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# **Ants of French Guiana: a DNA barcode dataset**

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## Abstract

This dataset represents a DNA barcode reference library for ants from French Guiana. A total of 3931 new sequences from 16S rRNA gene has been generated. The reference library covers 344 species distributed in 57 genera. 3920 sequences are assigned at the species level and 11 at the genus level. All these sequences were submitted to DDBJ/EMBL/GenBank databases in the Bioproject: PRJNA779056: 16S French Guiana Ants (Hymenoptera: Formicidae), sequence identifier KFFS00000000.

## Keywords

DNA sequencing, 16S rRNA, molecular identification, Formicidae, NGS, Neotropics

## Introduction

The current biodiversity crisis calls for efforts to reach more rapid biodiversity characterization. Indeed, our global knowledge of biodiversity is still largely unknown, with ca. 80% of species to be described and more than 20 years on average for the description of a new species following its discovery (Fontaine et al. 2012). Moreover, the classical taxonomical identification of specimens relies most often on subtle morphological criteria and expert knowledge, which is confronted to the shortage of taxonomists (Engel et al. 2021). Such issues are not only valid for the description of extant species diversity, but also to answer ecological questions such

as how communities are assembled and how they respond to global change. In this context, DNA barcoding has proved its effectiveness and has been successfully applied in taxonomical and ecological studies (Kress et al. 2015). DNA barcoding allows identifying specimens at species level using a short sequence of DNA as species tag (Hubert and Hanner 2015). If DNA barcoding is a robust and rapid technology with applications in many scientific areas from taxonomy to ecology, its accuracy and reliability relies on the completeness of a reference library.

With more than 16,000 described species to date (California Academy of Science 2022 (AntWeb)), ants constitute a moderately diversified group among insects. They are, however, a major component of terrestrial ecosystems, being ecologically dominant in all strata and involved in key ecological functions (Lori et al. 2010). Within tropical forests, ants can make up to 25% of the total animal biomass (Hölldobler and Wilson 1990). Their study provided so far important insights into community ecology, global biodiversity patterns or impacts of global change, which make them of the keystone taxa for studying ecological patterns and processes (Lori et al. 2010).

French Guiana, the largest French overseas territory, is located in the Guiana shield on the north-eastern coast of South America. Covered with primary forest on more than 90% of its surface, it is part of the largest block of tropical forest worldwide, hosting a large diversity of species. As an example, the recent checklist of ants from French Guiana highlighted the presence of 659 valid species and subspecies from 84 genera and 12 subfamilies, representing ca. 10% of the ant diversity known in the Neotropical realm (Franco et al. 2019). Here we provide a DNA barcoding database for ants of the region using a short DNA marker (16S rRNA gene, 250-300 bp in length) that can be used to describe and monitor ant

biodiversity in the neotropical area using Next Generation Sequencing methods.

## Methods

### Sampling

#### Geographic range

Ants were sampled from 2013 onwards in a diversity of sites covering most of the major forest habitats represented in French Guiana (Guitet et al. 2015b) and topography: terra-firme (29 plots distributed in 9 sites), swamp (16 plots / 6 sites), white-sand forests (11 plots / 5 sites), transitional forests (1 plot) on the slope of inselbergs, costal savannahs (10 plots / 5 sites), cloud forest (9 plots / 2 sites) and pastures (4 plots / 2 sites) (Fig. 1).

#### Collecting method

Sampling was performed following the Ants of Leaf Litter Protocol (Agosti and Alonso 2000). At each site, 0.12-ha plots (30 × 40 m) were established in the different habitats locally represented. Within each plot, 20 sampling points were established on a grid, with a 10 m distance between each point. At each point, two sampling methods were used: pitfall traps and mini-winkler (Bestelmeyer et al. 2000). Pitfall traps were 6 cm diameter containers placed in the ground with an opening at surface level, partially filled with a soap and salt water solution and left open for 72h. At the same sampling point, 1 m<sup>2</sup> of leaf litter was also sifted and then placed in mini-winkler extractors during 48h (Bestelmeyer et al. 2000).

#### Sample processing

Specimens were preserved in 95% ethanol and then sorted to morphospecies in the lab. One individual of each morphospecies was then mounted for morphological identification to species using taxonomic resources available in the literature and the expertise of taxonomy specialists. Voucher specimens were deposited in the Laboratorio de Mirmecologia, Cocoa Research Centre CEPEC/CEPLAC (Itabuna, BA, Brazil) and at EcoFoG in Kourou.

Although, the mitochondrial gene encoding the cytochrome c oxidase subunit 1 (COI) has been accepted as the consensus marker (Kress et al. 2015), its sequence length, i.e., about 650 bp, turned out to be problematic when using High-Throughput sequencing

technology. Indeed, Illumina technology, the most used and accurate sequencing technology, provides reads of 100 to 500 bp. As an alternative, an informative region of 16S rRNA gene of 135-276 bp has been shown as a suitable alternative to COI for DNA barcoding in insects (Elbrecht et al. 2016). This short 16S fragment has been sequenced here as described below.

