

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

Venuste Nsengimana, Ann Vanderheyden, Sophie Gombeer, Nathalie Smitz, Kenny Meganck, Marc De Meyer, Thierry Backeljau, Brian L Fisher & Wouter Dekoninck

To cite this article: Venuste Nsengimana, Ann Vanderheyden, Sophie Gombeer, Nathalie Smitz, Kenny Meganck, Marc De Meyer, Thierry Backeljau, Brian L Fisher & Wouter Dekoninck (2021): First record of the ant *Pheidole megatron* Fischer and Fisher, 2013 (Hymenoptera: Formicidae) from Rwanda, *African Zoology*, DOI: [10.1080/15627020.2021.1901605](https://doi.org/10.1080/15627020.2021.1901605)

To link to this article: <https://doi.org/10.1080/15627020.2021.1901605>



Published online: 23 Apr 2021.



Submit your article to this journal [↗](#)



Article views: 7



View related articles [↗](#)



View Crossmark data [↗](#)

## Short communication

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

Venuste Nsengimana<sup>1,2\*</sup> , Ann Vanderheyden<sup>3</sup>, Sophie Gombeer<sup>3</sup> , Nathalie Smitz<sup>4</sup> , Kenny Meganck<sup>4</sup> , Marc De Meyer<sup>4</sup> , Thierry Backeljau<sup>4,5,7</sup>, Brian L Fisher<sup>6</sup>  and Wouter Dekoninck<sup>7</sup>

<sup>1</sup> Department of Mathematics, Science and Physical Education, College of Education, University of Rwanda, Kigali, Rwanda

<sup>2</sup> Centre of Excellence in Biodiversity and Natural Resources Management (CoEB), College of Science and Technology, University of Rwanda, Kigali, Rwanda

<sup>3</sup> Royal Belgian Institute of Natural Sciences (BopCo), Brussels, Belgium

<sup>4</sup> Royal Museum for Central Africa (BopCo), Tervuren, Belgium

<sup>5</sup> Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium

<sup>6</sup> Entomology, California Academy of Sciences, San Francisco, California, United States

<sup>7</sup> Royal Belgian Institute of Natural Sciences (Scientific Heritage Service), Brussels, Belgium

\*Correspondence: [venusteok@gmail.com](mailto:venusteok@gmail.com)

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)

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<sub>2</sub>O. The PCR products were checked on a 1.5% agarose gel. Furthermore, the positive amplifications were purified using the ExoSAP-IT™ 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

