International Journal of Tropical Insect Science (2022) 42:403–413
https://doi.org/10.1007/s42690-021-00557-w

ORIGINAL RESEARCH ARTICLE

Morphological reassessments and DNA barcoding of *Pheidole rugaticeps* Emery and *Pheidole decarinata* Santschi collected in Nigeria

Mohammed Ahmed Ashigar[1,2] · Abdul Hafiz Ab Majid[1]

Received: 16 November 2020 / Accepted: 19 May 2021 / Published online: 8 June 2021

Abstract
Studies have revealed that the genus *Pheidole* (Formicidae: Myrmicinae) is the most diverse group of ants and highly distributed around the globe. Species delimitation through DNA-based approaches has lately received increased attention to overcome the taxonomic hurdle. This study reassessed the morphology and barcode *Pheidole rugaticeps* Emery and *Pheidole decarinata* Santschi collected in Nigeria. *P. rugaticeps* and *P. decarinata* are the most abundant ant species in the area sampled. Taxonomy of both *Pheidole* spp were reassessed using morphometrics. The morphological diagnosis of the worker castes was consistent with *P. rugaticeps* Emery, 1877 specimen (CASENT0281618), and *P. decarinata* Santschi specimen (CASENT0913301) from AntWeb.org. For the DNA-based identification, LCO1490/HCO2198 and LepF1/LepR1 primer pairs that consistently amplified a 710-bp fragment of Cytochrome oxidase subunit 1 (CO1) of invertebrates were used. The Basic Local Alignment Search Tool (BLAST) program of the NCBI database was used to confirm the genus as *Pheidole*. However, the species identity produced less than 90% match with the available CO1 sequences in the database. DNA sequences of *P. rugaticeps* and *P. decarinata* were not previously submitted at NCBI or other online databases. Hence, DNA sequences of *P. rugaticeps* (accession no; MT309805) and *P. decarinata* (accession no; MT308581) were added to the NCBI database for the first time and will be additional diagnostic tools for future studies. The phylogenetic result suggested that both *Pheidole* species were distinct. The study encourages the combination of morphological and molecular practices to identify this diverse ant group.

Keywords Identification · Mitochondrial gene (COI) · Molecular · Morphological · *Pheidole decarinata* Santschi · *P. rugaticeps* Emery

Introduction

Ants (Formicidae: Hymenoptera) are a diverse group of insects and play a key role in the ecosystems (Hölldobler and Wilson 1990). They sometimes outnumbered other insect taxa significantly representing a large part of the global biodiversity (Mora et al. 2011). Ants have a worldwide distribution, and the tropical regions have diverse and abundant taxonomic groups (Ward 2000; Wilson 2003). Furthermore, the Afrotropical region has a high proportion of unidentifiable morphospecies (Belshaw and Bolton 1993; Deblauwe and Dekoninck 2007; Fisher 2004; Hita Garcia et al. 2009). Studies have attributed several factors such as countless undescribed species, lack of modern-day revisionary treatments, and even the described species' inadequate identification keys to the high proportion of unidentifiable morphospecies (Fischer et al. 2012). The genus, *Pheidole* with high species richness worldwide (Longino 2009) and evolutionary success regarding ecology and diversity, is one of the ant group with largely unidentifiable morphospecies due to lack of the aforementioned factors.

Lately, the taxonomic study of the genus *Pheidole* has received a heightened interest. Especially after the work by Wilson (2003) on the fauna of the New World which increases the number of the *Pheidole* species described greatly (Fischer et al. 2012). Furthermore, several other studies have provided references for the taxonomic treatment of this group of ants (Longino 2009; Eguchi 2000,

✉ Abdul Hafiz Ab Majid
abdhafiz@usm.my

[1] Household & Structural Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia

[2] Department of Zoology, Federal University Lafia, Nasarawa State, Nigeria

2001a, 2001b, 2008; Eguchi and Bui 2005; Eguchi et al. 2007; Sarnat 2008; Salata and Fisher 2020). However, in the Afrotropical region, the genus *Pheidole* has received less attention from ant taxonomists and attempts to identify specimens using taxonomic literature was frustrating (Fischer et al. 2012) and more difficult (Wetterer 2012).

Most of the present *Pheidole* species and subspecies in this region were described within 150 years to date, mostly by F. Santschi, C. Emery, and few others. However, some of their descriptions lack accuracy and insufficient for identification due to poor documentation of the differences between subspecies and relatively little resemblance of subspecies described (Fischer et al. 2012). The most recent works on described species of *Pheidole* in the Afrotropical region were conducted by Bernard (1953), Arnold (1960), Wilson (1984), Fischer et al. (2012), and Salata & Fisher (2020). A large volume of new materials, undescribed species, and long neglection of this ant group in this region appeals for extensive taxonomic treatment, and the recent review of many species by Fischer et al. (2012) has revived the study in the region.

Several species have been mentioned in previous studies in Nigeria including *Pheidole minima* catella, *Pheidole nigeriensis* (Santschi 1914), *Pheidole stephensi* Taylor (Taylor 1980a), *Pheidole crassinoda*, currently known as *Pheidole costauriensis* Santschi, (Sylvanus et al. 1997), *Pheidole semidea* (Fischer et al. 2012), *Pheidole rugaticeps* Emery (AntWeb 2020), *Pheidole megacephala* (Wheeler 1922) and many more (Taylor 1976, 1978, 1979, 1980a, 1980b). This study reassessed the taxonomy and phylogeny of *Pheidole rugaticeps* Emery and *Pheidole decarinata* Santschi by integrating morphological taxonomic and DNA-based (COI gene) identification approaches. A study by Ng'endo et al. (2013) incorporated two methods in the taxonomic diagnosis of *Pheidole* ants and described it as a highly effective identification system. Therefore, the current study adopted the PCR assay of Ng'endo et al. (2013) as it might be a valuable tool for the identification of *Pheidole* spp. in the Afrotropical region. This study provides taxonomic descriptions and biological sequences of two of the commonly observed *Pheidole* species in the Nasarawa state of Nigeria, namely *Pheidole rugaticeps* Emery and *Pheidole decarinata* Santschi.

Materials and methods

Study area and insect collection

The study was performed to identify the species of the hyperdiverse genus, *Pheidole* (Formicidae; Myrmicinae) collected in Nasarawa, Nigeria. The ant samples were collected scavenging on dead insects from different locations in this study area (Table 1). Most of them were collected using *Periplaneta Americana* as bait, while others were found foraging on other insects such as butterfly, grasshopper, termites, and housefly maggot. Cadavers of American cockroaches were placed along their trail routes or at their nest entrance which leads to the emergence of both minor and major workers from the nest. The foraging ants were then collected and preserved in 70% ethanol for further analysis. The ant foragers were gently collected into a vial container and transferred into a separate jar containing 70% alcohol solution for preservation before further analysis. Ant specimens used for DNA extraction were preserved in RNAlater solution.

