naturae

2025 • 4

The tropical ant *Pheidole megacephala* (Fabricius, 1793) in France and discrimination with the local *Pheidole pallidula* (Nylander, 1849)

Alain LENOIR, Elfie PERDEREAU,
Simon DUPONT, Romain LIBBRECHT, Pierre PICQUET,
Christophe GALKOWSKI & Jean-Luc MERCIER

art. 2025 (4) — Publié le 2 avril 2025
www.revue-naturae.fr

OFB OFFICE FRANÇAIS DE LA BIODIVERSITÉ
PUBLICATIONS SCIENTIFIQUES
MUSÉUM NAL HIST NATURELLE

Directeur de la publication / *Publication director* : Gilles Bloch,
Président du Muséum national d'Histoire naturelle

Rédacteur en chef / *Editor-in-chief* : Jean-Philippe Siblet

Assistante de rédaction / *Assistant editor* : Sarah Figuet (naturae@mnhn.fr)

Mise en page / *Page layout* : Sarah Figuet

Comité scientifique / *Scientific board* :
Luc Barbier (Parc naturel régional des caps et marais d'Opale, Colembert)
Aurélien Besnard (CEFE, Montpellier)
Hervé Brustel (École d'ingénieurs de Purpan, Toulouse)
Thierry Dutoit (UMR CNRS IMBE, vignon)
Éric Feunteun (MNHN, Dinard)
Romain Garrouste (MNHN, Paris)
Grégoire Gautier (DRAAF Occitanie, Toulouse)
Frédéric Gosselin (Irstea, Nogent-sur-Vernisson)
Patrick Haffner (PatriNat, Paris)
Frédéric Hendoux (MNHN, Paris)
Xavier Houard (MNHN, Paris)
Isabelle Le Viol (MNHN, Concarneau)
Francis Meunier (Conservatoire d'espaces naturels – Hauts-de-France, Amiens)
Serge Muller (MNHN, Paris)
Francis Olivereau (DREAL Centre, Orléans)
Laurent Poncet (PatriNat, Paris)
Nicolas Poulet (OFB, Vincennes)
Jean-Philippe Siblet (PatriNat, Paris)
Julien Touroult (PatriNat, Paris)

Couverture / *Cover* :
Alligator Bay (Beauvoir). A view of the heated greenhouse housing differents species of Crocodilians, Credit : P. Picquet /
Alligator Bay (Beauvoir). Photographie de la serre tropicale abritant plusieurs espèces de crocodiliens. Crédit photo : P. Picquet.

Naturae est une revue en flux continu publiée par les Publications scientifiques du Muséum, Paris
Naturae *is a fast track journal published by the Museum Science Press, Paris*

Les Publications scientifiques du Muséum publient aussi / *The Museum Science Press also publish:*
***Adansonia*, *Zoosystema*, *Anthropozoologica*, *European Journal of Taxonomy*, *Geodiversitas*, *Cryptogamie* sous-sections *Algologie*, *Bryologie*, *Mycologie*, *Comptes Rendus Palevol*.**

Diffusion – Publications scientifiques Muséum national d'Histoire naturelle
CP 41 – 57 rue Cuvier F-75231 Paris cedex 05 (France)
Tél. : 33 (0)1 40 79 48 05 / Fax : 33 (0)1 40 79 38 40
diff.pub@mnhn.fr / https://sciencepress.mnhn.fr

© Cet article est sous licence Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/)
ISSN (électronique / electronic) : 2553-8756

The tropical ant *Pheidole megacephala* (Fabricius, 1793) in France and discrimination with the local *Pheidole pallidula* (Nylander, 1849)

Alain LENOIR
Elfie PERDEREAU
Simon DUPONT
Romain LIBBRECHT
Institut de Recherche sur la Biologie de l'Insecte (IRBI), UMR 7261 CNRS,
Université de Tours, Faculté des Sciences,
Parc de Grandmont, F-37200 Tours (France)
alain-cataglyphis@orange.fr
elfie.perdereau@univ-tours.fr
simon.dupont@univ-tours.fr
romain.libbrecht@univ-tours.fr

Pierre PICQUET
Zoological Institution Alligator Bay,
62 Route du Mont Saint-Michel, F-50170 Beauvoir (France)
picquet.vet@gmail.com

Christophe GALKOWSKI
104 route de Mounic, F-33160 Saint Aubin de Médoc (France)
chris.gal@wanadoo.fr

Jean-Luc MERCIER
Institut de Recherche sur la Biologie de l'Insecte (IRBI), UMR 7261 CNRS,
Université de Tours, Faculté des Sciences,
Parc de Grandmont, F-37200 Tours (France)
jean-luc.mercier@univ-tours.fr

Submitted on 29 April 2024 | Accepted on 6 August 2024 | Published on 2 April 2025

Lenoir A., Perdereau E Dupont S, Libbrecht R., Picquet P., Galkowski C. & Mercier J.-L. 2025. — The tropical ant *Pheidole megacephala* (Fabricius, 1793) in France and discrimination with the local *Pheidole pallidula* (Nylander, 1849). *Naturae* 2025 (4): 35-43. https://doi.org/10.5852/naturae2025a4

ABSTRACT
Introduced ants appear to be more and more frequent in many countries, becoming sometimes invasives. Recently, *Pheidole megacephala* (Fabricius, 1793) was signaled in greenhouses near Mont-Saint-Michel and Paris. As the International Union for Conservation of Nature (IUCN) has identified it as one of the 100 worst invasive alien species worldwide, it was interesting to verify the identity of this species. We used morphological measures, cuticular hydrocarbons and genetical analyses (COI) compared to the local *Pheidole pallidula* (Nylander, 1849). All the items confirmed the presence of *P. megacephala*. Now, it does not appear to be invasive, but it needs to be surveilled.

KEY WORDS
Invasive ants,
chemosystematics,
Pheidole,
COI,
heated greenhouses.

 © Publications scientifiques du Muséum national d'Histoire naturelle, Paris.

RÉSUMÉ

La fourmi tropicale Pheidole megacephala *(Fabricius, 1793) en France et sa discrimination avec la fourmi locale* Pheidole pallidula *(Nylander, 1849).*

Les fourmis introduites apparaissent de plus en plus fréquemment dans de nombreux pays avec tous les problèmes que cela peut poser. En France, *Pheidole megacephala* (Fabricius, 1793), une espèce classée dans le top100 des envahissantes par l'IUCN, vient d'être signalée en France dans des serres chauffées à Alligator Bay (Beauvoir, près du Mont Saint-Michel) et à Paris (Muséum national d'Histoire naturelle). Nous avons voulu confirmer la détermination de cette espèce comparée à l'espèce locale *Pheidole pallidula* (Nylander, 1849). Pour cela nous avons utilisé les critères morphologiques, les hydrocarbures cuticulaires et la génétique (gènes COI). Tous confirment bien la présence de *P. megacephala* dans ces serres en France. Cette espèce ne paraît pas envahissante pour le moment mais il faudra la surveiller de près dans les années à venir.

