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► To cite this version:

Julia Centanni, Bernard Kaufmann, Rumsais Blatrix, Olivier Blight, Adeline Dumet, et al.. High resolution mapping in Southern France reveals that distributions of supercolonial and monodomous species in the *Tapinoma nigerrimum* complex (Hymenoptera: Formicidae) are related to sensitivity to urbanization. *Myrmecological News*, 2022, 32, pp.41-50. 10.25849/myrmecol.news_032:041 . hal-03739534

HAL Id: hal-03739534

<https://hal.science/hal-03739534>

Submitted on 27 Jul 2022

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High resolution mapping in Southern France reveals that distributions of supercolonial and monodomous species in the *Tapinoma nigerrimum* complex (Hymenoptera: Formicidae) are related to sensitivity to urbanization

Julia CENTANNI, Bernard KAUFMANN, Rumsais BLATRIX, Olivier BLIGHT, Adeline DUMET, Pierre JAY-ROBERT & Alan VERGNES

Abstract

Urbanization modifies environments worldwide creating opportunities for the establishment of invasive species, natives or non-natives, a main driver of global change. To understand the process of biological invasion, we need to decipher the interactions between urbanization and species traits. Within the Mediterranean *Tapinoma nigerrimum* species complex (Hymenoptera, Formicidae), *Tapinoma darioi* SEIFERT & al., 2017 and *Tapinoma magnum* MAYR, 1861 have spread in continental European cities and exhibit “tramp”-species traits. In contrast, *T. nigerrimum* (NYLANDER, 1856) is not expanding its range and does not exhibit characteristics of tramp ant species. We investigated whether the distribution of these species differed in relation to land use. We chose Montpellier (Mediterranean France) as a study site because it combines the presence of all three species with rapid urbanization. Occurrence data were generated by systematically sampling all *Tapinoma* colonies over 926 sites, > 500m away from one another, over a 60 × 25 km area. Species were identified using 15 microsatellite markers. Four land-cover classes (urbanization, agricultural, natural, water) were used for landscape analyses based on 250m-radius buffer zones. *Tapinoma nigerrimum* was found at 197 sites (21.27% of occurrences), *T. darioi* at 78 sites (8.42%), and *T. magnum* at 6 sites (0.65%). The probability of occurrence of *T. darioi* was positively correlated with urbanization up to a threshold of 75% impervious land-cover, negatively correlated with natural land-cover, and was higher near the coast. In contrast, *T. nigerrimum* occurred less often at sites with impervious land-cover and near the coast, and more frequently in natural areas. These distinct distributions support an association between urbanization and species exhibiting tramp traits, and highlight the importance of adapting land management to control biological invasions.

Key words: *Tapinoma darioi*, urban landscape, Montpellier, *Tapinoma magnum*, Mediterranean region, microsatellite.

Received 23 June 2021; revision received 30 November 2021; accepted 1 December 2021

Subject Editor: Andrew V. Suarez

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Introduction

Urbanization is land conversion leading to total or partial irreversible soil replacement by impervious surfaces (e.g., buildings, roads, parking lots, large-scale infrastructures) (MCKINNEY 2006). It has mainly negative consequences on native biodiversity (MCKINNEY 2002, 2006, HETERICK & al. 2013, CADOTTE & al. 2017), while creating opportunities for the establishment of some species which are adapted to disturbance caused by human activities (VITOUSEK & al. 1997, MCKINNEY 2006, CADOTTE & al. 2017). As

urban areas are hubs of human activities, they are also a main point of species entry, release or escape and spread (PADAYACHEE & al. 2017).

According to VALÉRY & al. (2008, 2009), invasive traits, which allow species to spread to new areas or habitats and reach high numbers or densities, can be acquired by both indigenous and non-indigenous species. Successful biological invasions may result from interactions between urbanization and species' ecological traits (CADOTTE &

al. 2017). One way to investigate these interactions is to compare species that are closely related (i.e., that differ by only a few traits) and that co-occur spatially within an urbanized landscape (CADOTTE & al. 2017). For example, a study of cryptic bat species showed that those two morphologically similar species may respond differently to urban landscapes (LINTOTT & al. 2016). One species is considered a generalist and tolerates moderate levels of urbanization (HALE & al. 2012, LINTOTT & al. 2016).

In the present study, we compared the distribution of cryptic ant species in the *Tapinoma nigerrimum* species complex (Hymenoptera, Formicidae) on a mosaic of land use. The *T. nigerrimum* species complex comprises four species found in the western Mediterranean region: *Tapinoma darioi* SEIFERT & al., 2017, *Tapinoma ibericum* SANTSCHI, 1925, *Tapinoma magnum* MAYR, 1861, and *Tapinoma nigerrimum* (NYLANDER, 1856), the delineation of which has been clarified only recently (SEIFERT & al. 2017). Although similar morphologically, these species have been separated by NUMOBAT (Numeric Morphology-Based Alpha-Taxonomy) and by mtDNA (mitochondrial DNA), and have different ecological traits (SEIFERT & al. 2017). All species are native to the Mediterranean region, but their precise native ranges need further investigations, except for *T. ibericum*, which is found in Southern Spain only, and *T. nigerrimum*, whose distribution is restricted to Spain and Southern France. *Tapinoma nigerrimum* forms monodomous colonies that are aggressive to one another (SEIFERT & al. 2017). *Tapinoma darioi* and *T. magnum* show “tramp”-species traits (SEIFERT & al. 2017) and have established populations outside their native range (DEKONINCK & al. 2015, SEIFERT & al. 2017). In addition to human-assisted dispersal and having a generalist diet, tramp species exhibit polygyny (i.e., multiple reproductive queens per nest), supercoloniality (i.e., numerous interconnected nests spread over a large area), mass recruitment (e.g., the capacity to rapidly gather foragers over a resource), and ecological dominance resulting in an exclusion of other ant species (PASSERA 1994, HOLWAY & al. 2002).