Morphological identification

The collected *Pheidole* ant species were identified based on their morphological features. Different sides (head, lateral, and dorsal views) of ten specimens from each of the minor and major workers of each species were studied, and photomontage of each species was made. Structure and shape of the head, mesosoma, petiolar and postpetiolar nodes as well as the gaster were studied and measured. All observations and measurements were taken using Olympus CZ61 light stereomicroscope (2× to 4.5× mg) and Olympus DP21 camera on a light stereomicroscope combined with software cellSens standard (version 1.4.1). Minimal and maximal values and averages of the

Table 1 Sampling information of two *Pheidole* ant populations in the Nasarawa state of Nigeria

S/No	Ants species	Locations	Codes	GPS Coordinates
1	*P. rugaticeps*	GRA, Keffi	3EE1	08°50′53.25″ N 07°53′08.48″ E
2		Low-cost Housing, Akwanga	LHE4	08°55′38.29″ N 08°24′46.91″ E
3		Kurikyo	KRK4	08°31′32.09″ N 08°35′51.59″ E
4		Gwandara	KDR4	08°34.11.95″ N 08°29′50.59″ E
5		PHC, Akwanga	PHC4	08°54′50.41″ N 08°24′51.86″ E
6		Dalhatu Specialist Hospital	DH4	08°30′08.95″ N 08°31′21.95″ E
7		GRA, Keffi	GRA4	08°50′53.25″ N 07°53′08.48″ E
8		Nasarawa State Polytechnic	POLY2	08°32.47.55″ N 08°32′09.81″ E
9	*P. decarinata*	Dalhatu Specialist Hospital	DH2	08°30′08.95″ N 08°31′21.95″ E
10		Lafia East	LE2	08°29′33.57″ N 08°32′27.10″ E
11		GRA, Keffi	GRA3	08°50′53.25″ N 07°53′08.48″ E

body sizes were measured in millimetres (mm) with three decimal digits. Measurements and most indices are derived from Fischer et al. (2012). The general morphological terminology followed was from Bolton (1994) and Longino (2009). Identification of the specimens was achieved by comparing specimens with coloured ant images available on www.AntWeb.org, antsofafrica.org, and taxonomic keys of Collingwood and Agosti (1996), Wheeler (1922), and other relevant literature. The photomontages of the ant specimens were produced by cutting the larger images taken and their scales were recorded using ImageJ software (Fiji) downloaded online.

Measurements

The general morphological terminologies of insect identification from Bolton (1994) and Longino (2009) were used in the morphological identification of the insect specimens.

FL Full length; outspread length of the insect from the mandible apex to the gaster apex.
HL Head length; the full head length except for the mandibles.
HW Head width; full head width behind the eyes in full-face view.
EL Eye length; full eye diameter.
PH Petiole height; the full height of a petiolar node in lateral view from the peak (middle) of the node to ventral outline.
PPH Postpetiole height; the full height of postpetiole measured in lateral view from the highest (median) point of the node to the ventral outline.
PL Petiole length; full length in dorsal view, from anterior to posterior margin.
PPL Postpetiole length; full length in dorsal view.
PPW Postpetiole width; full width in dorsal view.
PRW Pronotal width, the full length of the width in dorsal view.
PW Petiole width; full width in dorsal view.
SL Scape length without the basal neck.

Indexes

CI Cephalic Index: HW / HL × 100
SI Scape Index: SL / HW × 100
FI metafemur index: MFL / HW × 100

Molecular analysis

PCR amplification and molecular identification

For molecular identification using PCR techniques, mitochondrial DNA Cytochrome oxidase subunit 1 (CO1) gene of the ants were sequenced using the primer pairs, LCO1490/HCO2198 and LepF1/LepR1 according to Ng'endo et al. (2013). The primers were commercially synthesized (Apical Scientific Sdn. Bhd., Malaysia) and used in both forward and reverse directions. The following amplification parameters were applied using the TaKaRa PCR Thermal Cycler Dice™ Mini (Takara Bio Inc. Japan); heated lid thermocycler was used to amplify DNA; 25 µl reactions were prepared by adding 8 pmol of each primer (1 µl of each primer), 4 µl of template DNA, 12.5 µl of Master Mix (EconoTaq PLUS GREEN 2X Master Mixes, Lucigen), and 6.5 µl of double-distilled water. All amplification reactions were heated at 105 °C for 5 min. The PCR protocol used with the HCO/LCO universal primers was i) 94 °C for 60 s, ii) 45 °C for 48 s, and iii) 72 °C for 60 s for 35 cycles, prior to a final extension step at 72 °C for 10 min and stored at 4 °C (Ng'endo et al. 2013). When LepF1/LepR1 were used, PCR parameters were the same as above except for annealing temperature which was set at 48 °C for 60 s.

Agarose gels containing ethidium bromide (0.1 lg per ml) with concentrations of 1.0–1.5% (w/v) was used to visualize the samples using SafeBlue illuminator Electrophoresis System, MBE-15. 100-bp DNA ladder was used as molecular weight markers. PCR product was cleaned up to reproduce a pure form for DNA sequencing at Apical Scientific Sdn. Bhd, Malaysia. Using the DNA sequence obtained from the service provider, the insect species identities were confirmed through the Basic Local Alignment Search Tool (BLAST) program from the NCBI database. A successful insect species identification is based on the presence of a band on an agarose gel in the PCR product, a high-quality sequencing and 95% similarities to the reference sequences in NCBI or any public databases. Selection of 95% as a similarity criterion is based on the recommendations by Lin et al. (2015) who suggested a 4–5% threshold as acceptable to delineate closely related species. Eventually, the sequences were submitted to the NCBI database.

Phylogenetic analysis

Sequence composition and analysis were estimated using Molecular Evolutionary Genetics Analysis version 7.0, MEGA7 (Kumar et al. 2016). All eleven DNA sequences were aligned via CLUSTAL-W (Thompson et al. 1994). The evolutionary history was deduced using the Neighbor-Joining method (Saitou and Nei 1987). An optimal tree with branch length of 0.22456576 was presented. Evolutionary distances were figured using the p-distance method (Nei and Kumar 2000) and presented as the number of base differences per site. The evolutionary analysis involved 12

nucleotide sequences (MT458705.1 *Pheidole parva* isolate USMBP was an outgroup). DNA sequence polymorphism estimation such as genetic differentiation and gene flow estimation were performed using DnaSP (Rozas et al. 2017).

Results

All insect samples have shown dimorphic ant species (Figs. 1, 2). Major workers have disproportionately enlarged heads and broad tridentate mandibles, but minor workers have a narrow head with large multidentate mandibles. A total of 1143 *Pheidole* ants were collected from 14 different locations, in which *P. rugaticeps* Emery (52%) and *P. decarinata* Santschi (16%) are the most abundant in the areas. Both species are highly scavenging ants collected in the indoors and outdoors, and forage during the day and night although *P. decarinata* Santschi is more active during the night.

Species identifications

Pheidole rugaticeps Emery, 1877

Description of workers

Major (n = 10). — HL 1.62–1.91 mm; HW 1.52–1.90 mm; SL 0.78–1.16 mm; FL 1.25–1.78 mm; EL 0.18–0.27 mm; MDL 0.41–0.66 mm; ML 1.27–1.48 mm; PSL 0.11–0.15 mm; PTL 0.31–0.45 mm; PTH 0.27–0.35 mm; PPL 0.27–0.32 mm; PPH 0.26–0.35 mm; PPW 0.31–0.37 mm; CI 94–99; SI 51–61; FI 77–94.