MOTS CLÉS
Fourmis invasives,
chémosystématique,
COI,
Pheidole,
serres chauffées.

INTRODUCTION

Introduced ants appear to be more and more successful in many countries, becoming invasives, and recently *Pheidole megacephala* (Fabricius, 1793) was signaled in greenhouses near Mont-Saint-Michel and Paris. As the International Union for Conservation of Nature (IUCN) has identified it as one of the 100 worst invasive alien species worldwide, it was interesting to verify the identity of this species as *Pheidole* is a very large genus with hundreds of different species in the world (Wilson 2003; Moreau 2008; Hoffmann & Parr 2008; Sarnat *et al.* 2015).

In Europe *Pheidole pallidula* (Nylander, 1849) is the main local species of this genus (Bernard 1968; Blatrix *et al.* 2013; Lebas *et al.* 2016). It is well-known in France as the "Fourmi à grosse tête", one rare species with soldiers in our country.

Cuticular hydrocarbons (CHCs) cover the cuticles of all insects, serving as a waterproofing agent (Gibbs 1998) and as a communication signal (Martin & Drijfhout 2009). The CHCs profile is also an indicator of the species identity and is used in chemotaxonomy. We used cuticular hydrocarbons to classify European species of *Pheidole* in a chemosystematic point of view. In Europe, *Pheidole pallidula* have been investigated previously only in one place in France (Fournier *et al.* 2016). *P. megacephala* CHCs have been studied in Cameroon (Fournier *et al.* 2012) and other places (Madagascar, South Africa, Australia and Mauritius (Fournier *et al.* 2009, 2016). Molecular data obtained by Fournier *et al.* (2016) revealed two cryptic species of *P. megacephala*, one inhabiting urban areas and the other rainforests. We want also to see if *P. megacephala* invaders have changed their chemical signature along their migration from the Africa as they have been introduced nearly globally in tropical regions (Wetterer 2012; Sarnat *et al.* 2015). With a molecular barcode based on the COI genetic marker, we also wanted to add additional, robust identification of certain samples.

Exotic tropical *Pheidole* species have been sometimes found in France like:

– *Pheidole anastasii* Emery, 1896 in a tropical greenhouse in Paris (Blatrix *et al.* 2018);

– *Pheidole bilimeki* Mayr, 1870 in heated greenhouse in a plant store in Bordeaux (Casevitz-Weulersse & Galkowski 2009; Blatrix *et al.* 2018), but according to C. Galkowski (pers. comm.) it has never been seen again;

– *Pheidole megacephala* (Fabricius, 1793) (African big-headed ant, *Fourmi africaine à grosse tête*) found in two heated buildings in Bordeaux and Lyon, and in Paris in the large tropical greenhouse of the Jardin des plantes (Blatrix *et al.* 2018). In Spain it was found in Barcelona in 2016 (Espadaler & Pradera 2016);

– *Pheidole indica* (= *teneriffana* Mayr, 1879) from Canary Islands, a tropical invasive species, is now on the Mediterranean coasts and islands, but not in France.

MATERIAL AND METHODS

Cuticular hydrocarbons

Chemical studies were performed via a GC/MS-TQ Agilent with a column compound of 5% Phenyl –95% Dimethylpolysiloxane. The temperature program was 2 min at 150°C, and then increasing at 5°C/min to 320°C, and 5 min hold at 320°C (for more details see Lenoir *et al.* 2023).

Compounds were identified by their fragmentation pattern, compared to standard alkanes, library data, and Kovats retention indices. All compounds were included in the analyses. When it was impossible to estimate the amount of each co-eluted compound, they were treated as a single compound. Sterols and other contaminants like phthalates were not included.

All the % of CHCs are provided as mean ± SD. The data were analyzed using cluster analysis on % with Euclidean distances and the Ward method (Statistica 8.0 program). We also calculated the equivalent chain length ECL, which indicates the mean of hydrocarbons length ECL = (Σ(%CnxXn))/100), where Cn is the x number of carbons and Xn is the % of this category. Martin *et al.* (2019) called it the Mean chain-length. ECL is not frequently used in chemical discrimination as it is insufficient to discriminate precisely species but is a good indication to classify them into different groups according to the length of hydrocarbons. This index allows separating easily some different species as it appeared in the Dolichoderinae (Lenoir *et al.* 2023). We did not analyze hydrocarbons under C25 to avoid possible volatile compounds from the internal glands.

Genetic studies

On different samples from Alligator Bay (Beauvoir) and Jardin des plantes (Paris) sites genomic DNA was extracted from whole individuals with the NucleoMag® Tissue kit (Macherey Nagel) following manufacturer instructions. The COI gene (787 bp) was then amplified from each individual using primers C1-J-2183 (Jerry) (5' - CAA CAT TTA TTT TGA TTT TTT GG - 3') and TL2-N-3014 (5' - CCA ATG CAC TAA TCT GCC ATA TTA - 3') adapted from Simon *et al.* (1994). PCR amplifications, sequencing and species identification were like the protocol detailed in Tourneur *et al.* (2022). The species status of each sample was identified from NCBI databases using the BLAST tool (Altschul *et al.* 1990) and confirmed with phylogenetic analyses. The BLAST results were ranked by percent identity and the reference sequences with at least 99% identity to our sequences were used to assign the species status. Two phylogenetic trees were constructed using Neighbor-Joining (NJ) and Bayesian Inference (BI) methods in Geneious® 9.1.8 (Biomatters Ltd.). The NJ method was applied using the Tamura-Nei distance model with 1000 bootstrap replicates. For BI method, MrAIC was used to find the appropriate sequence evolution model for the data (GTR+I+G) (Nylander 2004). BI was carried out using MrBayes (Huelsenbeck & Ronquist 2001); the model was run for 100 000 generations. These phylogenetic analyses employed 12 *Pheidole* reference sequences: those for *Pheidole allani* Bingham, 1903 (GenBank Accession Number OQ731736), *P. taipoana* Wheeler, 1928 (NC_080222), *P. fervens* Smith, 1858 (NC_080217), *P. sagei* Forel, 1902 (OQ731740), *P. antipodum* (Smith, 1858) (BK012122), *P. sinica* (Wu & Wang, 1992) (OQ731741), *P. smythiesii* Forel, 1902 (NC_080219 and OQ731742) and *P. megacephala* (Fabricius, 1793) (NC_080221, KF171410, KF171414 and KJ141874). *Cardiocondyla elegans* Emery, 1869 (DQ023071) was used as outgroup. All sequences obtained in this study have been submitted to GenBank; their accession numbers are PP709021, PP709022, PP709023, PP709024 and PP709025.