While recent invasions of *Tapinoma darioi* and *Tapinoma magnum* have been documented in the Netherlands, Germany, and Belgium (DEKONINCK & al. 2015, SEIFERT & al. 2017), these species also express invasive behaviours in Southern France (BLIGHT & al. 2010, BERVILLE & al. 2013). Coastal areas of Southern France have seen rapidly growing urbanization since the 1960's, and now feature contrasted land uses with juxtaposition of natural and urban areas. This is one of the few places where *Tapinoma nigerrimum*, the native species, occurs sympatrically with two invasive species of the complex, *T. darioi* and *T. magnum* (SEIFERT & al. 2017). The goal of our study was to study the contribution of land use to the spatial distribution of each species and interpret this contribution in light of the invasiveness-related traits known to differ between the species. To do so, we first had to develop a fast and reliable method based on microsatellite markers for the unambiguous identification of the

four species within the *T. nigerrimum* species complex. These microsatellite markers also allowed the identification of other species outside this complex (e.g., *Tapinoma erraticum*, LATREILLE, 1798 and *Tapinoma madeirense*, FOREL, 1895).

Then, we made a high-resolution mapping of the distribution of each species of the complex and linked their occurrences with urbanization levels, measured as the proportions of land-cover classes, that is, impervious, agricultural, and natural. We hypothesize a positive relationship between level of urbanization and the occurrence of *Tapinoma darioi* and *Tapinoma magnum*.

Materials and methods

Study area

The study area is located in Southern France, along the Mediterranean coast. Sampled sites are distributed from the sea-shore to the hills in the North of Montpellier, in a broad 20 × 60 km area parallel to the coast (Fig. S1, as digital supplementary material to this article, at the journal's web pages). The climate is characterized by mild, wet winters and hot, dry summers (LEROUX 2001). From South to North, the landscapes consist of (1) alluvial coastal plains, with sandy dunes which separate large saltwater lagoons from the Mediterranean Sea, and (2) plateaux and hills made of layers of marine sedimentary limestone that underwent folding and faulting during the Pyrenean orogeny, dominated by Mediterranean shrublands and mostly oak forests. Agricultural areas (e.g., fields, vineyards, orchards, olive groves) are present between the lagoons and the hills, wherever urbanization has not taken hold (BORNAND & al. 1997, MYERS & al. 2000).

The sampled areas include the urban areas of Sète (43° 24' N, 3° 42' E; 90,720 inhabitants), Montpellier (43° 36' N, 3° 52' E; 457,839 inhabitants), Lunel (43° 40' N, 4° 08' E; 48,672 inhabitants), and Nîmes (43° 50' N, 4° 21' E; 268,785 inhabitants) (Fig. 1; INSEE 2017, 2018). Since 1968, the population of all the urban areas combined has doubled (INSEE 2020) due to the simultaneous urbanization of the shoreline for mass tourism and the uncontrolled urban spread as a result of new inhabitants (DIRECTION DÉPARTEMENTALE DES TERRITOIRES ET DE LA MER DE L'HÉRAULT 2016). There are, however, strong heterogeneities in population dynamics among the four urban areas, with Montpellier and Lunel showing the strongest population growth (2.5 and 2.4-fold growth, respectively) and Sète and Nîmes well below (1.5 and 1.7-fold, respectively).

Biological model

Species identification in the field is impossible within the *Tapinoma nigerrimum* complex and is made even more difficult by the presence of other species of the genus *Tapinoma*, such as *T. erraticum* or *T. madeirense*. *Tapinoma simrothi* KRAUSSE, 1911, which can be found in Southern Spain and Italy, belongs to another species complex but is morphologically very similar to the species of