Pheidole rugaticeps Emery is large and has medium reddish-brown to dark colour. The major workers are long-limbed (Fig. 1) with a subquadrate head and lengthy yet parallel longitudinal rugae/striate in a full-face view (Fig. 1a). The face lacks frontal carinae & antennal scrobes (Fig. 1a). Posterior margin of the head is slightly free of distinct rugae. The promesonotum has two convexities in profile view: a large anterior dome and a distinct prominence on the posterior slope (Fig. 1b). The mesonotum and propodeum are densely punctate and shiny (Fig. 4), while propodeal spines are short and erect (Fig. 3). Both petiole and postpetiole are densely dotted/punctate anteriorly and shiny (Fig. 3), although the latter is slightly or not wider than the length. The postpetiole is also conspicuously enlarged and dome-like with bulging anteroventral and posterodorsal (Fig. 3). The gaster is dark and shiny with a densely punctate first tergite segment. The whole body is covered with dispersed erect hair including the antennae (Fig. 2e-f).

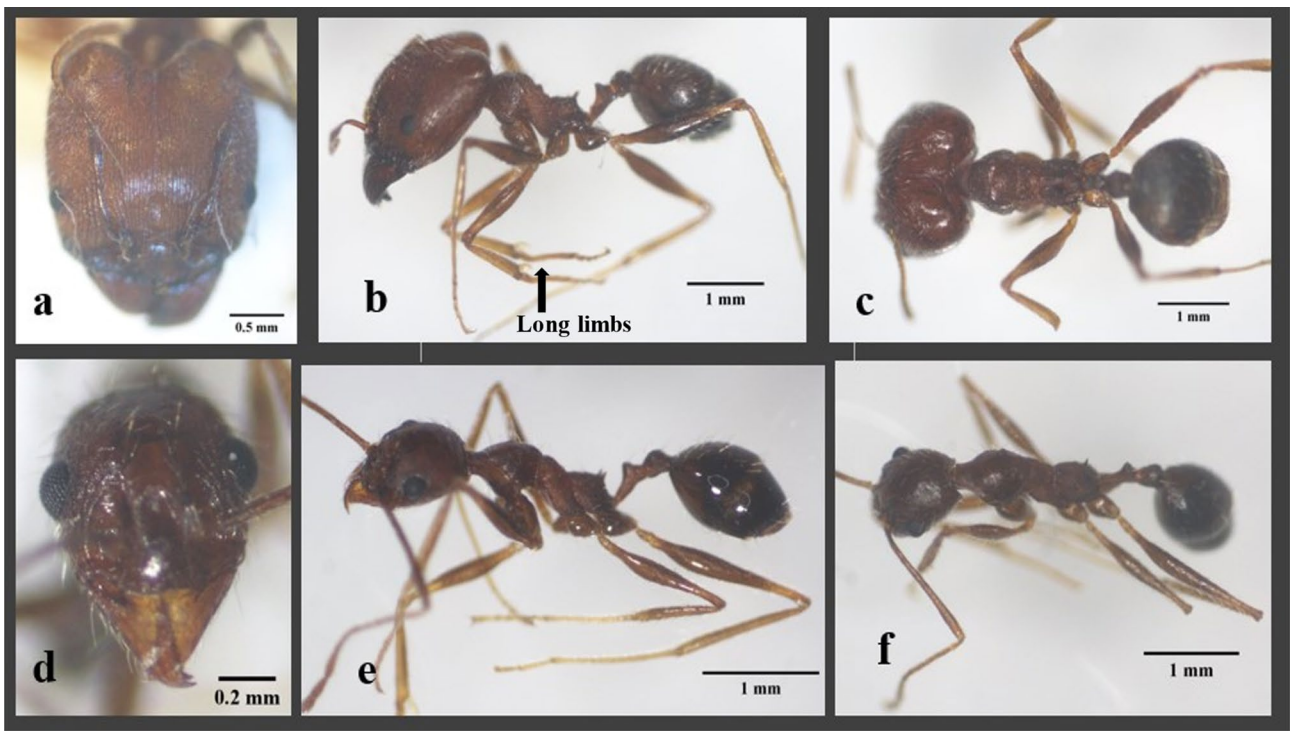

Fig. 1 Head, lateral, and dorsal views of workers of *P. rugaticeps* Emery. **a** Full-face view of major worker. **b** Lateral view of major worker, the arrow showing the long-limbed. **c** Dorsal view of the major worker. **d** Full-face view of minor worker. **e** Lateral view of minor worker. **f** Dorsal view of the minor worker

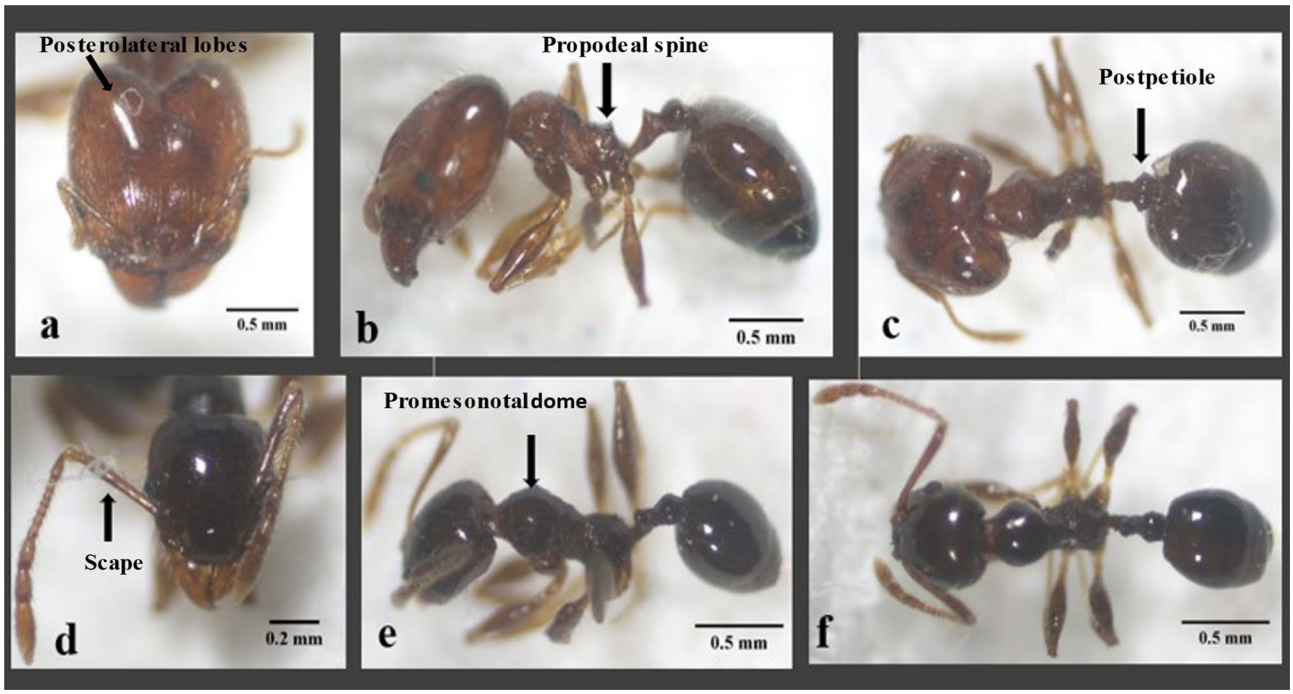

Fig. 2 Head, lateral, and dorsal views of workers of *P. decarinata* Santschi. **a** Full-face view of major worker, the posterolateral lobes showing lack of rugae, foveolate and carinae. **b** Lateral view of major worker, the arrow indicating the short propodeal spines. **c** Dorsal view of the major worker, arrow indicating postpetiole wider than the petiole. **d** Full-face view of minor worker, showing slightly longer scape compared to that of the major workers. **e** Lateral view of minor worker, arrow showing a round and slightly angulate promesonotal dome. **f** Dorsal view of the minor worker