Abbreviations

Collector names

A. L.	Alain Lenoir;
C. G.	Christophe Galkowsky;
C. L.	Claude Lebas;
D. F.	Denis Fournier;
E. M.	Emmanuella Maurizi;
J.-L. M.	Jean-Luc Mercier;
L. B.	Laurence Berville;
M. J.	Maxime Jacquot;
P. F.	Pierre Froidevaux;
P. P.	Pierre Picquet;
P. W.	Philippe Wegnez;
Q. R.	Quentin Rome;
R. B.	Rumsaïs Blatrix.

LIST OF *PHEIDOLE* SPECIES AND SAMPLES

Samples are indicated according to the rule number of the Department and name of the country, date of collect, GPS (latitude, longitude), altitude, name of collector (for example A. L., Alain Lenoir), number of samples. It is not representative of the distribution of the species, but only indication of the samples used in this work.

Famille Formicidae Latreille, 1809
Genre *Pheidole* Westwood, 1839

Pheidole pallidula (Nylander, 1849)
(Figs 1B; 2B; 3B)

Specimen distribution. — 27 specimens in 14 zones, 19 places.

Observed and examined material. — **France** • 1 observed specimen; Corrèze, Nespouls; 45°3'9"N, 1°30'44"E; 318 m alt.; 2.IX.2020; A. L. • 1 obs. specimen; Corsica, Foce; 41°37'43"N, 9°1'9"E; 610 m alt.; 21.V.2016; C. L. • 1 obs. specimen; Corsica, Sartène; 41°41'8"N, 9°2'8"E; 151 m alt.; 16.V.2016; C. L. • 1 obs. specimen; Corsica, Portigliolo; 41°47'48"N, 8°44'43"E; 49 m alt.; 15.V.2016; C. L. • 1 obs. specimen; Gard, Villeneuve-lès-Avignon; 43°57'12"N, 4°47'21"E; 50 m alt.; 16.VII.2017; A. L. • 1 obs. specimen; Hérault, Mauguio; 43°34'43"N, 3°57'32"E; 6 m alt.; 11.VIII.2017; A. L. • 1 obs. specimen; Hérault, Vic-la-Gardiole; 43°30'2"N, 3°47'6"E; 7 m alt.; 22.V.2018; A. L. • 1 obs. specimen; Hérault, Carnon; 43°33'16"N, 4°0'54"E; 2 m alt.; 22.V.2018; A. L. • 3 obs. specimens; Isère, Crolles; 44°54'11"N, 5°37'9"E; 25 m alt.; 22.VIII.2014; A. L. • 1 obs. specimen; Landes, Pimbo; 43°34'20"N, 0°22'11"W; 158 m alt.; 17.V.2020; A. L. • 3 obs. specimens; Pyrénées-Orientales, Canohès; 42°38'53"N, 2°51'14"E; 15.X.2016; C. L.• 1 obs. specimen; Pyrénées-Orientales, Banyuls; 45°27'24"N, 3°5'9"E; 72 m alt.; 8.V.2021; A. L. • 1 obs. specimen; Tarn et Garonne, Bruniquel; 44°3'0"N, 1°39'22"E, 248 m alt.; 26.IV.2010; D. F. (Fournier *et al.* 2016).
Italy • 1 obs. specimen; Bomarzo; 42°35'59"N, 2°9'15"E; 139 m alt.; 9.VI.2017; A. L. • 1 obs. specimen; Fiazole; 43°3'36"N, 0°30'3"E; 25 m alt.; 11.VI.2017; A. L. • 1 obs. specimen; Roma; VII.2011; E. M. • 2 obs. specimens; Sicilia, San Teodoro; 37°52'46"N, 14°1'3"E; 1760 m alt.; 24.IV.2018; R. B.
Spain • 2 obs. specimens; North Spain, Aisa; 42°39'36"N, 0°37'6"E; 967 m alt.; 11.V.2010; A. L. • 1 obs. specimen; North Spain, Aisa; 42°41'17"N, 0°37'16"W; 1048 m alt.; 12.V.2018; A. L. • 1 obs. specimen; Andalusia, Sevilla; 37°28'52"N, 5°56'33"W; 17 m alt.; 26.X.2018; A. L.
Croatia • 1 obs. specimen; Rovinj; 45°5'1"N, 45°5'1"E; 24 m alt.; 18.VII.2018; P. W.

Remarks

Pheidole pallidula is very frequent in the South of France (Bernard 1968 ; Blatrix *et al.* 2013). It has been found in 36 departments (Antarea, 24 June 2024).

Pheidole megacephala (Fabricius, 1793)
(Figs 1A; 2A; 3A)

Specimen distribution. — 29 specimens in 12 sites)

Observed and examined material. — **France** • 8 observed specimens; Manche, Beauvoir, Alligator Bay (near Saint-Malo); 48°36'3"N, 1°30'41"W; 7 m alt.; 29.II.2016 and XII.2023; P. P. (veterinarian) and J.-L. M. It was already found in 2009 by Elena Angulo and Xim Cerdá (pers. comm.) • 2 obs. specimens; Paris, Jardin des plantes, Muséum national d'Histoire naturelle, Tropical greenhouse; 48°50'35"N, 2°21'27"E; 36 m alt.; 11.XII.2023; P. F.

and Q. R. • 2 obs. specimens; La Réunion, Ravine des Cabris; 21°17'7"S, 55°28'45"E; 280 m alt.; 15.III.2017; M. J. • 3 obs. specimens; La Réunion, L'étang salé; 21°17'7"S, 55°28'45"E; 30 m alt.; 15.III.2017; M. J. • 3 obs. specimens; La Réunion, Saint-Pierre; 21°19'14"S, 55°29'8"E; 152 m alt.; 23.III.2017; M. J.
Spain • 2 obs. specimens; Canary Islands, Las Galletas; 28°0'25"N, 16°39'25"W; 2 m alt.; 2.XI.2023; C. G. • 2 obs. specimens; Canary Islands, Las Galletas; 28°0'27"N, 16°39'29"W; 2 m alt.; 2.XI.2023; C. G.
Australia • 2 obs. specimens; Darwin, Coconut Grave; 5.IV.2015; L. B. • 2 obs. specimens; Brisbane; 8.III.2017; L. B.
Indonesia • 1 obs. specimen; Ubud; 8°31'27"S, 115°15'30"E; 175 m alt.; 6.X.2018; A. L.
Vietnam • 1 obs. specimen; Vinh Long; 10°16'45"N, 106°0'8"E; 2 m alt.; 29.XI.2019; A. L.
Cambodge • 1 obs. specimen; Angkor; 13°21'34"N, 108°53'7"E; 15 m alt.; 3.XII.2019; A. L.