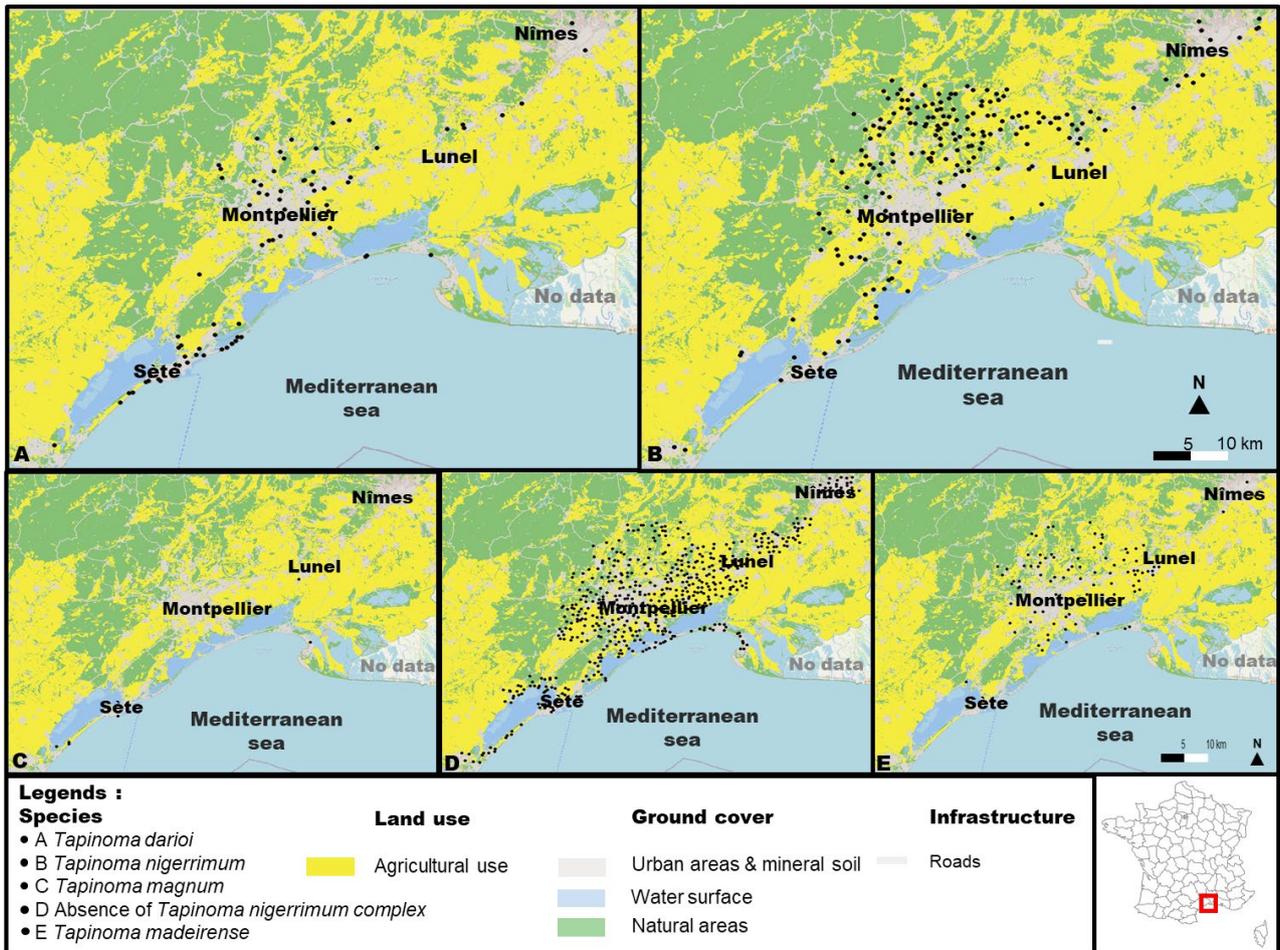


Fig. 1: Distribution maps of the three species of the *Tapinoma nigerrimum* complex found in the sampling area. Map D shows all sampled sites where no species of the *T. nigerrimum* complex could be found. Map E shows the occurrence of *Tapinoma madeirense* for information, although it is not part of the *Tapinoma nigerrimum* complex. *Tapinoma ibericum* was only present in Mèze (% urban = 0.86, close to the coast) and therefore not shown.

the *T. nigerrimum* complex. Although these other species have never been recorded in our study area, all *Tapinoma* colonies, regardless of species group, were sampled in the field for identification in the laboratory.

Sampling scheme

Based on a 1 km² mesh grid, 926 sampling points were semi-randomly positioned using the "random point under the influence of a layer" tool in QGIS version 3.10.6 (QGIS DEVELOPMENT TEAM 2019) (excluding aquatic surfaces) and sampled once from March to July 2019 in dry weather, between 15 °C and 30 °C. This climate corresponds to the highest activity period of most ants, and it is optimal for *Tapinoma* (BLATRIX & al. 2013).

Ants were collected in nests or on dense trails with an entomological aspirator or flexible forceps within a radius of 15 m around each sampling point (following GIPPET & al. 2017) and along four 50 m transects (oriented North, South, East, West), when the configuration allowed it. In sites where *Tapinoma* occurred, 30 individuals from one nest were collected and preserved in 96% ethanol.

Genetic identification by microsatellites

SEIFERT & al. (2017) used NUMOBAT to define species within the *Tapinoma nigerrimum* species complex and to identify individuals to the species level. They also used mtDNA (Cytochrome Oxidase gene) sequencing, which supported the NUMOBAT taxonomy. However, a number of disagreements between NUMOBAT and mtDNA arose for *Tapinoma magnum* and *Tapinoma darioi* from Italy and from France.

NUMOBAT is time consuming (KLARICA & al. 2011), and mtDNA markers do not give exact identifications in species complexes where hybridization or incomplete lineage sorting occur (as is the case in *Tapinoma nigerrimum* complex, SEIFERT & al. 2017). Following the methods developed in CORDONNIER & al. (2019), 15 microsatellite markers designed for *Tapinoma magnum* to genotype one individual per colony collected were used. The details for microsatellite development, sequences, and primers can be found in Appendix S1, as digital supplementary material to this article, at the journal's web pages. The rationale behind identification by genotyping is that mutations that accumulate in microsatellite sequences should

Tab. 1: Results of Generalized Linear Models showing the effects of the variables on the occurrence of *Tapinoma nigerrimum*. % nat: percentage of natural areas, % urban: percentage of urbanization, % agri: percentage of area for agricultural use, distance: distance to the coast, interaction % urban distance: interaction between % urban and distance to the coast. Bold lines correspond to the best models by variable according to the Akaike Information Criteria (AIC).