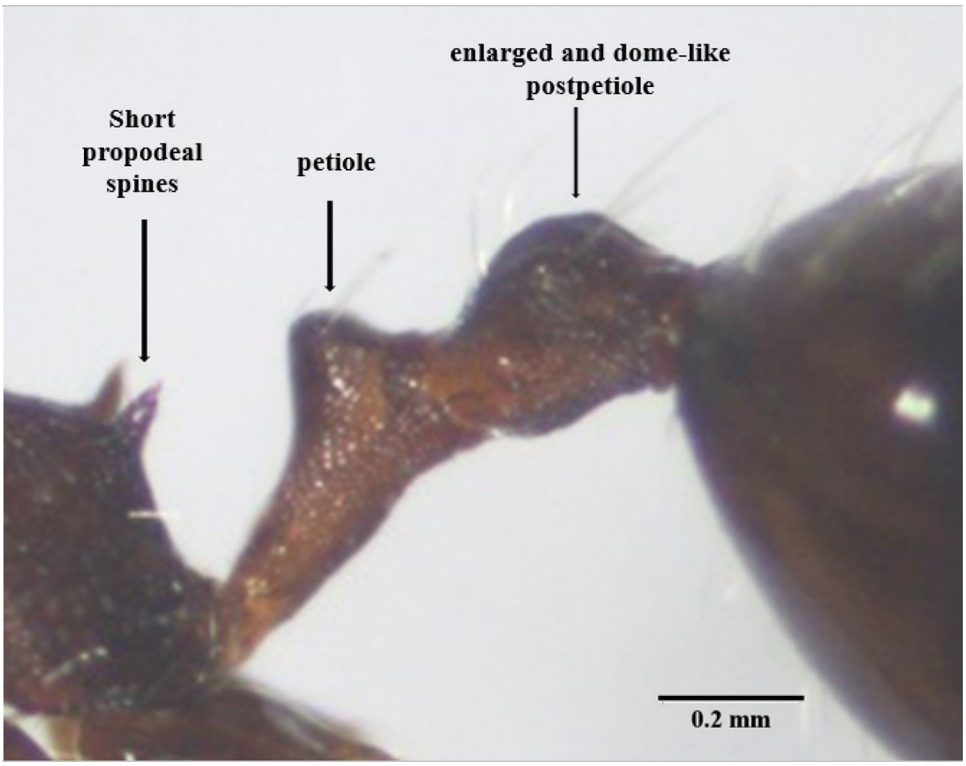

Fig. 3 Lateral view of *P. rugaticeps* major showing the short spines, punctation, anteroventral and posterodorsal bulge of the postpetiole

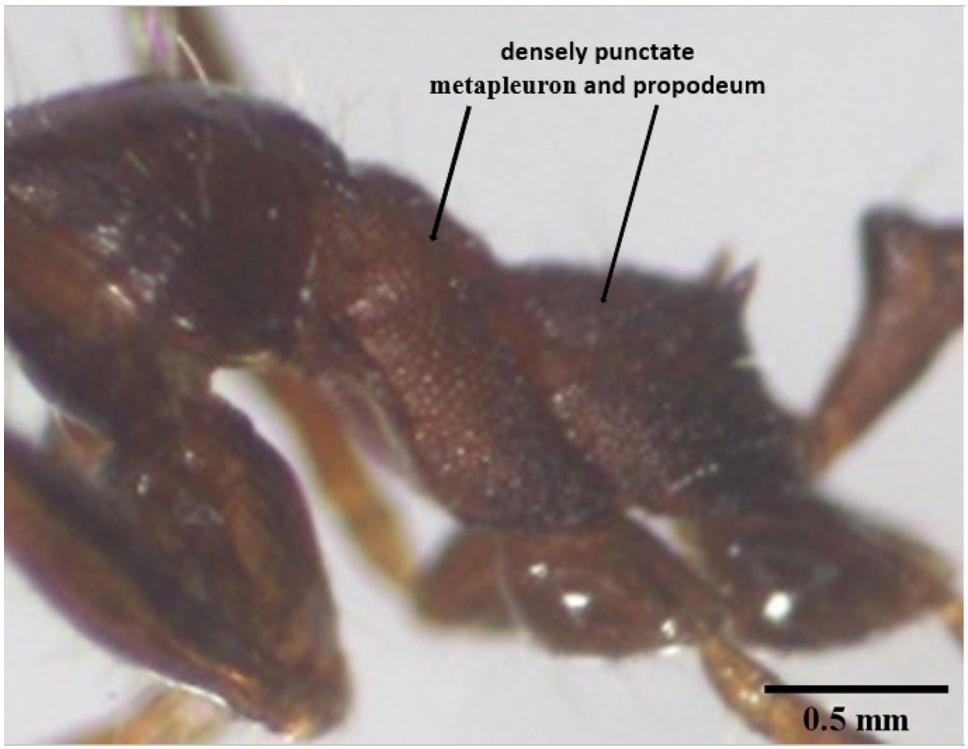

Fig. 4 Lateral view of the mesopleuron *P. rugaticeps* minor showing the punctation on the mesopleuron and a pair of short propodeal spines

Minor (n = 10). — HL 0.64–0.84 mm; HW 0.58–0.76 mm; SL 0.91–1.22 mm; FL 1.01–1.39 mm; EL 0.17–0.22 mm; MDL 0.28–0.38 mm; ML 0.98–1.26 mm; PSL 0.07–0.09 mm; PTL 0.24–0.34 mm; PTH 0.16–0.21 mm; PPL 0.18–0.25 mm; PPH 0.14–0.22 mm; PPW 0.17–0.23 mm; CI 84–90; SI 157–161; FI 174–183.

In full-face view, the head shape is broadly rounded to slightly flattened posterior margin (see Fig. 2d). It is completely glossy with extremely long and hairy antennal scapes (Fig. 1d). In lateral view, the promesonotum forms two convexities, namely a large anterior dome and a distinct prominence on the posterior slope (Fig. 2e). Mesopleuron is entirely punctate with a moderately produced pair of short propodeal spines (Fig. 4). Postpetiole has a slender dorsal but slightly wider than petiole. It is relatively more swollen compared to petiole and anteroventrally bulged (Fig. 2e). The gaster is shiny and densely dotted/punctate anteriorly identical to the major worker. The body is covered with erect hair including the antennae (Fig. 2e-f).

Pheidole decarinata santschi

Description of workers

Major (n = 10). — HL 1.62–1.91 mm; HW 0.95–1.20 mm; SL 0.56–0.73 mm; FL 0.69–0.90 mm; EL 0.12–0.15; MDL 0.24–0.49; ML 0.80–0.97 mm; PSL 0.03–0.05 mm; PTL 0.23–0.31 mm; PTH 0.20–0.21 mm; PPL 0.16–0.20 mm; PPH 0.16–0.20 mm; PPW 0.24–0.32 mm; CI 102–108; SI 59–61; FI 72–75.

The major workers are relatively small, having short scapes and legs, and reddish-brown coloured with a dark gaster (Fig. 2). Average body length obtained from the morphological studies is 2.89 mm (2.59–3.07 mm, n = 10). Their integument is shining brightly (Fig. 2). The head is longer than the width and relatively large compared to the rest of their body. It also has a subquadrate shape and glossy, with the posterolateral lobes is lacking rugae, foveolate and carinae (Fig. 2a). Mesosoma, petiole and postpetiole are similar to minor workers in lateral view but the postpetiole of the major is slightly angled in dorsal view (see Fig. 3b-f). In lateral view, the promesonotum forms a single dome (Fig. 2b) and lacks a distinct prominence on the posterior slope (Fig. 2b). Both metapleuron and petiolar nodes have some punctation (Fig. 5). The propodeal spine is short for both major and minor workers, and the bodies are covered with relatively abundant long and fine erect hairs.