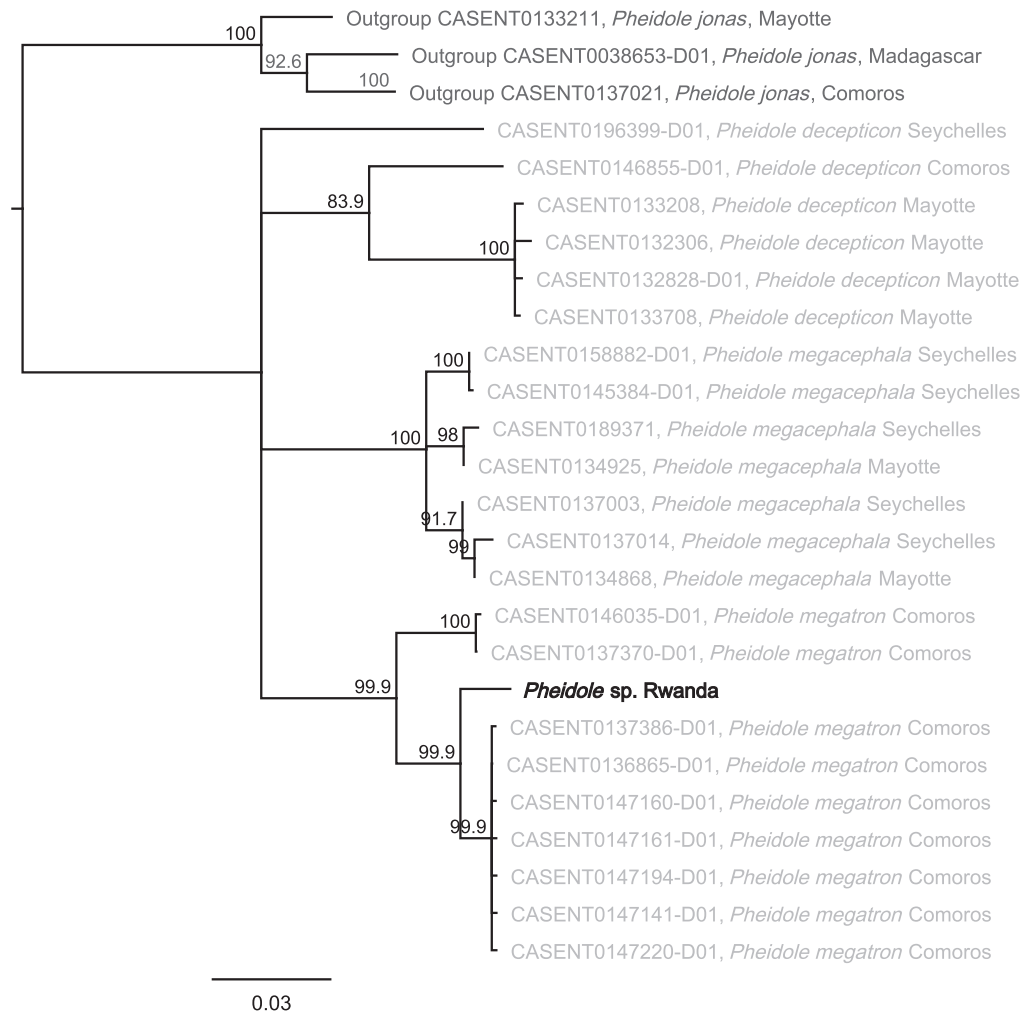


**Figure 1:** Frontal and lateral views of a minor worker of *Pheidole megatron* collected by Nsengimana Venuste in July 2019 at 2°3'15.01" S, 29°47'53.02" E, at an elevation of 1 826 m, in central Rwanda

(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*.

**Acknowledgments** — This research was funded by the Belgian General Directorate for Development and Cooperation, part of the Global Taxonomy Initiative within the framework of Capacities for Biodiversity and Sustainable Development (CEBioS). The molecular analysis was done by the Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo), supported by the Belgian Science Policy Office (Belspo) as part of the Belgian Federal in-kind contribution to the European Research Infrastructure Consortium LifeWatch. We thank the Centre of Excellence in Biodiversity and Natural Resources Management (CoEB) based in the College of Science and Technology, University of Rwanda, for assistance during the writing and review of the manuscript

**Funding declaration** — This research was funded by the Belgian General Directorate for Development and Cooperation (DGD),



part of the Global Taxonomy Initiative (GTI) within the framework of Capacities for Biodiversity and Sustainable Development (CEBioS). The molecular analysis was done by the Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo), supported by the Belgian Science Policy Office (Belspo) as part of the Belgian Federal in-kind contribution to the European Research Infrastructure Consortium LifeWatch.

**Ethics and permits** — With submission of this article the authors have complied with the institutional and/or national policies governing the human and ethical treatment of the experimental subjects, and they are willing to share original data and materials if so requested. All research pertaining to this article did not require any research permits.