| Models | Formula | AIC | Estimate | Standard error | P-Value | Figures |
|-------------------------------------|-------------------------------------|---------------|-------------------|------------------|------------------|-------------------|
| % nat linear | % natural | 922.45 | 0.017987 | 0.002833 | <0.001 | Fig. 2. A |
| % nat quadratic | % natural | 924.44 | 1.710e-02 | 1.011e-02 | 0.08933 | Not shown |
| | %natural ² | | 9.588e-06 | 1.052e-04 | 0.92741 | |
| % nat cubic | % natural | 925.68 | -1.435e-03 | 2.346e-02 | 0.9512 | Not shown |
| | % natural ² | | 5.212e-04 | 5.958e-04 | 0.3825 | |
| | % natural ³ | | -3.567e-06 | 4.092e-06 | 0.3838 | |
| % urban linear | % urban | 911.69 | -0.025989 | 0.004051 | <0.001 | Not shown |
| % urban quadratic | % urban | 902.94 | -0.0613954 | 0.0115195 | <0.001 | Not shown |
| | % urban ² | | 0.0005376 | 0.0001566 | <0.01 | |
| % urban cubic | % urban | 890.79 | -1.513e-01 | 2.811e-02 | <0.001 | Fig. 2. C |
| | % urban² | | 3.807e-03 | 9.598e-04 | <0.001 | |
| | % urban³ | | -2.820e-05 | 8.513e-06 | <0.001 | |
| % agri linear | % agri | 957.67 | 0.005635 | 0.002534 | <0.05 | Not shown |
| % agri quadratic | % agri | 937.64 | 0.0462602 | 0.0091596 | <0.001 | Fig. S3. A |
| | % agri² | | -0.0004993 | 0.0001095 | <0.001 | |
| % agri cubic | % agri | 935.64 | 8.226e-02 | 2.019e-02 | <0.001 | Not shown |
| | % agri ² | | -1.650e-03 | 5.822e-04 | <0.01 | |
| | % agri ³ | | 8.780e-06 | 4.340e-06 | <0.05 | |
| distance linear | distance | 901.67 | 6.884e-05 | 9.134e-06 | <0.001 | Fig. 2. E |
| Interaction % urban distance | distance | 853.49 | 8.375e-05 | 1.147e-05 | <0.001 | Fig. S4. A |
| | % urban³ | | 5.278e-06 | 1.367e-06 | <0.001 | |
| | % urban | | -3.589e-02 | 7.698e-03 | <0.001 | |
| | % urban³:distance | | -1.683e-10 | 6.293e-11 | <0.01 | |

result in strong differentiation over time, especially in species which are reproductively isolated. Microsatellites are, however, known to converge by homoplasy. To overcome this obstacle, several markers have to be analyzed together. In CORDONNIER & al. (2019), five species of the ant genus *Tetramorium* MAYR, 1855 were identified with 17 highly polymorphic microsatellite markers, which also revealed numerous interspecific hybrids between two species (CORDONNIER & al. 2018). In the present paper, the same approach was used, with one major difference: DNA of individuals that had been subjected to both NUMOBAT and mtDNA identification in SEIFERT & al. (2017) was used to assign species names to genotype groups defined by microsatellite analysis.

Microsatellite development

Fifteen markers were developed from *Tapinoma magnum* individuals: The total genomic DNA was isolated from the ants collected in Lyon in 2014. The DNA was extracted following crushing (3 min at 30 Hz in a Retsch MM200 ball mill, Haan, Germany), standard digestion (proteinase K (75 µg) + 200 µl of TNES buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, 0.5% SDS)), and salt-chloroform purification. The total genomic DNA was sent to GenoScreen, Lille, France, where 1 µg was used for the development of microsatellite libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as described in

MALAUSA & al. (2011). The total DNA was mechanically fragmented and enriched for AG, AC, AAC, AAG, AGG, ACG, ACAT, and ATCT repeated patterns. The enriched fragments were subsequently amplified. The PCR products were purified, quantified, and the GS-FLX libraries were then carried out following the manufacturer's protocols and sequenced on a GS-FLX PTP. Details for microsatellite development, sequences, and primers can be found in Appendix S1. Primer characteristics and marker accession numbers can be found in Appendix S2, Table 1.

DNA extraction

The DNA from one individual per colony collected in the field was extracted by grinding each ant in proteinase K (10 µL at 15 mg / ml) and adding Chelex 7% (150 µL), followed by a 2-hour incubation at 55 °C and an additional 15 min at 90 °C to inactivate the proteinase K.

Genotyping

Fifteen microsatellite markers were organized into three genotyping mixes (Appendix S2, Tab. 1) and run through a 3730xl DNA Analyzer (Applied Biosystems) by a service provider (GENTYANE, Clermont-Ferrand, France). Forward primers were end-labelled with universal tails following BLACKET & al. (2012); four different tails were used with the following fluorescent dyes – Tail_A_6FAM [6FAM]GCCTCCCTCGGCCA, Tail_B_

Tab. 2: Results of Generalized Linear Models with binomial logistic “error”, showing the effects of the variables on the occurrence of *Tapinoma darvici*. % nat: percentage of natural areas, % urban: percentage of urbanization, % agri: percentage of area for agricultural use, distance: distance to the coast, interaction % urban distance: interaction between the % urban and distance to the coast. Bold lines correspond to the best models by variable according to the Akaike Information Criteria (AIC).

| Models | Formula | AIC | Estimate | Standard error | P-Value | Figures |
|-------------------------------------|-------------------------------------|---------------|-------------------|------------------|------------------|-------------------|
| % natural linear | % natural | 528.94 | -0.016144 | 0.005462 | <0.01 | Fig. 2. B |
| % natural quadratic | % natural | 528.81 | 0.0068496 | 0.0170569 | 0.6861 | Not shown |
| | %natural ² | | -0.0003186 | 0.0002321 | 0.1438 | |
| % natural cubic | % natural | 530.38 | -1.357e-02 | 3.573e-02 | 0.7039 | Not shown |
| | % natural ² | | 3.847e-04 | 1.117e-03 | 0.7286 | |
| | % natural ³ | | -5.905e-06 | 9.336e-06 | 0.5137 | |
| % urban linear | % urban | 504.39 | 0.025342 | 0.004307 | <0.001 | Not shown |
| % urban quadratic | % urban | 487.71 | 0.0941229 | 0.0180119 | <0.001 | Fig. 2. D |
| | % urban² | | -0.0008457 | 0.0002137 | <0.001 | |
| % urban cubic | % urban | 485.42 | 1.694e-01 | 4.173e-02 | <0.001 | Not shown |
| | % urban ² | | -3.016e-03 | 1.065e-03 | <0.01 | |
| | % urban ³ | | 1.651e-05 | 7.740e-06 | <0.05 | |
| % agri linear | % agri | 509.48 | -0.026834 | 0.005841 | <0.001 | Fig. S3. B |
| % agri quadratic | % agri | 511.31 | -0.0204340 | 0.0163521 | 0.2129 | Not shown |
| | % agri ² | | -0.0000992 | 0.0002400 | 0.6750 | |
| % agri cubic | % agri | 511.57 | -6.089e-02 | 3.586e-02 | 0.07868 | Not shown |
| | % agri ² | | 1.523e-03 | 1.307e-03 | 0.22644 | |
| | % agri ³ | | -1.453e-05 | 1.184e-05 | 0.18748 | |
| distance linear | distance | 518.93 | -0.0000662 | 0.0000157 | <0.001 | Fig. 2. F |
| Interaction % urban distance | distance | 483.12 | -3.876e-05 | 2.346e-05 | 0.09478 | Fig. S4. B |
| | % urban² | | -8.232e-04 | 2.168e-04 | <0.001 | |
| | % urban | | 9.036e-02 | 1.872e-02 | <0.001 | |
| | % urban²:distance | | -1.327e-09 | 7.982e-09 | 0.86660 | |