Minor (n = 10). — HL 0.49–0.58 mm; HW 0.44–0.56 mm; SL 0.49–0.66 mm; FL 0.51–0.70 mm; EL 0.11–0.14; MDL 0.16–0.21; ML 0.61–0.73 mm; PSL 0.01–0.02 mm; PTL 0.15–0.21 mm; PTH 0.13–0.15 mm; PPL 0.13–0.16 mm; PPH 0.13–0.14 mm; PPW 0.14–0.18 mm; CI 90–97; SI 111–118; FI 116–125.

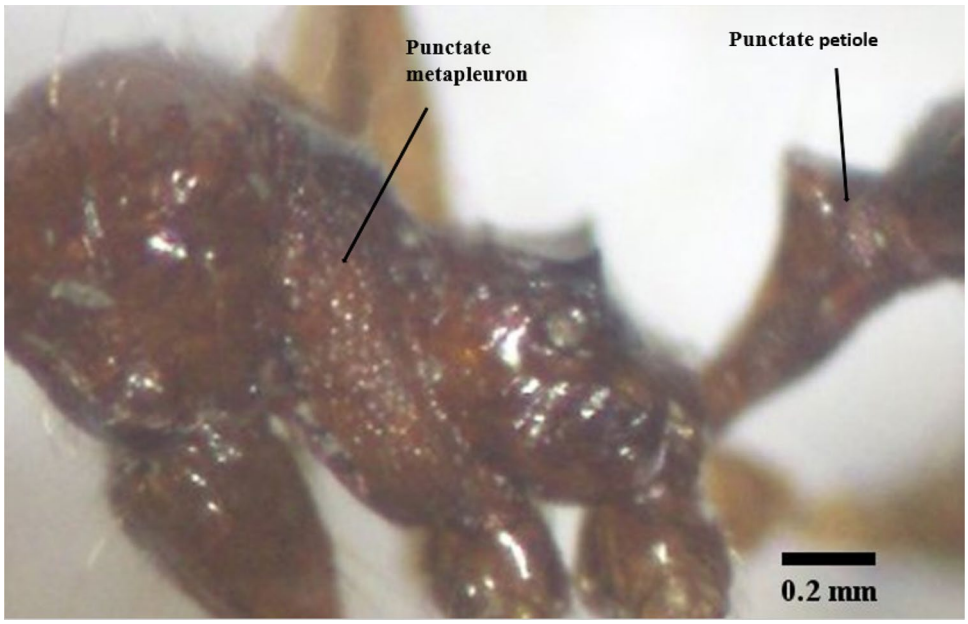

Fig. 5 Lateral view of the mesopleuron *P. decarinata* major showing the punctation on the mesopleuron

Minors of the *P. decarinata* are smaller with an average full length of 1.85 mm (1.73–2.02 mm, n = 10). Their appearances are dark reddish-brown (see Fig. 2d-f) and have a slightly longer scape compared to the major workers. The posterior head margin is compressed or flattened (Fig. 2d). The head is moderate size relative to the rest of their body. Promesonotal dome is round and slightly angulate in profile view (see Fig. 2e). The spines of the minors are relatively short with some punctation on the metapleuron and petiolar node (Fig. 5). Both petiole and postpetiole are relatively short but the latter is raised in lateral view and wider in dorsal view (Fig. 2c). Postpetiole also lack ventral process and not angulate dorsally. The propodeal spine is short in both major and minor workers (Fig. 2). The whole body is covered with relatively abundant long and fine erect hairs.

Molecular and phylogenetic result

Eleven genomic DNA sequences of the mitochondrial COI gene (700 base pairs) between 403 – 565 bp from *P. rugaticeps* and *P. decarinata* were successfully recovered and submitted to the National Center for Biotechnology Information (NCBI). The accession number and percentage similarity match are shown in Table 2. A sequence matched of 89.83% and 87.96% were obtained with *Pheidole annemariae* voucher CASENT0198029-D01 (HM419524.1) and *Pheidole ampla* voucher RA0358 (accession no EF518309.1) (Moreau 2008), respectively. The blast result of the *P. rugaticeps* sequences showed 87.03% match with *Pheidole colaensis* isolate PH409 (KJ141886.1) and 86.55% match with the *Pheidole roosevelti* voucher EMS2343 (HM144368.1).

Table 2 Organisms, accession number and percentage similarity with available sequences in GenBank database

S. No	Organism	Code/ Isolate	Query Sequence Accession No	% Similarity	Database Sequence
1	*Pheidole rugaticeps*	3EE1	MT309805	87.03%	KJ141886.1
2	*Pheidole rugaticeps*	LHE4	MW080378	86.55%	HM144368.1
3	*Pheidole rugaticeps*	KRK4	MW080379	86.55%	HM144368.1
4	*Pheidole rugaticeps*	KDR4	MW080380	86.55%	HM144368.1
5	*Pheidole rugaticeps*	PHC4	MW080381	86.54%	HM144368.1
6	*Pheidole rugaticeps*	DH4	MW080382	86.19%	HM144368.1
7	*Pheidole rugaticeps*	GRA4	MW080383	86.55%	HM144368.1
8	*Pheidole rugaticeps*	POLY2	MW080384	86.55%	HM144368.1
9	*Pheidole decarinata*	DH1	MT308581	89.83%	HM419524.1
10	*Pheidole decarinata*	LE2	MW080385	87.96%	EF518309.1
11	*Pheidole decarinata*	GRA3	MW080386	87.96%	EF518309.1