Pheidole sp. from Cuba.

OBSERVED AND EXAMINED MATERIAL. — **Cuba** • 1 observed specimen; Viñales; 21°48'7"N, 82°18'19"W; 143 m alt.; 22.III.2017; A. L. • 1 obs. specimen; Topes de Collantes; 22°37'45"N, 83°41'40"W; 735 m alt.; 25.IV.2017; A. L.

REMARKS

It was impossible to determine this species according to Jacques Delabie.

RESULTS

MORPHOLOGICAL DIFFERENCE BETWEEN THE TWO SPECIES

Pheidole megacephala is a species of sub-Saharan origin, a member of a species complex, the megacephala group defined by Fischer *et al.* (2012). This group contains a high number of described species and infraspecific taxa that would require taxonomic revision. The first step was achieved by Fischer & Fisher (2013) by fixing a neotype for the species *Pheidole megacephala*.

Pheidole megacephala can be easily distinguished from the only species of the genus *Pheidole* existing in France (*P. pallidula*) by several morphological characteristics (Figs 1-3). The most apparent is the presence of a lobe under the postpetiole in *P. megacephala* (see the yellow arrow in Figure 2), which is absent in *P. pallidula*.

GENETICS

The five COI sequences obtained are similar to the following GenBank sequences: 100% identity with the *Pheidole megacephala* sequence (GenBank Accession NC_080221) for the three samples from the Alligator Bay (Beauvoir) site, 100% identity with the *Pheidole megacephala* sequence (GenBank Accession KJ141874) for the two samples from the Jardin des plantes (Paris) site. The phylogenetic trees reconstructed using the two methods (NJ and BI) had congruent topologies and always revealed the presence of one well-supported monophyletic group for *Pheidole megacephala* (Fig. 4). The sequences of the five samples fit well into this monophyletic group. The COI analyses revealed that all samples (3/3) from the Alligator Bay (Beauvoir) site and from Jardin des plantes (Paris) (2/2) belonged to the species *Pheidole megacephala*.

PHEIDOLE MEGACEPHALA AT ALLIGATOR BAY (BEAUVOIR) AND IN THE JARDIN DES PLANTES OF PARIS

Four figures show the green house where *P. megacephala* is present in Alligator Bay (Fig. 5).

At the Zoological Institution Alligator Bay, ants were found in all year-round heated buildings and green houses. They are spread in all the buildings. Colonies are sometime in reptile cages, for example in earth or under food plates, or sometime in the concrete of buildings. Main problem is that they quickly feed on insects and vegetables which are offered every day to the reptiles. Most of time, there is quickly a big number of ants on food. Consequently, that food is not consumed by reptiles. We had few cases of very young, small, and weak lizards attacked by ants.

In the greenhouse of the Jardin des plantes in Paris, the ants are not a problem as they simply remove small particles of earth according to the responsible Pierre Froidevaux (Quentin Rome, pers. comm.).

HYDROCARBONS ANALYSIS

Three species appeared clearly on the dendrogram: *Pheidole* sp. from Cuba, *Pheidole megacephala* and *Pheidole pallidula* (Fig. 6). It confirms that several species can be recognized with hydrocarbons.

P. megacephala appears to be very homogenous in all the countries studied in the tropical world. It confirms that the ants from Beauvoir and Paris are indeed of the species *P. megacephala* but now it is not possible to know their origin. The snakes of Beauvoir coming from the entire world and the plants coming also from many places we need genetic data to have more information. Two cryptic *megacephala* species have been described (Fournier *et al.* 2012), but they do not appear here; we need more samples.

Concerning *P. pallidula*, the group is also very homogeneous in South France, Italy, Spain, and Croatia.

It appeared respectively (*P. megacephala* and *P. pallidula* ± SD; Fig. 6) that:

– the species are comparable for alkanes (14.84±9.93, 15.24±6.12, $p = 0.749$), mono-methyl (43.31±11.21, 42.02±8.90, $p = 0.60$) and tri-methyl alkanes (1.77±2.23, 1.53±2.09, $p = 0.44$);

– they differed for dimethyl-alkanes (8.59±5.71, 23.61±10.88, $p<0.000$) and for alkenes (41.18±12.24, 17.60±10.34, $p = 0.0002$);

– the two species differed a little for the hydrocarbon length ECL, but it was not significant (29.83±1.15, 30.41±0.97, $p = 0.55$).

DISCUSSION

P. pallidula have also a very homogeneous hydrocarbons profiles in all their repartition. According Seifert (2016), four cryptic species can be found in Europe: *P. pallidula*, *P. balcanica* nov. sp. (Bosnia, Bulgaria, Croatia, Greece, Montenegro and Turkey), *P. koshewnikovi* Ruzsky, 1905 (Azerbaijdshan, Cyprus, Greece,

Fig. 1. — Morphological comparison of *Pheidole megacephala* (Fabricius, 1793) (**A**, worker in profile view, scale bar = 0.5 mm) and *Pheidole pallidula* (Nylander, 1849) (**B**, worker in profile view, scale bar = 0.5 mm) major workers. Credit: C. Galkowsky.