HEX [HEX]GCCTTGCCAGCCCGC, Tail_C_ATTO550 [ATTO550]CAGGACCAGGCTACCGTG, Tail_D_ATTO565 [ATTO565]CGGAGAGCCGAGAGGTG. The primer and tail correspondence are indicated in Appendix S2, Table 1. All three PCR mixes had a total volume of 12 µl with 1 × Master Mix Type-it microsatellite PCR kit (QIAGEN, 206246, Hilden, Germany) and 2 µl extracted DNA with 0.02 - 0.1 µM of primer forward, 0.04 - 0.2 µM of primer reverse, and 0.1 - 0.2 µM of tail. All PCRs were run with the same cycling program: 5 min, annealing at 95 °C, 36 cycles with denaturation (30 s) at 95 °C, annealing (3 min) at 60 °C, extension (30 s) at 72 °C, and a final 30 min extension at 60 °C. Electropherograms were read and interpreted with Genemarker 1.95 (Softgenetics, State College, PA, USA).

Genetic analyzes

Bayesian methods such as STRUCTURE (PRITCHARD & al. 2000) require data that conform to several conditions, in particular the Hardy-Weinberg equilibrium within a cluster, the sample-size equilibrium of clusters, and the allelic-diversity equilibrium between clusters (PUECHMAILLE 2016). As basic population genetics (i.e., allelic richness, heterozygosities, F indices, null allele frequency) could not be computed before identification of species, none of these three conditions could be verified before clustering. Species identification was therefore made by using three methods concurrently: simple PCA, discriminant PCA (dACP, JOMBART 2008, JOMBART & al. 2010), and

GENECLASS 2 (PIRY & al. 2004). To assign species names to genotypic groups defined by microsatellite analysis, the original DNA of individuals (“reference individuals”) that were subjected to both NUMOBAT and mtDNA identification in SEIFERT & al. (2017) was used. As our samples might have included individuals from distant clades such as *Tapinoma erraticum* and *Tapinoma simrothi* species groups, a two-step approach was used. All three methods were applied on all the individuals sampled, with an addition of 15 reference individuals per described species. Each detected species group separately was then reanalyzed, again with 15 reference individuals per described species. Identification results were subsequently used as priors in Bayesian analysis (STRUCTURE, PRITCHARD & al. 2000) for confirmation. The detailed identification methods and results can be found in Appendix S1 to 4, respectively.

Spatial analyses

Spatial analyses were based on 2015 landscape data from the OCS GE (Occupation du sol à Grande Echelle) Version 1.1 for Hérault and Gard departments from IGN (Institut National de l’Information Géographique et Forestière). The scale of use is 1:2500. OCS GE vector files have 14 land-cover classes and 17 land-use classes. Minimal polygon areas are 200 m² within built-up areas, 500 m² within urban zones, and 2500 m² outside urban zones.

In a 250 m radius buffer around each sampling site, the percentage of urbanization (% urban, comprising two land-cover classes: impervious built-up CS (“Couverture du sol” in French or land cover) 1.1.1.1 and impervious none built-up CS 1.1.1.2), the percentage of agricultural land-use (% agri, land-use class Agriculture US (“usage du sol” in French or land use) 1.1), and the percentage of natural areas (% natural, land-cover classes Forests CS 2.1.1 and Scrublands CS 2.1.2) were calculated; other classes in this category were not taken into account as they include anthropic land uses such as gardens, lawns, or crops). Water surfaces (CS 1.2.2) were subtracted from the analyses.

The distance to the coast (in meters) was also measured using the coastline and the “Join attributes by nearest” tool (QGIS DEVELOPMENT TEAM 2019). The distance to the coast was used to represent a habitat and niche gradient, from sand-dunes to sedimentary agricultural plain to dry, forested hillsides. All analyses were performed using QGIS 3.10.6 (QGIS DEVELOPMENT TEAM 2019).