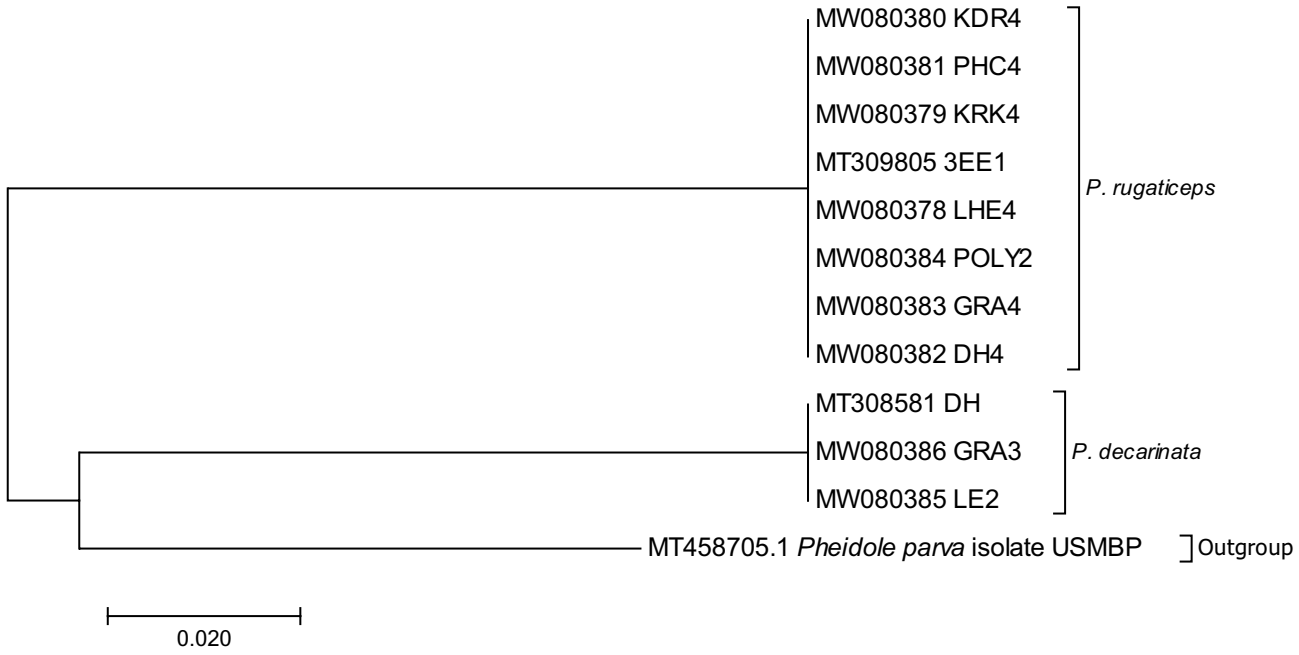

Fig. 6 Phylogenetic relationships of *P. rugaticeps* Emery and *P. decarinata* Santschi collected from 11 different locations

The molecular identification of the specimen sequence revealed two distinct species of *Pheidole* separated by Neighbour-Joining tree as represented in Fig. 6. Molecular phylogenetic tree indicated that both *Pheidole* species are genetically distinct to each other since the populations are visibly distributed in two major groups. The pairwise genetic distance (p-distance) within each population is 0.000 whereas the p-distance between both population groups is 0.166 (Table 3). Result of the gene flow estimation between the two *Pheidole* species showed a complete divergence (FST = 1.0000 (Nm = 0.00)) and a highly significant genetic differentiation (Chi2: 11.000, df = 1. *P-value* 0.0009). Overall haplotype and nucleotide diversity found from eleven sequences of both *Pheidole* species populations are shown in Table 4. The haplotype diversity (Hd) of eleven samples is 0.43636. Individually, the haplotype diversity (Hd) and nucleotide diversity (Pi) of each haplotype group are 0.00000.

Discussion

Ants are the only eusocial hymenopteran that produces morphologically complex worker castes. Among ants, *Pheidole* is one of the few ecologically successful genera that produce highly polymorphic workers (Collingwood and Agosti 1996; Dolezal 2019). In most *Pheidole* species, there is a worker polymorphism which mostly bimodal (trimodal in rare cases) which develop into small minor workers or

Table 3 Pairwise genetic distance (p-distance) within and between the two species studied

	Acc no/	1	2	3	4	5	6	7	8	9	10	11
1	MT309805_3EE1 {1}											
2	MW080384_POLY2 {1}	0.000										
3	MW080383_GRA4 {1}	0.000	0.000									
4	MW080382_DH4 {1}	0.000	0.000	0.000								
5	MW080381_PHC4 {1}	0.000	0.000	0.000	0.000							
6	MW080380_KDR4 {1}	0.000	0.000	0.000	0.000	0.000						
7	MW080379_KRK4 {1}	0.000	0.000	0.000	0.000	0.000	0.000					
8	MW080378_LHE4 {1}	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
9	MT308581_DH {2}	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166			
10	MW080386_GRA3 {2}	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.000		
11	MW080385_LE2 {2}	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.000	0.000	

Table 4 Overall Haplotype/nucleotide diversity found in two different populations of *Pheidole* species

S. No	Statistical Data Name	Total Data Estimates
1	Number of sequences	11
2	Number of segregating sites (S)	67
3	Number of haplotypes (h)	2
4	Haplotype diversity (Hd)	0.43636
5	Average number of nucleotide differences (Kt)	29.23636
6	Nucleotide diversity (p)	0.07255

*Government Residential Area (GRA), Kwandare (KDR), Primary Healthcare Akwanga (PHC), Polytechnic Lafia (Poly), Housing Estate Akwanga (HE), Kurikyo (KRK), Dalhatu Arab Specialist Hospital (DH) and Lafia East (LE)

large major workers (Dolezal 2019). Morphological identification of collected specimens to genus *Pheidole* was less demanding due to the dimorphism in head and body sizes and shapes of the worker caste. Hence the *Pheidole* samples collection and identification to genus level was done without challenges. However, species-level identification is highly demanding and difficult, especially the Afrotropical species with vast unidentifiable morphospecies (Belshaw and Bolton 1993; Deblauwe and Dekoninck 2007; Fisher 2004; Hita Garcia et al. 2009). Morphological diagnosis of the worker castes was consistent with *P. rugaticeps* Emery, 1877 specimen (CASENT0281618) and *P. decarinata* Santschi specimen (CASENT0913301) from www.AntWeb.org.

From morphological examination and comparison of the *Pheidole* samples collected with the online images from www.AntWeb.org, antsofafrica.org and taxonomic keys of Emery (1877), Wheeler (1922), Santschi (1929), Collingwood and Agosti (1996), and other relevant literature, the specimens (3EE1, LHE4, KRK4, KDR4, PHC4, DH4, GRA4 and POLY2) were found to be consistent with *P. rugaticeps* Emery specimen (CASENT0281618), whereas specimens from DH1, LE2 and GRA3 were consistent with *P. decarinata* Santschi specimen (CASENT0913301). Both species were valid and native to the Afrotropical region (Bolton 2020). *P. rugaticeps* has been reported to originates from the Afrotropical region of Central African (Menozzi 1926), Congo (Wheeler 1922), Eritrea, Ethiopia, Nigeria, Senegal (Bolton 2020; Diame et al. 2017; Madl 2019), Gambia (Borowiec and Salata 2018), Mali (Taylor et al. 2016), and Somalia (Menozzi 1930). Similarly, a subspecies known as *Pheidole rugaticeps* Arab from the Arabian Peninsula and some parts of North Africa have also been reported (Collingwood and Agosti 1996; Sharaf et al. 2018; Borowiec 2014). Likewise, *P. decarinata* has also been reported recently in Mali, Sudan (Bolton 2020).

The morphological features provided by Emery (1877), and Wheeler (1922), and Collingwood and Agosti (1996), as well as online images available on www.AntWeb.org and www.antsofafrica.org, made it possible to identify *P. rugaticeps* with ease particularly the head striation/sculpture and form of the major workers' postpetiole. However, other features such as the two convexities along the promesonotum slope of major workers that form a large anterior dome and a distinct prominence on the posterior slope are similar to of the invasive species, *P. noda* described by Sarnat et al. (2015). This study utilizes the taxonomic description by Sarnat et al. (2015) and observed punctuation on the mesopleuron and propodeum of the *P. rugaticeps* major workers. This feature was described by Emery (1877) as "subtly grainy". Similarly, the lateral view of minor workers also shows punctation on both mesopleuron and propodeum. However, the propodeum of the major workers also has rugose sculpture as well. Nonetheless, the sculptured and punctate face with a large glossy medium section of *P. rugaticeps* minor workers resembles the minor workers of *P. rugosula* described by Sarnat et al. (2015).