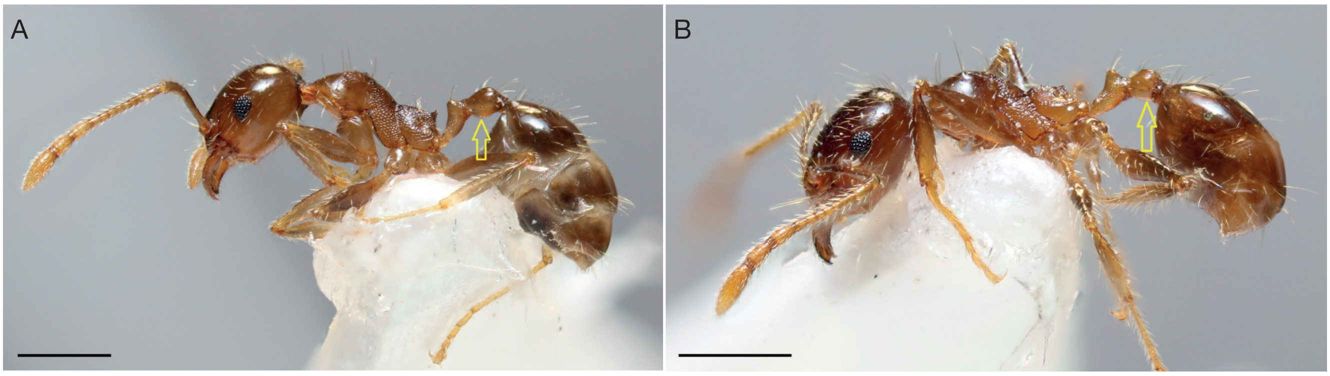

Fig. 2. — Morphological comparison of *Pheidole megacephala* (Fabricius, 1793) (**A**, worker in profile view, scale bar = 0.5 mm) and *Pheidole pallidula* (Nylander, 1849) (**B**, worker in profile view, scale bar = 0.5 mm) minor workers. Credit: C. Galkowsky.

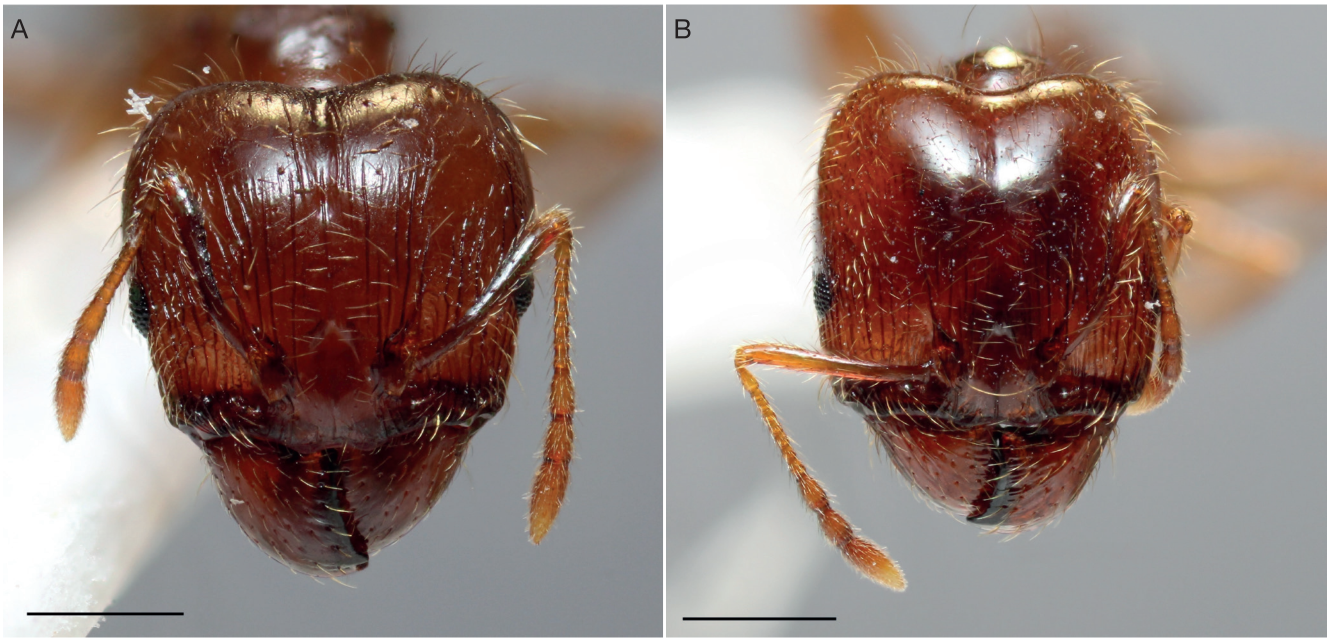

Fig. 3. — Morphological comparison of *Pheidole megacephala* (Fabricius, 1793) (**A**, worker head in full-face view, scale bar = 0.5 mm) and *Pheidole pallidula* (Nylander, 1849) (**B**, worker head in full-face view, scale bar = 0.5 mm) major workers. Credit: C. Galkowsky.