Statistical analyses

Normality distributions of the four landscape variables were tested using a Shapiro-Wilk-Test. As the data were non-normal, we measured the correlations between the four variables using a Spearman rank correlation test (BORCARD & al. 2018). To study the probability of occurrence of the detected species as a function of explanatory variables, generalized linear models (GLMs) with binomial logistic error (also called logistic models) were used. These models are suitable for presence / absence data (CRAWLEY 2009). The effect and shape of effects were tested for all three land-cover variables, for the distance to the coast, and for the interaction between % urban and distance to the coast (Tabs. 1 and 2). For land-cover variables, linear, quadratic (i.e., by combining parameter + parameter²), and cubic models (parameter + parameter² + parameter³) were tested. For all models, except for the distance to the coast one, a model selection protocol was followed using the Akaike Information Criteria (AIC). The best model selected was the one with the lowest AIC depending on the number of parameters studied (adding one parameter must decrease the AIC by two unities to improve the model). The AIC thus achieved the best compromise between data adjustment and model parsimony (CRAWLEY 2009). For each model, the significance of the parameters was calculated using a type III Analysis of variance, adapted to logistic regressions (package “car”; FOX & WEISBERG 2019). The display of the models was produced using the “effect” package (FOX & al. 2020). Different types of models (linear, quadratic, and cubic) were tested because along this gradient environmental parameters could show linear but also more complex responses, with thresholds and hump-shaped curves (MCDONNELL & HAHS 2008, CRAWLEY 2009).

All statistical analyses were computed using R version 3.6.2 (R DEVELOPMENT CORE TEAM 2019), and R studio (R STUDIO TEAM 2020).

Results

Species occurrences

From the 926 sampling sites, individuals from 373 *Tapinoma* colonies were collected. DNA analyses identified 197 *Tapinoma nigerrimum* (21.27% of occurrences), 78 *Tapinoma darioi* (8.42%), 6 *Tapinoma magnum* (0.65%), 1 *Tapinoma ibericum* (0.11%) and 91 *Tapinoma madeirense* (9.83%). We did not detect any *Tapinoma* in the remaining 553 points (59.72%) (Fig. 1). As *T. magnum* and *T. ibericum* were uncommon and *T. madeirense* does not belong to the *T. nigerrimum* species group, they were not included in the statistical analyses.

Correlations between variables and study area description

All variables were weakly but significantly correlated (p-value < 0.001), except for the % urban versus % agri (correlation coefficient = -0.6) and % agri versus % natural (correlation coefficient = -0.5) which were highly correlated (Fig. S2.). The total buffers represent 29.56% natural areas, 21.73% urban areas, and 29.02% for agricultural use.

Effects of land cover and land use on the probability of occurrence for *Tapinoma nigerrimum* and *Tapinoma darioi*

Natural areas: Our study area comprised 35.24% natural areas that contained 9 *Tapinoma darioi*, 1 *Tapinoma magnum*, 65 *Tapinoma nigerrimum*, 21 *Tapinoma madeirense*, and 0 *Tapinoma ibericum* colonies. Natural areas had significant effects on the distribution of both species. For *T. nigerrimum*, GLMs showed a positive effect of % natural (Fig. 2A). Conversely, for *T. darioi*, GLMs showed a negative effect of % natural (Fig. 2B). The best models according to the AIC for % natural were the linear models (Tabs. 1 and 2).

Urban areas: Our study area comprised 13.30% urban areas containing 37 *Tapinoma darioi*, 1 *Tapinoma magnum*, 38 *Tapinoma nigerrimum*, 26 *Tapinoma madeirense* and 1 *Tapinoma ibericum* colonies. For *T. nigerrimum*, the best selected model (AIC = 890.79) was the cubic model (Tab. 1; Fig. 2C). The probability of occurrence of *T. nigerrimum* was very high (0.5 to 0.1) from 0 to 20% of % urban followed by a plateau (probabilities between 0.1 and 0.15) from 20 to 70% of % urban. Above 70%, the probabilities dropped to 0.

For *Tapinoma darioi*, the best model according to AIC was the quadratic effect of % urban on the probability of occurrence (Tab. 2; Fig. 2D). The probabilities of occurrences were maximized around 75% of % urban, and dropped below 0.05 below 20% and above 95% of % urban (Tab. 2; Fig. 2D).

Agricultural land use: Our study area comprised 30.31% agricultural use areas containing 7 *Tapinoma darioi*, 0 *Tapinoma magnum*, 58 *Tapinoma nigerrimum*, 23 *Tapinoma madeirense* and 0 *Tapinoma ibericum* colonies.

