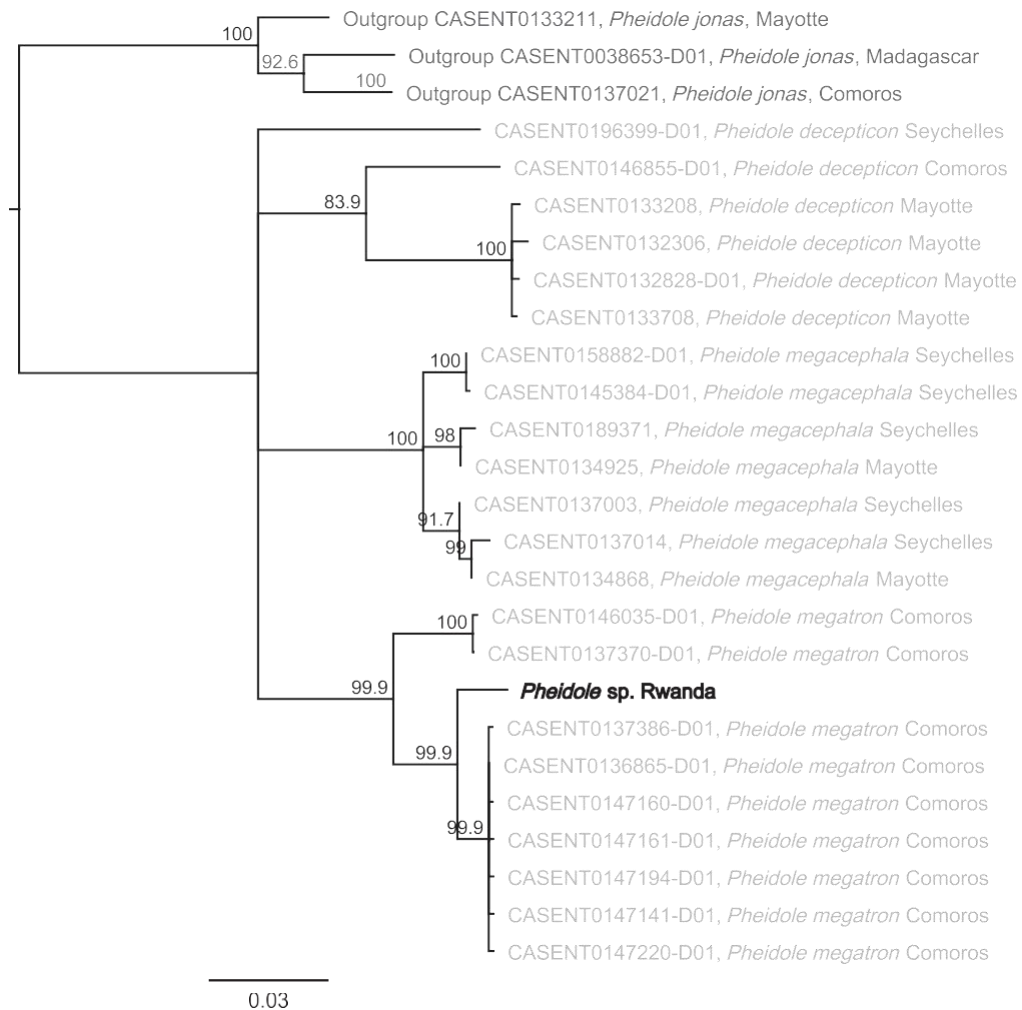
Figure 2: Neighbor-Joining tree of *Pheidole megatron* specimens from the Comoros, Mayotte and Seychelles. The specimen discussed in this study is indicated in bold black

all so-called *P. megacephala* found in the region would be needed to investigate the origin of the species.

Given that *P. megatron* is closely related to the highly invasive *P. megacephala*, it is possible that they both share similar traits. The success of *P. megacephala* as a pantropic invasive species is based on its generalist omnivorous diet involving scavenging for food on the ground (Riginos et al. 2015). *Pheidole megacephala* is also a good predator with efficient nest mate recruitment, enabling the species to dominate baits and retrieve prey too large for single workers to carry (Sarnat et al. 2015). The adaptation mechanisms of *P. megatron* have not yet been compared with *P. megacephala*. However, our personal observation during data collection indicated high spread of the species during the dry season. Workers were aggressive to other ants, unicolonial, and had a general diet; characteristics found in other invasive ant species (Bertelsmeier et al. 2015; Fournier et al. 2019), bolstering the argument that *P. megatron* is exotic in Rwanda and may become problematic.

We recommend additional studies focusing on the biology and the ecology of *P. megatron*. Future studies should focus also on ecological adaptations and the effects of *P. megatron* on native biodiversity to assess whether it is exotic in Rwanda and whether it can exert extreme ecological pressure warranting it being labelled as an invasive species. Furthermore, the screening of the *P. megacephala* by other finer genetic markers with higher mutation rates than mitochondrial sequences is recommended to determine the origin of *P. megatron*.

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