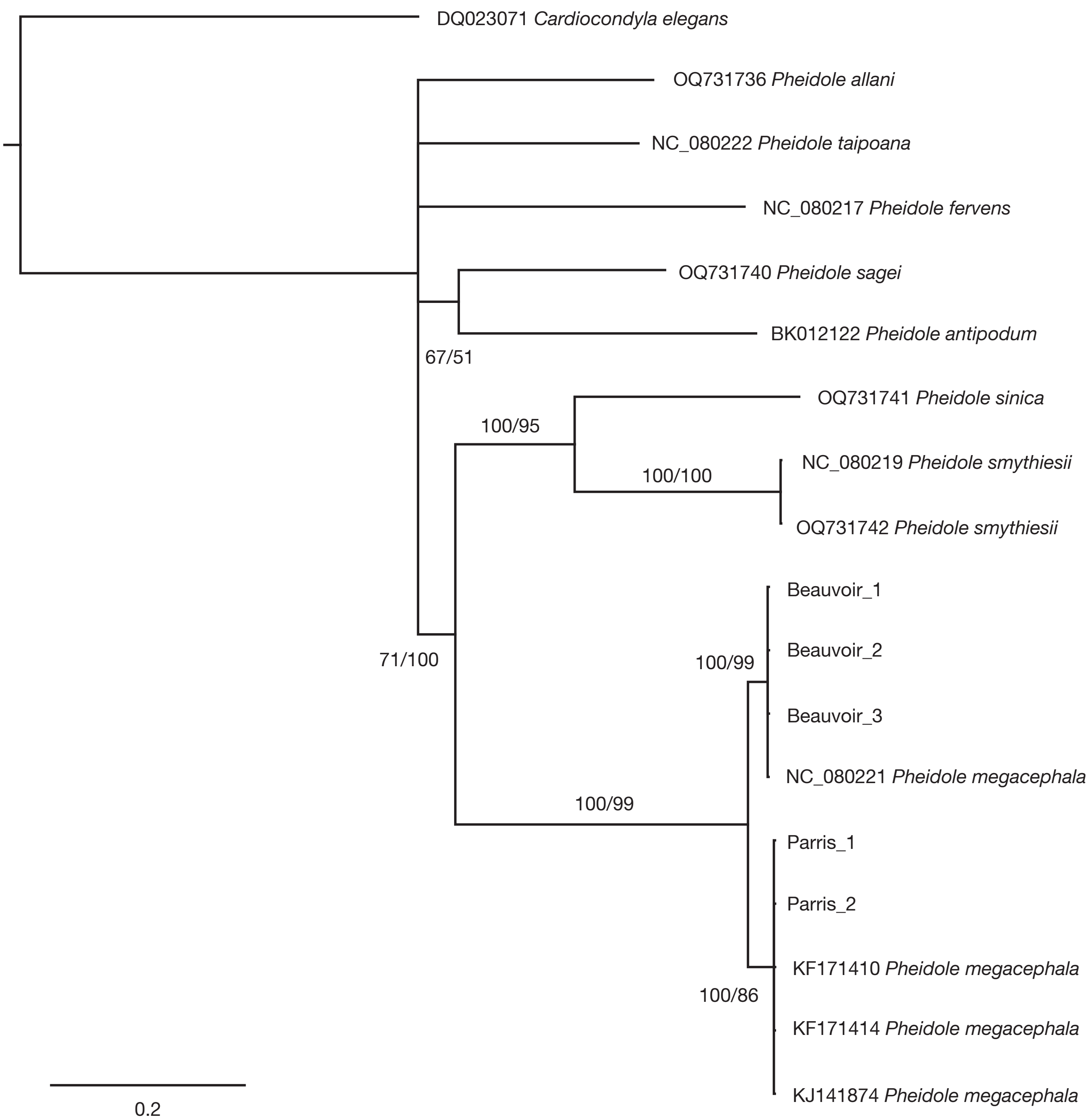

Fig. 4. — Consensus phylogenetic tree for the COI gene obtained using Bayesian Inference and Neighbor-Joining methods. Branch support values (> 50%) are Bayesian posterior probabilities followed by NJ consensus support percentage. Reference sequences show the GenBank accession number followed by the species name.

Kazakhstan, Kyrghystan, Montenegro, Turkey and Uzbekistan) and *P. cicatricosa* Stitz, 1917 from Algeria. So, in our collects there was a very small probability to find them, except in Croatia, but we had there only one sample. It would be interesting to search more in these countries to find these new species and to verify that hydrocarbons can identify cryptic species.

Pheidole megacephala have also a very homogeneous hydrocarbons profile in all their world repartition (Fournier *et al.* 2009). We cannot confirm the existence of cryptic species as indicated in Fournier *et al.* (2016). This invasive species is originating from Africa and will probably extend its repartition In the future, as indicated by Bertelsmeier & Courchamp (2014). For example, recently it has invaded savannas in Kenya by killing *Crematogaster* associated with acacias and it modified completely the ecosystem (Kamaru *et al.* 2024).

Are HCs profiles changing with geographical dispersion? There is not a general rule. Some species like *Lasius niger* (Linnaeus, 1758) do not change their cuticular hydrocarbons profile according to all their large European distribution

Fig. 5. — Pictures of the different stuctures colonized by the big-headed ant *Pheidole megacephala* (Fabricius, 1793) at Alligator Bay. **A**, Non planted terrarium housing an australian water dragon (*Intellagama* (*Physignathus*) *lesueurii*) (Gray, 1831) and a central african rock python (*Python sebae*) (Gmelin, 1789); **B**, planted terrarium housing a panther chameleon (*Furcifer pardalis*) (Cuvier, 1829); **C**, heated building not attached to the main building and housing different species of tortoises like the Burmese star tortoise (*Geochelone platynota*) (Blyth, 1863) and the red-footed tortoise (*Chelonoidis carbonarius*) (Spix, 1824); **D**, tropical heated greenhouse housing different species of Crocodilians. Credits: P. Picquet.

(Lenoir *et al.* 2009). Concerning invasive ants, they generally change their profile. For example, the Argentine ant CHCs changes rapidly according to the food they find ("You are what you eat") (Liang & Silverman 2000; Abril *et al.* 2018). In *Tapinoma magnum* Mayr, 1861 it is intermediary, no dramatic changes appeared with migration, but the ants progressively modify their profile (Lenoir *et al.* 2023).

Comparison of hydrocarbon classes

The two species are comparable for alkanes, mono-methyl and tri-methyl alkanes. They differed for dimethyl-alkanes and for alkenes. This point is very difficult to analyze and we do not have clear information. Menzel *et al.* (2017) found that species from wet climates had more alkenes and fewer dimethyl alkanes than those from drier habitats, which can be explained by different waterproofing capacities of these compounds, and it is clear also for *Pheidole* as *P. pallidula* lives in drier regions than *P. megacephala*.

Comparison of hydrocarbon length (ECL)

They are generally thought to enhance desiccation resistance when they are higher and support very dry climates (reviewed, for example, by Gibbs 1998) and it is confirmed for the two species *P. pallidula* and *P. megacephala* with an ECL of 30.

Is *P. megacephala* an invasive species in France? For the moment, it does not appear to be the case as it has been found only in two greenhouses, but it needs to be monitored. Temperature seems to be the primary factor affecting the distribution of this species (Ke *et al.* 2024). Western Europe including all the occidental cost in France is one of the potential suitable areas for the future global distribution of *P. megacephala* according to climate changes. As some other ant species confined in greenhouses now, *P. megacephala* may disperse outside in the future. For example, in the Jardin des plantes of Paris, *P. megacephala* were observed in large quantities outside the greenhouse in March 2024