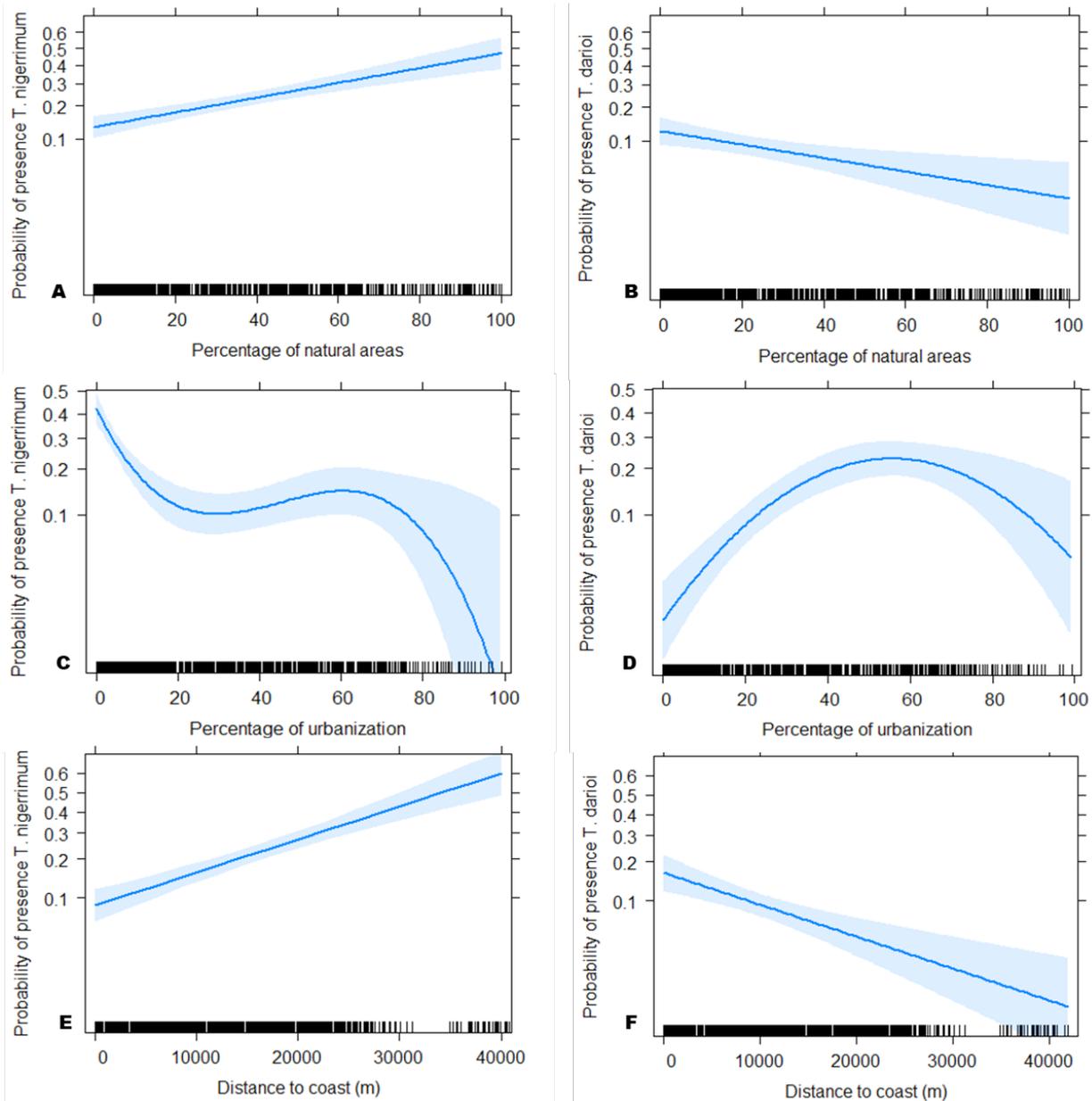


Fig. 2: Probabilities of occurrence for (A) *Tapinoma nigerrimum* (linear model, p-value < 0.001) and (B) *Tapinoma darioi* (linear model, p-value < 0.001) as a function of percentage of natural landscapes in a 250 m radius circular buffer around sampling sites (% natural). Probabilities of occurrence for (C) *Tapinoma nigerrimum*, (cubic model, p-value < 0.001) and (D) *T. darioi*, (quadratic model, p-value < 0.001), as a function of percentage of urban landscapes in a 250 m radius circular buffer around sampling sites (% urban). Probabilities of occurrence for (E) *T. nigerrimum* and (F) *T. darioi* as a function of the distance to the coast in meters with a p-value < 0.001. The light blue area represents the standard error. The rug plot at the bottom of the graph shows the number of sampling points corresponding to % urban values.

For *Tapinoma nigerrimum*, the best selected model was the quadratic model (Tab. 1; Fig. S3A). The probability of occurrence of *T. nigerrimum* was maximized at around 50% of % agri at 0.3 and dropped below 0.15 below 30% and above 80% of % agri (Tab. 1; Fig. S3A).

For *Tapinoma darioi*, GLMs showed a negative effect of % agri (Fig. S3B). The best model according to the AIC for % agri was the linear model (Tab. 2).

Distance to coast: The probability of occurrence of *Tapinoma nigerrimum* was positively and linearly

correlated with the distance to the coast (Tab. 1; Fig. 2E). Conversely, the probability of occurrence of *Tapinoma darioi* decreased linearly as we moved away from the coast (Tab. 2; Fig. 2F).

Interactions between distance to the coast and portion of urban land use:

For *Tapinoma nigerrimum*, the best model was the interaction (Tab. 1; Fig. S4A), with the probability of occurrence higher in urbanized areas close to the coast, decreasing

as we moved away from the coast. The best model was the interaction model also for *Tapinoma darioi* (Tab. 2). However, the interaction was not significant (Tab. 2). As shown in Figure S4B, the same response to % urban was observed independently of the distance to the coast: The probability of occurrence of *T. darioi* peaks at 60% of % urban whatever the distance to the coast. However, far from the coast, the probabilities for *T. darioi* decreased to < 0.05 at low values of % urban, while near the coast, this decrease was less pronounced at 0.12 but the standard error was larger (Fig. S4B).

Discussion

Species occurrence in the *Tapinoma nigerrimum* complex

Tapinoma nigerrimum was found in 21% of the sampled points, making it the most frequently observed species of the complex, far beyond *Tapinoma darioi* (8%), *Tapinoma magnum* (1%), and *Tapinoma ibericum* (1 site). Indeed, it is considered a fairly common species in Southern France (BLATRIX & al. 2013), described in 1856 on the basis of specimens from the surroundings of Montpellier, although it may have been confused with other species of the complex (SEIFERT & al. 2017). It can also be noted that *Tapinoma* supercolonies (*magnum* or *darioi*) were observed in Valras-Plage (15 km South-East of sampling zone) and Port-Leucate (120 km South-East) in 1991 (B. Kaufmann, unpubl.).

Our results are comparable with those obtained by GIPPET & al. (2017) in the urban area of Lyon for the genus *Lasius* FABRICIUS, 1804, where native species are much more common than the invasive *Lasius neglectus*. Interestingly, the relative rarity of *Tapinoma magnum* in the urban area of Montpellier was not expected because SEIFERT & al. (2017) had observed this species along the coast nearly as often as *Tapinoma darioi*. Further studies would allow us to understand the causes of this difference, such as inspecting museum collections to determine the date of introduction, studying how the two species are introduced and dispersed in the region to determine the routes of invasion and vectors, and conducting competition experiments in the field or in the laboratory. *Tapinoma ibericum* was only observed once, which is consistent with the observations of SEIFERT & al. (2017), who only reported one single invaded site outside the Iberian Peninsula, on the Isle of Wight.