## ORCID

Nsengimana Venuste: <https://orcid.org/0000-0001-5963-8314>

Sophie Gombeer: <https://orcid.org/0000-0003-3450-1375>

Nathalie Smitz: <https://orcid.org/0000-0001-5155-0801>

Kenny Meganck: <https://orcid.org/0000-0002-0270-5560>

Marc De Meyer: <https://orcid.org/0000-0003-0755-2898>

Brian L Fisher: <https://orcid.org/0000-0002-4653-3270>

## References

- Agarwal VM, Rastogi N. 2008. Deterrent effect of a guild of extrafloral nectary visiting and species on *Raphidopalpa foveicollis* a major insect pest sponge gourd, *Luffa cylindrica*. *Entomology Experimental and Applied* 128: 303–310.
- AntWeb. 2020. California Academy of Sciences. <https://www.Antweb.Org/Taxonomicpage>. [Accessed 2 January 2020].
- Bagyaraj DJ, Nethravathi CJ, Nitin KS. 2016. Soil biodiversity and Arthropods: Role in soil fertility. In: Chakravarthy a, Sridhara S. (Eds) Economic and Ecological Significance of Arthropods in diversified ecosystems. Springer, Singapore. [https://doi.org/10.1007/978-981-10-1524-3\\_2/](https://doi.org/10.1007/978-981-10-1524-3_2/).
- Barcode of Life Data System (BOLD). 2020. <https://www.boldsystems.org>. [Accessed 6 January 2020].
- Bertelsmeier C, Avril A, Blight O, Confais A, Diez L, Jourdan H, Orivel J, Saint Germès N, Courchamp F. 2015. Different behavioral strategies among seven highly invasive ant species. *Biological Invasions* 17: 2491–2503. <https://doi.org/10.1007/s10530-015-0892-5>.
- Blaxter ML. 2004. The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 359: 669–679. <https://doi.org/10.1098/rstb.2003.1447>.
- Borowiec ML. 2016. Generic revision of the ant subfamily Dorylinae (Hymenoptera, Formicidae). *ZooKeys* 608: 1–280. <https://doi.org/10.3897/zookeys.608.9427>.
- Brecko J, Mathys A, Dekoninck W, Leponce M, Vandenspiegel D, Semal P. 2014. Focus stacking: Comparing commercial top-end set-ups with a semi-automatic low budget approach. A possible solution for mass digitization of type specimens. *ZooKeys* 464: 1–23. <https://doi.org/10.3897/zookeys.464.8615>.
- Cammeraat WLH, Risch AC. 2008. The impact of ants on mineral soil properties and processes at different spatial scales. *Journal of Applied Entomology* 132: 285–295. <https://doi.org/10.1111/j.1439-0418.2008.01281.x>.
- Casadei-Ferreira A, Chaul JCM, Feitosa RM. 2019. A new species of *Pheidole* (Formicidae, Myrmicinae) from Dominican Amber with a review of the fossil records for the genus. *ZooKeys* 866: 117–125. <https://doi.org/10.3897/zookeys.866.35756>.
- Eguchi K, Viet BT, Yamane S. 2011. Generic synopsis of the Formicidae of Vietnam (Insecta: Hymenoptera), Part I – Myrmicinae and Pseudomyrmecinae. *Zootaxa* 2878: 1–61. <https://doi.org/10.11646/zootaxa.2878.1.1>.
- Fatima MSM, Huising EJ, Bignell DE. 2008. *A Handbook of tropical biology: Sampling and characterization of below-ground biodiversity*. London: Earth Scan.
- Fischer G, Fisher BL. 2013. A revision of *Pheidole* Westwood (Hymenoptera: Formicidae) in the Islands of the Southwest Indian Ocean and designation of a neotype for the invasive *Pheidole megacephala*. *Zootaxa* 3683: 301–56. <https://doi.org/10.11646/zootaxa.3683.4.1>
- Fisher BL, Bolton B. 2016. *Ants of Africa and Madagascar: A Guide to the Genera*. Berkeley: University of California Press.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of Mitochondrial Cytochrome c Oxidase Subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Fournier A, Penone C, Pennino MG, Courchamp F. 2019. Predicting future invaders and future invasions. *Proceedings of the National Academy of Sciences of the United States of America* 116 (16): 7905–10. <https://doi.org/10.1073/pnas.1803456116>.
- Graham JH, Krzysik AJ, Kovacic DA, Duda JJ, Freeman DC, Emlen M, Zak JC, Long RW, Wallace MP, Chamberlin-Graham, et al. 2009. Species richness, equitability, and abundance of ants in disturbed landscapes. *Ecological Indicators* 9: 866–877.
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings. Biological Sciences* 270: 313–32. <https://doi.org/10.1098/rspb.2002.2218>.
- Heethoff M, Laumann M, Weigmann G, Rasputnig G. 2011. Integrative taxonomy: Combining morphological, molecular and chemical data for species delineation in the parthenogenetic *Trhypochthonius tectorum* complex (Acari, Oribatida, Trhypochthoniidae). *Frontiers in Zoology* 8: 2. <https://doi.org/10.1186/1742-9994-8-2>.
- Hillis DM. 1987. Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics* 18: 23–42. <https://doi.org/10.1146/annurev.es.18.110187.000323>.
- Hita Garcia F, Fischer G, Peters MK, Roy Snelling MK, Wägele JW. 2009. A preliminary checklist of the Ants (Hymenoptera: Formicidae) of Kakamega forest (Kenya). *Journal of East African Natural History* 98: 147–65. <https://doi.org/10.2982/028.098.0201>.
- Lavelle P, Decaens T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P, Rossi JP (2006). Soil invertebrates and ecosystem services. *European Journal of Soil Biology* 42: 3–15. <https://doi.org/10.1016/j.ejsobi.2006.10.002>.
- Majalakshimi BR, Channaveerappa H. 2016. Diversity of ant species (Hymenoptera: Formicidae) in the campus of Maharani's Science College for Women: a mini model of habitat persistence. *International Journal of Pure and Applied Zoology* 4: 277–281. <https://www.alliedacademies.org/articles/diversity-of-ant-species-hymenoptera-formicidae-in-the-campus-of-maharanis-science-college-for-women-a-mini-model-of-habitat-persi.html>.
- National Center for Biotechnology Information (NCBI) 2020. <https://www.ncbi.nlm.nih.gov>. [Accessed 5 January 2020].
- Nsengimana V, Dekoninck W. 2020. A preliminary checklist of ants from Rwanda (Hymenoptera, Formicidae). *Belgian Journal of Entomology* 101: 1–22.
- Nsengimana V, Kaplin BA, Francis F, Kouakou MML, Dekoninck W, Nsabimana D. 2018. Use of soil and litter ants (Hymenoptera: Formicidae) as biological indicators of soil quality under different land uses in southern Rwanda. *Environmental Entomology* 47: 1394–1401. <https://doi.org/10.1093/ee/invy144>.
- Pirk GI, di Pasquo F, Lopez de Casenave J. 2009. Diet of two sympatric *Pheidole* spp. ants in the central Monte desert: implications for seed-granivores interactions. *Insect Sociology* 56: 277–283. <https://doi.org/10.1007/s00040-009-0021-5>.
- Rastogi N. 2004. Ecologically dominant, predatory ant species as a