As mentioned earlier, *P. rugaticeps* Arab is a subspecies from Saudi Arabia, Oman, and Yemen (Collingwood and Agosti 1996) that shared the same features with *P. rugaticeps* Emery except for a slightly smoother head sculpture. All other features are shared by this sister species. However, the findings of this study obtained head width (HW) between 1.62–1.90 mm whereas Collingwood & Agosti (1996) established it to be greater than 2 mm. The head is full of wrinkled striate at both sides up to occiput which agrees with the description by Collingwood & Agosti (1996). But the peak of the posterolateral lobes appears to be smooth and shiny. Another Afrotropical species that slightly resemble *P. rugaticeps* is the *Pheidole rebeccae* described by Fischer et al. (2012), but they can be easily distinguishable by the length of the propodeum spines. *P. rugaticeps* workers have shorter propodeum spines (Fig. 3) compared to *Pheidole rebeccae* (Fischer et al. 2012). Images of *P. rugaticeps* taken in this study matched with images of *P. rugaticeps* Emery from AntWeb.org, antsofafrica.org, and Borowiec & Salata (2018). As observed in *P. megacephala* (Sarnat et al. 2015), the postpetiole of *P. rugaticeps* also has both anteroventral and posterodorsal bulges.

Only a few taxonomic information available on *P. decarinata* Santschi. However, features observed from *P. decarinata* specimen in this study were similar to the taxonomic description of Santschi (1929) as well as the images from www.AntWeb.organd www.antsofafrica.org (Taylor 2020). These are the only sources for taxonomy information of *P. decarinata* can be found. Hence, the comparative taxonomic analysis of *P. decarinata* specimens in this study with different species, having similar sizes and colouration recorded in Nigeria and other Afrotropical countries, shows a very close resemblance to the ones described by Santschi (1929), online images from www.AntWeb.org (casent0913301) and other *P. decarinata* images from www.antsofafrica.org (Taylor 2020).

Phylogenetic results suggested that *P. rugaticeps* Emery and *P. decarinata* Santschi are two distinct *Pheidole* species, and both sequences showed less than 90% match to the available *Pheidole* sequences in the public reference libraries (Table 2). According to the recommendations of Lin et al. (2015), a threshold of 4–5% is appropriate to delineate closely related species. *Pheidole colaensis* isolate PH409 (KJ141886.1) and *Pheidole roosevelti* voucher EMS2343 (HM144368.1) are the closest match to the *P. rugaticeps* sequences based on the blast results. However, both *P. colaensis* and *P. roosevelti* are from different geographic regions (Sarnat 2008) in Fiji with distinct morphological features especially the shape of their head, mesosoma and spine as observed in Sarnat & Moreau (2011).

Moreover, sequences obtained from the *Pheidole decarinata* specimens are closely matched to *Pheidole annemariae* voucher CASENT0198029-D01 (HM419524.1) and *Pheidole ampla* voucher RA0358 (accession no EF518309.1) submitted by Moreau (2008). Geographically, *Pheidole annemariae* are native to the Afrotropical region but morphologically dissimilar to the *Pheidole decarinata* while *P. ampla* is native to Australasian Region (Moreau 2008). Morphologically, Salata and Fisher (2020) described *P. annemariae* to be dissimilar with *P. decarinata* specimens. Therefore, COI sequences of both *P. rugaticeps* and *P. decarinata* were submitted to the NCBI database for the first time and accession numbers are shown in Table 2. These sequences will serve as additional diagnostic tools for future studies.

The pairwise genetic distance from the eleven samples (8 from *P. rugaticeps* and 3 from *P. decarinata*) and the value within each of the two populations are 0.000 and 0.166 respectively. Previously, Fournier et al. (2009) established no genetic differentiation between nests of *Pheidole megacephala.* The study also reported a weak genetic differentiation between populations of *Pheidole megacephala* primarily from private alleles (Fournier et al. 2009). The haplotype analysis of all sequences uncovered two haplotypes groups (Table 3). Eight sequences formed the haplotype 1 and three sequences make up the haplotype 2 (Table 4). Additionally, the FST value (1.00000) revealed the sequences from the two haplotype populations are highly different. Similarly, previous studies on genetic diversity of ants also revealed a high genetic diversity between ants (Schmidt et al. 2010; Szalanski et al. 2010). The used of DNA barcoding alongside the traditional taxonomic studies can go a long way in limiting the insect identification restrictions faced by taxonomists especially those of the hyper-diverse genus like *Pheidole*.

Acknowledgements This research was funded under the Universiti Sains Malaysia (USM) Bridging Fund (304/PBIOLOGI/6316510)

Declarations

Conflicts of Interest The authors declare no conflict of interest.

References

AntWeb (2020) *Pheidole decarinata* Santschi, 1929. https://www.antweb.org/description.do?name=decarinata&genus=Pheidole&rank=species. Accessed 22 October 2020

Arnold G (1960). New species of African Hymenoptera. No. 15. Occasional Papers of The National Museum of Southern Rhodesia 3 (No. 24B): 452–488

Belshaw R, Bolton B (1993) The effect of forest disturbance on the leaf litter ant fauna in Ghana. Biodivers Conserv 2:656–666. https://doi.org/10.1007/BF00051965

Bernard F (1953) La réserve naturelle intégrale du Mt Nimba. XI. Hyménoptères Formicidae. Mémoires de l'Institut Français d'Afrique Noire 19: 105

Bolton (2020) An Online Catalog of the Ants of the World. Retrieved from https://www.antcat.org/catalog/429833

Bolton B (1994) Identification Guide to the Ant Genera of the World. Harvard University Press

Borowiec L (2014) Catalog of ants of Europe, the Mediterranean Basin, and adjacent regions (Hymenoptera: Formicidae). Genus (wroclaw) 25(1–2):1–340

Borowiec L, Salata S (2018) Notes on ants (Hymenoptera: Formicidae) from Gambia (Western Africa). Annals of the Upper Silesian Museum in Bytom Entomology 27:1–13. https://doi.org/10.5281/zenodo.1481794.Accessed 22 October 2020

Collingwood CA, Agosti D (1996) Formicidae (Insects: Hymenoptera) of Saudi Arabia (Part 2). Fauna of Saudi Arabia 15:300–385

Deblauwe I, Dekoninck W (2007) Diversity and distribution of ground-dwelling ants in a lowland rainforest in southeast Cameroon. Insectes Soc 54:334–342. https://doi.org/10.1007/s00040-007-0951-8

Diame L, Taylor B, Blatrix R, Vayssieres JF, Rey JY, Grechi I, Diarra K (2017) A preliminary checklist of the ant (Hymenoptera, Formicidae) fauna of Senegal. Journal of Insect Biodiversity 5(15):1–16

Dolezal AG (2019) Caste determination in arthropods. In Encyclopedia of Animal Behavior (2nd edn, Vol. 4). https://doi.org/10.1016/B978-0-12-809633-8.20815-7

Eguchi K, Bui TV (2005) A new South Vietnamese species of the genus *Pheidole* with a truncated head in the major and queen. Sociobiology 45:721–730

Eguchi K (2000) Two new *Pheidole* species with a 5-segmented antennal club. Entomological Science 3:687–692

Eguchi K (2001a) A revision of the Bornean species of the ant genus *Pheidole*. Tropics 2:1–155

Eguchi K (2001b) A taxonomic study on Asian *Pheidole* (Hymenoptera, Formicidae): New synonymy, rank Changes, lectotype designations, and redescriptions. Insecta Koreana 18:1–35

Eguchi K (2008) A revision of Northern Vietnamese species of the ant genus *Pheidole*. Zootaxa 1902:1–118