Distinct spatial patterns between non-invasive and invasive species

Neither *Tapinoma nigerrimum* nor *Tapinoma darioi* showed a linear response to urbanization. While *T. nigerrimum* was mainly observed in natural areas on limestone plateaus and hills mostly covered with Mediterranean forests, the tramp species *T. darioi* peaked at intermediate levels of urbanization. These results support our hypothesis of a positive relation between urbanization and the occurrence of species that exhibit traits common in invasive ants. These results are also consistent with

SEIFERT & al. (2017), who suggested that *T. darioi* is more abundant in sites impacted by human activities, whereas *T. nigerrimum* prefers natural and semi-natural habitats.

Three non-exclusive hypotheses can be proposed to explain these distinct distributions. First, the observed distribution may result from the historical presence of both species in the area before the expansion of the city of Montpellier. *Tapinoma darioi* is commonly found in open sandy habitats along the coast and might have persisted despite urbanization. Second, like most invasive species, *T. darioi* could have been introduced and spread in urban areas where most native species usually disappear (CADOTTE & al. 2017). While for *Tapinoma nigerrimum* most new founding queens probably disperse on the wing, in *T. darioi*, it is more likely that passive human-mediated dispersal occurs (SEIFERT & al. 2017). As has been shown in fire ants (KING & al. 2009) or in Argentine ants (SUAREZ & al. 2001), dispersal of ants occurs at medium and long distances through the transport of soil containing colony fragments with adult or larval queens (e.g., SUHR & al. 2019). GIPPET & al. (2017) found that *Lasius neglectus* was detected at significantly higher probabilities in major road embankments, which was interpreted as the impact of landscaping generating important transportation of soil.

Moreover, intermediate levels of urbanization corresponding to the green suburbs of the largest cities, as well as in urban parks and tourist towns close to the coast, offer a heterogeneous environment (MCKINNEY 2006, CADENASSO & al. 2007, CLERGEAU 2007). This environment could provide habitat patches for invasive species such as *Tapinoma darioi*, while filtering against ecological traits exhibited by *Tapinoma nigerrimum*. *Tapinoma nigerrimum* does not have the characteristics of a tramp species (PASSERA 1994): monodomous (only one nest per colony, and colonies behave aggressively to one another; SEIFERT & al. 2017). Its social system is therefore similar to *Lasius niger* and *Tetramorium immigrans*, which are the most urban tolerant species in most of Europe. As it builds 20 - 40 cm high large dome-shaped nests in open areas, it might be negatively affected by constant mowing and soil compaction found in urban greenspaces, which may explain this absence. Conversely, *T. darioi*'s supercolonial organization, where many interconnected nests make up the whole of the colony, can facilitate the access to numerous nesting sites in the heterogeneous suburban landscape, where parts of the colony can concentrate depending on the season and available resources, as has been observed in the Argentine ant as well as in *Tapinoma sessile* (HELLER & GORDON 2006, MENKE & al. 2010).

The rarity of *Tapinoma darioi* in dense city centers might be explained by the absence of both suitable nesting sites and food resources. It is also possible that *T. darioi* has established itself in disturbed habitats with less interspecific competition (CADOTTE & al. 2017). Indeed, potential competitors such as *Tetramorium immigrans* SANTSCHI, 1927, *Lasius niger* (LINNAEUS, 1758), or *Pheidole pallidula* (NYLANDER, 1849) are present in denser parts of the urban gradient (MADRE & al. 2013). Unfortu-

nately, as stated above, it is impossible to know whether *T. darioi* was already present before the expansion of the city, making the current distribution of the *Tapinoma nigerrimum* species complex difficult to explain.

Third, dense urban areas are characterized by urban heat islands, where heat-radiating buildings and canyon effects impose a steep, rising temperature gradient from rural areas to city centers, with the effect being stronger in larger cities. *Tapinoma darioi* could be better adapted to the higher temperatures of urban areas or in the middle of dunes close to the coast compared with *Tapinoma nigerrimum*. Indeed, even closely related ant species may differ in their thermal tolerance (e.g., CERDÁ & al. 1998). Moreover, ANGILLETTA & al. (2007) showed that ectotherms such as ants in urban areas are more heat tolerant than those in rural areas. BUJAN & al. (2021) have recently shown that *Tapinoma magnum* can shift its thermal niche, a capacity for thermal plasticity that *T. darioi* might also possess.

Conclusion

We used microsatellites to confirm species identities in the *Tapinoma nigerrimum* species complex to improve our knowledge of the occurrence and distribution of species in this complex in Southern France. Biodiversity does not only provide services, and this must be taken into account in the management of cities. As other invasive ants have been shown to decrease local ant and soil arthropod diversity (e.g., *Lasius neglectus*; see NAGY & al. 2009), further research should examine the ecological consequences of *Tapinoma darioi* and *T. nigerrimum* on the richness and abundance of the local arthropod and plant communities.

Acknowledgements

We thank Bernhard Seifert and Massimiliano Centorame for providing part of the reference individuals from SEIFERT & al. (2017) which were used in this study. We thank Annick Lucas for her collaboration and her availability. We also thank all collaborators who actively participated in the collecting of *Tapinoma* ants and more specifically Yannick Juvé, Célian Diblasi, Pierre-Louis Duarte, Célia Lecomte, Ambre Mautuit, Corentin Sagnet, and Julien Tchilingrian. We also thank Cyril Bernard, assistant engineer at the CNRS, for his advice given on QGIS. We are grateful for the funding provided by Doctoral School 60, CNRS-PEPS, as well as the LABEX IMU (ANR-10-LABX-0088) of Université de Lyon.

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