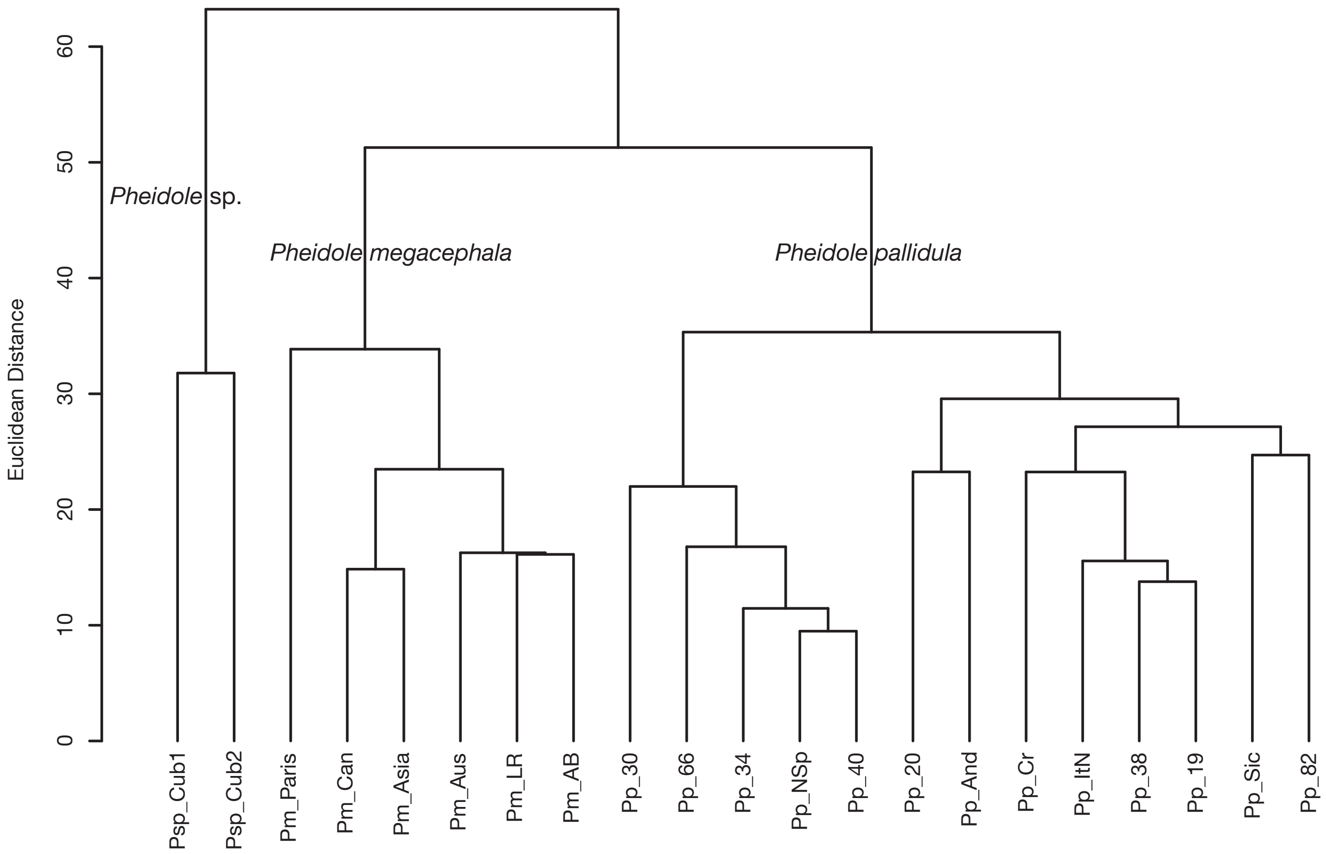

Fig. 6. — Dendrogram of all *Pheidole*. Abreviations: **Pm**, *Pheidole megacephala* from Paris, Canarias (**Can**), Asia, Australia (**Aus**), La Réunion (**LR**), Alligator Bay Beauvoir, 50) (**AB**); **Pp**, *Pheidole pallidula* from Gard (**30**), Pyrénées-Orientales (**66**), Hérault (**34**), North Spain (**NSp**), Landes (**40**), Corsica (**20**), Andalusia (**And**), Croatia (**Cr**), North Italia (**ItN**), Isère (**38**), Corrèze (**19**), Sicila (**Sic**), Tarn et Garonne (**82**); **Psp_Cub1-2**, *Pheidole* sp. from Cuba.

(Quentin Rome, pers. comm.). It needs to be followed. Peng *et al.* (2023) showed that urban forest populations of *P. megacephala* in Taiwan exhibit a lower number of queens, a higher worker relatedness and larger workers than in peri urban forest habitats, suggesting that this species can adapt easily to the habitats they can invade.

Author contributions

Alain Lenoir and Jean-Luc Mercier were responsible of the research; Elfie Perdereau and Alain Lenoir made chromatography; Simon Dupond genetic analysis; Alain Lenoir and Romain Libbrecht the analysis of chromatograms, Christophe Galkowski determined many ants. Pierre Picquet observed and collected the ants with Jean-Luc Mercier in Alligator Bay Zoological Institution. All the authors participated and approved the redaction.

Acknowledgments

Thanks to Benoît Guénard who determined some Asiatic ants. We thank particularly Quentin Rome and Pierre Froidevaux for the collection of ants in Paris Museum. Thanks to Laurence Berville, Rumsais Blatrix, Jacques Delabie, Denis Fournier, Maxime Jacquot, Claude Lebas, Emmanuella Maurizi and Philippe Wegnez who found many ants. Thanks to an anonymous referee and Alain Dejean for their constructive comments. And finally, thanks to Sarah Figuet for her help in finalizing this article.

REFERENCES

Abril S., Diaz M., Lenoir A., Ivon Paris C., Boulay R. & Goâmez C. 2018 — Cuticular hydrocarbons correlate with queen reproductive status in native and invasive Argentine ants (*Linepithema humile*, Mayr). *PLoS ONE* 13 (2): e0193115. https://doi.org/10.1371/journal.pone.0193115

Altschul S. F., Gish W., Miller W., Myers E. W. & Lipman D. J. 1990. — Basic local alignment search tool. *Journal of Molecular Biology* 215 (3): 403-10. https://doi.org/10.1016/S0022-2836(05)80360-2

Bernard F. 1968. — *Les fourmis (Hymenoptera Formicidae) d'Europe occidentale et septentrionale*. Masson, Paris, 411 p.

Bertelsmeier C. & Courchamp F. 2014. — Future ant invasions in France. *Environmental Conservation* 41(2): 217-228. https://doi.org/10.1017/S0376892913000556

Blatrix R., Galkowski C., Lebas C. & Wegnez P. 2013. — *Fourmis de France*. Delachaux et Niestlé, Luçon, 288 p.

Blatrix R., Colin T., Wegnez P., Galkowski C. & Geniez P. 2018. — Introduced ants (Hymenoptera: Formicidae) of mainland France and Belgium, with a focus on greenhouses. *Annales de la Société entomologique de France* (N.S.) 54: 293-308. https://doi.org/10.1080/00379271.2018.1490927

Casevitz-Weulersse J. & Galkowski C. 2009. — Liste actualisée des fourmis de France (Hymenoptera, Formicidae). *Bulletin de la Société entomologique de France* 114 (4): 475-510. https://doi.org/10.3406/bsef.2009.2724

Espadaler X. & Pradera C. 2016. — *Brachymyrmes patagonica* Mayr, 1868 y *Pheidole megacephala* (Fabricius, 1703), two new exotic ants in Spain (Hymenoptera, Formicidae). *Iberomyrmex* 8: 4-10.