Eguchi K, Yamane S, Zhou SY (2007) Taxonomic revision of the *Pheidole rinae* Emery complex. Sociobiology 50:257–284

Emery C (1877) Catalog of the ants existing in the collections of the Civic Museum of Genoa. Part one. Ants from the journey of the Antinori, Beccari and Issel lords in the Red Sea and in the land of the Bogos [concluded]. Annals of the Civic Museum of Natural History 9:369–381

Fischer G, Garcia FH, Peters MK (2012) Taxonomy of the ant genus *Pheidole* Westwood (Hymenoptera: Formicidae) in the Afrotropical zoogeographic region: Definition of species groups and systematic revision of the *Pheidole* pulchella group. Zootaxa, 3232(1):1–43. https://doi.org/10.11646/zootaxa.3232.1.1

Fisher BL (2004) Diversity patterns of ants (Hymenoptera: Formicidae) along an elevational gradient on Monts Doudou in Southwestern Gabon. California Academy of Sciences Memoir 28:269–286

Fournier D, De Biseau JC, Aron S (2009) Genetics, behaviour and chemical recognition of the invading ant Pheidole megacephala. Mol Ecol 18:186–199. https://doi.org/10.1111/j.1365-294X.2008.04018x

Hita Garcia F, Fischer G, Peters MK, Wägele JW (2009) A preliminary checklist of the ants (Hymenoptera: Formicidae) of Kakamega Forest (Kenya). J East Afr Nat Hist 98:147–165. https://doi.org/10.2982/028.098.0201

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lin X, Stur E, Ekrem T (2015) Exploring genetic divergence in a species-Rich insect genus using 2790 DNA barcodes. PLoS ONE 10:e0138993

Longino JT (2009) Additions to the taxonomy of New World *Pheidole* (Hymenoptera: Formicidae). Zootaxa 2181(2181):1–90. https://doi.org/10.11646/zootaxa.2181.1.1

Madl M (2019) Notes on the ant fauna of Eritrea (Insecta: Hymenoptera: Formicidae): type specimens deposited in the Natural History Museum Vienna (Austria) and a preliminary checklist. Annalen Des Naturhistorischen Museums in Wien B 121:9–18

Menozzi C (1926) Central African ants. Bulletin of the Italian Entomological Society 58:36–41

Menozzi C (1930) Ants from southern Italian Somalia. Memoirs of the Italian Entomological Society 9:76–130

Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011) How Many Species Are There on Earth and in the Ocean? PLoS Biol 9(8):e1001127. https://doi.org/10.1371/journal.pbio.1001127

Moreau CS (2008) Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). Mol Phylogenet Evol 48(1):224–239. https://doi.org/10.1016/j.ympev.2008.02.020

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford university press

Ng'endo RN, Osiemo ZB, Brandl R (2013) DNA barcodes for species identification in the hyperdiverse ant genus *Pheidole* (Formicidae: Myrmicinae). Journal of Insect Science (online) 13:27. https://doi.org/10.1673/031.013.2701

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Mol Biol Evol 34(12):3299–3302. https://doi.org/10.1093/molbev/msx248

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

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

Santschi F (1929) Mélange Myrmécologique Wien Entomol Ztg 46:84–93

Santschi F (1914) Formicides de l'Afrique occidentale et australe du voyage de Mr. le Professeur F. Silvestri. Bollettino del Laboratorio di Zoologia Generale e Agraria della Reale Scuola Superiore d'Agricoltura. Portici 8:309–385

Sarnat EM (2008) A taxonomic revision of the *Pheidole roosevelti*-group (Hymenoptera: Formicidae) in Fiji. Zootaxa 1767:1–36

Sarnat EM, Moreau CS (2011) Biogeography and morphological evolution in a Pacific island ant radiation. Mol Ecol 20(1):114–130. https://doi.org/10.1111/j.1365-294X.2010.04916.x

Sarnat EM, Fischer G, Guénard B (2015) Economo EP (2015) Introduced *Pheidole* of the world: Taxonomy, biology and distribution. ZooKeys 543:1–109. https://doi.org/10.3897/zookeys.543.6050

Schmidt AM, d'Ettorre P, Pedersen JS (2010) Low levels of nestmate discrimination despite high genetic differentiation in the invasive pharaoh ant. Front Zool 7:20. https://doi.org/10.1186/1742-9994-7-20

Sharaf MR, Fisher BL, Al Dhafer HM, Polaszek A, Aldawood AS (2018) Additions to the ant fauna (Hymenoptera: Formicidae) of Oman: an updated list, new records and a description of two new species. Asian Myrmecology 10:e010004

Sylvanus CE, Badejo MA, Ajayi OO (1997) Ants of forest and fallow plots in Nigeria. Biotropica 29(1):93–99

Szalanski AL, McKern JA, Solorzano C, Austin JW (2010) Genetic Diversity of Ants (Hymenoptera: Formicidae) from the Ozark-St. Francis National Forest, Arkansas, USA. Sociobiology 56:1–10

Taylor B (2020). The Ants of Africa, Genus Pheidole, Pheidole decarinata Santschi. http://antsofafrica.org/ant_species_2012/pheidole/pheidole_decarinata/pheidole_decarinata.htm. Accessed 22 October 2020

Taylor B (1976) Ants of the Nigerian Forest Zone (Hymenoptera: Formicidae). I. Ponerinae, Cerapachyinae, Pseudomyrmecinae. Cocoa Research Institute of Nigeria Technical Bulletin Series 4:1–41

Taylor B (1978) Ants of the Nigerian Forest Zone (Hymenoptera: Formicidae). II. Formicinae, Dolichoderinae. Cocoa Research Institute of Nigeria Research Bulletin 5:1–57

Taylor B (1979) Ants of the Nigerian Forest Zone (Hymenoptera: Formicidae). III. Myrmicinae (Cardiocondylini to Meranoplini). Cocoa Research Institute of Nigeria Research Bulletin 6:1–65

Taylor B (1980a) Ants of the Nigerian Forest Zone (Hymenoptera: Formicidae). IV. Myrmicinae (Myrmecinini to Tetramoriini). Cocoa Research Institute of Nigeria Research Bulletin 7:1–63

Taylor B (1980b) Ants of the Nigerian Forest Zone (Hymenoptera: Formicidae). V. Dorylinae, Leptanillinae. Cocoa Research Institute of Nigeria Research Bulletin 8:1–33

Taylor B, Yefremova ZA, Kravchenko VD, Muller GC (2016) The ants of Mali (Hymenoptera: Formicidae) - a historical review and new records. Entomofauna 37(35):545–564

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Ward PS (2000) Broad-scale patterns of diversity in leaf-litter ant communities. In D. Agosti, D., Majer, J. D., Alonso, L. E., & Schultz, T. R. (Eds.), Ants: Standard methods for measuring and monitoring biodiversity. Smithsonian Institution Press, Washington, pp. 99–121

Wetterer JK (2012) Worldwide spread of the African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). Myrmecological News 17:51–62

Wheeler WM (1922) Ants of the American Museum Congo expedition. A contribution to the myrmecology of Africa. IX. A synonymic list of the ants of the Malagasy region. Bulletin of the American Museum of Natural History 1005–1055

Wilson EO (1984) Tropical social parasites in the ant genus *Pheidole*, with an analysis of the anatomical parasite syndrome. Insectes Soc 31:316–334

Wilson EO (2003) *Pheidole* in the new world: A dominant, hyperdiverse ant genus. Rev Biol Trop 53(1–2):297–304