- resource for sustainable insect pest management in plantations. In: Ignacimuthu S, Jayaraj S (Eds), *Sustainable Insect Pest Management*. New Delhi: Narvosa Publication. pp 118–128.
- Riginos C, Karande MA, Rubenstein DI, Palmer T. 2015. Disruption of a protective ant-plant mutualism by an invasive ant increases elephant damage to savanna trees. *Ecology* 96: 654–661. <https://doi.org/10.1890/14-1348.1>.
- Salata S, Fisher BL. 2020. *Pheidole* Westwood, 1839 (Hymenoptera, Formicidae) of Madagascar – an introduction and a taxonomic revision of eleven species groups. *ZooKeys* 905: 1–235. <https://doi.org/10.3897/zookeys.905.39592>
- Sarnat EM, Fischer G, Guénard B, Economu EP. 2015. Introduced *Pheidole* of the World: Taxonomy, Biology and Distribution. *ZooKeys* 543: 1–109. <https://doi.org/10.3897/zookeys.543.6050>.
- Shukla RK, Singh H, Rastogi N, and Agarwal VM. 2013. Impact of abundant *Pheidole* ant species on soil nutrients in relation to the food biology of the species. *Applied Soil Ecology* 71: 15–23. <https://doi.org/10.1016/j.apsoil.2013.05.002>.
- Sreedevi K, Meshram N, Shashank PR. 2015. Insect taxonomy - Basics to barcoding. In: Chakravarthy AK (Ed.), *New horizons in insect science: Towards sustainable pest management*. Switzerland: Springer Nature. pp 3–12.
- Wortley AH, Scotland RW .2006. The effect of combining molecular and morphological data in published phylogenetic analyses. *Systematic Biology* 55: 677–85. <https://doi.org/10.1080/10635150600899798>.