DNA extraction was performed from single leg or whole specimen for the smallest, with at least 3 specimens per species. Each extract was amplified by PCR with the 16S rRNA primer Ins16S\_1 (Clarke et al. 2014) (TRRGACGAGAAGACCCTATA / TCTTAATCCAACATCGAGGTC), using the "HotShot" protocol (Truett et al. 2000) with the following cycles: 15min at 95°C, 38 cycles of 95°C for 20 s (denaturation), 49°C for 30 s (hybridation) and 72°C for 30 s, (elongation) and a final extension at 72°C for 5min for the 6 first runs and with the cycle: 15 min at 95°C, 40 cycles of 95°C for 30 s (denaturation), 50°C for 30 s (hybridation) and 72°C for 30 s (elongation) and a final extension at 72° C for 10 min for the 2 last runs. Samples were multiplexed with tagged primers to identify sequences from each specimen. Products were verified and visualized by electrophoresis on 0.8% agarose gels. Sequences shorter than 100 bp were removed by purification from PCR reaction with the GeneClean Turbo Kit (MP Biomedicals, LLC, Sante Ana, CA, USA). Finally, amplicon sequencing was performed using Illumina Miseq technology (2 × 250 bp) by Fasteris (Plan-les-Ouates, Switzerland) or at the Genotoul platform ([www.genotoul.fr](http://www.genotoul.fr)).

## Data processing

Sequence data (Suppl. material 1) were analyzed using Obitools, Obitools3 (Boyer et al. 2015) and dada2 (Callahan et al. 2016) packages in R (R Core Team 2020). The two approaches provided complementary results despite their different strategy of data processing and assignation. In Obitools and Obitools3 (Boyer et al. 2015), paired-end read assembly, read demultiplexing and read dereplication were first performed. Then, low-quality sequences (i.e., shorter than expected - under 80bp), singletons and sequences not assigned to samples were discarded. Chimera sequences were also excluded using the uchime3\_denovo algorithm from usearch tools (Edgar 2016). Remaining sequences were assigned using the EMBL invertebrate database (Baker 2000) with the Obitools assignation process. In dada2 (Callahan et al. 2016), sequences were trimmed and demultiplexed using cutadapt (Martin 2011) and deML tools (Renaud and Schmidt 2017), respectively. Then, low-quality sequences were

discarded and remaining sequences dereplicated. An error model was generated from data themselves and used for creating amplicon single variants (ASVs). Finally, chimeras were deleted using the “removeBimeraDenovo” function from dada2 and remaining sequences were identified using the 16 rRNA sequences of the EMBL invertebrate database (Baker 2000) with the RDP classifier algorithm implemented directly in dada2 (Wang et al. 2007). Finally, results from the two workflows were assembled, the most abundant sequence was kept for each sampled specimen and the molecular identification was compared with the morphological one. Only groups of similar sequences corresponding to identical morphological taxonomic assignation were conserved.

### Quality Checking

The quality of the sequences (Suppl. material 1) was checked using a taxonomic congruence approach. For each species, multiple specimens were sequenced and the corresponding sequences were expected to form a monophyletic group. Sequences were aligned using Muscle (Edgar 2004) and a distance tree was performed using the BloNJ (Gascuel 1997) algorithm in phym1 (Guindon et al. 2010). For species for which only a single specimen was available, we considered the sequence to be correct if it was placed in the correct genus and significantly different from the remaining species.

## Taxonomy

### Taxonomic coverage

This dataset (Suppl. material 1) complements the Genbank library with Ants from French Guiana sequences. Most of the sequences are from species that have not been sequences so far using this maker, or even sequenced at all. A total of 3931 sequences have been deposited, representing 344 species

distributed in 57 genera. Most of the sequences ( $n = 3920$ , 99.7%) have been assigned at the species level and the remaining ( $n = 11$ ) were at the genus level (i.e., close enough to sequence groups belonging the same genus, but not close enough to a sequence group forming a species). New sequences will be added periodically the dataset when available.

### Temporal coverage

Notes: 2013-present

### Data Resources

This Targeted Locus Study project has been deposited at DDBJ/EMBL/GenBank under the accession KFFS000000000. The version described in this paper is the first version, KFFS01000000.

### Resource 1

#### Download URL

<https://www.ncbi.nlm.nih.gov/Traces/wgs/?val=KFFS01>

#### Resource identifier

KFFS01000001-KFFS01003931

#### Data format

FASTA

### Usage Rights

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## Conflicts of interest

The authors declare no conflicts of interests.

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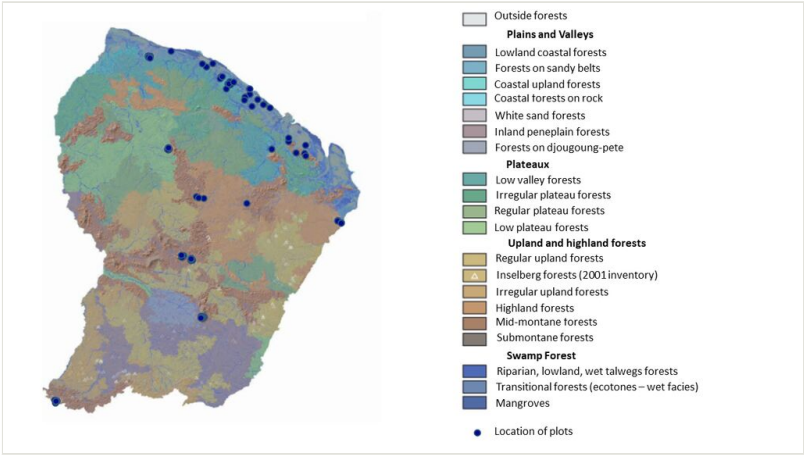


Figure 1.

Distribution of the sampling plots across French Guiana. Background colors represent the main forest habitats in the region and a topographic layer from a 30 m resolution SRTM radar image produced by NASA resolution *sensu* Guitet et al. (2015a).

## Supplementary material

### Suppl. material 1: Sequences file linked to this publication

Authors: Gaëtan Rongier

Data type: FASTA

[Download file](#) (1.02 MB)