Fischer G., Hita Garcia F. & Peters M. K. 2012. — Taxonomy of the ant genus *Pheidole* Westwood 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

Fischer G. & Fisher B. L. 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: 301-356. https://doi.org/10.11646/zootaxa.3683.4.1

Fournier D., Biseau J.-C. de & Aron S. 2009. — Genetics, behaviour and chemical recognition of the invading ant *Pheidole megacephala*. *Molecular Ecology* 18 (2): 186-199. https://doi.org/10.1111/j.1365-294X.2008.04018.x

Fournier D., Tindo M., Kenne M., Mbenoun Masse P. S., Van Bossche V., Coninck E. de & Aron S. 2012. — Genetic structure, nestmate recognition and behaviour of two cryptic species of the invasive big-headed ant *Pheidole megacephala*. *PLoS ONE* 7 (2): e31480. https://doi.org/10.1371/journal.pone.0031480

Fournier D., Biseau J.-C. de, Laet S. de, Lenoir A., Passera L. & Aron S. 2016. — Social structure and genetic distance mediate nestmate recognition and aggressiveness in the facultative polygynous ant *Pheidole pallidula*. *PLoS ONE* 11 (5): e0156440. https://doi.org/10.1371/journal.pone.0156440

Gibbs A. G. 1998. — The role of lipid physical properties in lipid barriers. *American Zoologist* 38 (2): 268-279. https://doi.org/10.1093/icb/38.2.268

Hoffmann B. D. & Parr C. L. 2008. — An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biological Invasions* 10: 1171-1181. https://doi.org/10.1007/s10530-007-9194-x

Huelsenbeck J. P. & Ronquist F. 2001. — MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17 (8): 754-755. https://doi.org/10.1093/bioinformatics/17.8.754

Kamaru D. N., Palmer T. M., Riginos C., Ford A. T., Belnap J., Chira R. M., Githaiga J. M., Gituku B. C., Hays B. R., Kavwele C. M., Kibungei A. K., Lamb C. T., Maiyo N. J., Milligan P. D., Mutisya S., Ng'weno C. C., Ogutu M., Pietrek A. G., Wildt B. T. & Goheen J. R. 2024. — Disruption of an ant-plant mutualism shapes interactions between lions and their primary prey. *Science* 383(6681): 433-438. https://doi.org/10.1126/science.adg1464

Ke Z., Mengfei M., Bamisile S., Zhigiang L. & Yijuan X. 2024. — Predicting the potential distribution of the *Pheidole megacephala* in light of present and future climate variations. *Journal of Economic Entomology* 117 (2): 457469. https://doi.org/10.1093/jee/toae013

Lebas C., Galkowski C., Blatrix R. & Wegnez P. 2016. — *Fourmis d'Europe occidentale*. Delachaux et Nietslé, Luçon, 416 p.

Lenoir A., Depickere S., Devers S., Christides J.-P. & Detrain C. 2009. — Hydrocarbons in the ant *Lasius niger*: from the cuticle to the nest and home range marking. *Journal of Chemical Ecology* 35 (8): 913-921. https://doi.org/10.1007/s10886-009-9669-6

Lenoir A., Perdereau E. & Berville L. 2023. — Chemotaxonomy of *Tapinoma* and some Dolichoderinae ants from Europe and North Africa. *Sociobiology* 70 (3): e9099. https://doi.org/10.13102/sociobiology.v70i3.9099

Liang D. & Silverman J. 2000. — "You are what you eat": diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* 87: 412-416. https://doi.org/10.1007/s001140050752

Martin S. & Drijfhout F. 2009. — A review of ant cuticular hydrocarbons. *Journal of Chemical Ecology* 35: 1151-1161. https://doi.10.1007/s10886-009-9695-4

Martin S., Drijfhout F. & Hart A. G. 2019 — Phenotypic plasticity of nestmate recognition cues in *Formica execta* ants. *Journal of Chemical Ecology* 45: 735-740. https://doi.org/10.1007/s10886-019-01103-2

Menzel F., Blaimer B. B. & Schmitt T. 2017. — How do cuticular hydrocarbons evolve? Physiological constraints and climatic and biotic selection pressures act on a complex functional trait. *Proceedings of the Royal Society B: Biological Sciences* 284 (1850): 20161727. https://doi.org/10.1098/rspb.2016.1727

Moreau C. S. 2008. — Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). *Molecular Phylogenetics Evolution* 48 (1): 224-239. https://doi.org/10.1016/j.ympev.2008.02.020

Nylander J. A. A. 2004. — *MrAIC.pl.* [Program distributed by the author]. Evolutionary Biology Centre, Uppsala University, Uppsala.

Peng M. H., Liu K. L., Tsai C. Y., Shiodera S., Haragushi T. F., Itoh M., Tseng S. P., Yang C. C. S., Singham G. V., Tay J. W. & NEOH K. B.2023. — Urbanization influences the trophic position, morphology, and colony structure of invasive African big-headed ants (Hymenoptera: Formicidae) in Taiwan. *Myrmecological News* 33: 197-209. https://doi.org/10.25849/myrmecol.news_033:197

Sarnat E. M., Fischer G., Guenard B. & Economo E. P. 2015. — Introduced *Pheidole* of the world: taxonomy, biology and distribution. *ZooKeys* 543: 1-109. https://doi.org/10.3897/zookeys.543.6050

Seifert B. 2016. — Inconvenient hyperdiversity – the traditional concept of "*Pheidole pallidula*" includes four cryptic species. *Soil Organisms* 88 (1): 1-17.

Simon C., Frati F., Bechenbach A., Crespi B., Liu H. & Flook P. 1994. — Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87 (6): 651-701. https://doi.org/10.1093/aesa/87.6.651

Tourneur J.-C., Cole C., Vickruck J., Dupont S. & Meunier J. 2022. — Pre- and post-oviposition behavioural strategies to protect eggs against extreme winter cold in an insect with maternal care. *Peer Community Journal* 2: e21. https://doi.org/10.24072/pcjournal.104

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

Wilson E. O. 2003. — Pheidole *in the New World: a dominant, hyperdiverse ant genus.* Harvard University Press, 818 p.

Submitted on 29 April 2024;
accepted on 6 August 2024;
published on 2 April 2025.