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Notes on some ants from Dalmatia, Croatia (Hymenoptera: Formicidae)

Christophe GALKOWSKI[1] · **Alain LENOIR**[2] · **Elfie PERDEREAU**[3] · **Simon DUPONT**[4]

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Abstract
The article presents a list of ant species observed during a journey to Seget Donji, a locality on the Adriatic coast of Croatia, in April 2023. A total of 30 species is presented, with brief notes on their ecology and distribution. One of these species, *Tapinoma magnum*, is reported for the first time in Croatia.

Keywords | *Tapinoma magnum* • checklist • Adriatic Sea • Balkans • Europe

Notes sur quelques fourmis de Dalmatie (Croatie) (Hymenoptera : Formicidae)

Résumé
L'article présente une liste d'espèces de fourmis observées lors d'un voyage à Seget Donji, une localité sur la côte adriatique de la Croatie, en avril 2023. Au total, 30 espèces sont présentées, avec de brèves notes sur leur écologie et leur distribution. Une de ces espèces, *Tapinoma magnum*, est signalée pour la première fois en Croatie.

Mots-clefs | *Tapinoma magnum* • liste d'espèces • mer Adriatique • Balkans • Europe

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INTRODUCTION

Dalmatia is a region of the Balkans along the coast of the Adriatic Sea within the Mediterranean basin, one of the planet's main biodiversity hotspots (MYERS *et al.*, 2000). In the context of the major ecological changes taking place today, understanding the biodiversity of these areas and how it is changing is of vital importance. Improving our knowledge of biodiversity components start modestly with simple naturalist inventories. Seget is a municipality located around 20 km west of Split, the capital of the Split-Dalmatia County (map 1). The municipality has a population of around 5,000 people divided into six localities, including Seget Donji (ANONYMOUS, 2024).

A visit to Seget Donji between 16 and 23 April 2023 enabled us to compile a brief inventory of the ant species present. Several sites were visited (map 2):

- (1) A fairly dense pine forest of Aleppo pines criss-crossed by numerous footpaths. Beneath the pine forest, the herbaceous cover is fairly dense and dotted with numerous limestone boulders and stones.
- (2) A small valley criss-crossed by a path that starts at the seaside and then winds its way up towards inhabited areas. The lower part of the valley has a different type of vegetation, dominated by deciduous trees (including oaks). The environment is wetter than the pine forest, as the center of the valley has to drain run-off water during rainfall. The vegetation is very dense with herbaceous plants and shrubs at the bottom of the valley. This environment dries out as you take the path upwards and after walking for around 200 m you come back to the pine forest. This valley is bordered by highly fissured limestone outcrops.

[1] [CG] 104 Route de Mounic, F – 33160 Saint Aubin, France • chris.gal@wanadoo.fr
https://orcid.org/0000-0002-1001-1470 • ZooBank https://zoobank.org/C480F514-D567-4787-AF55-2BE00FB79706
[2] [AL] IRBI, Institut de recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Faculté des Sciences, Parc de Grandmont, F – 37200 Tours, France • alain.lenoir@univ.tours.fr
https://orcid.org/0000-0001-5829-0096 • ZooBank https://zoobank.org/0B03EB0F-06EB-413C-A91E-B57CBB22C623
[3] [EP] IRBI, Institut de recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Faculté des Sciences, Parc de Grandmont, F – 37200 Tours, France • perdereau@univ-tours.fr
https://orcid.org/0000-0002-0923-0377 • ZooBank https://zoobank.org/46366561-D448-4A23-A069-4AF82D3BB721
[4] [SD] IRBI, Institut de recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Faculté des Sciences, Parc de Grandmont, F – 37200 Tours, France • simon.dupont@univ-tours.fr
https://orcid.org/0000-0003-4760-6811 • ZooBank https://zoobank.org/A7788F53-7EE9-45E7-862F-21F7FD60E8C4

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Map 1. Geographical location of the studied locality (from https://umap.openstreetmap.fr/fr/, modified, consulted 15 February 2024).

Map 2. Stations surveyed during the visit. **1.** Fairly dense pine forest. **2.** Damp valley bordered by limestone outcrops. **3.** Limestone hillside. **4.** Anthropogenic areas: hotel gardens, pavements and vacant lots in town, beachside paths (from https://umap.openstreetmap.fr/fr/, modified, consulted 15 February 2024).

- (3) Above the coastal pine forest, the environment becomes much drier and more open and becomes limestone hillside environment with low scrubland-type vegetation.
- (4) Environments with varying degrees of human activity, such as paved beachside paths, hotel gardens, pavements and wasteland in towns.

MATERIAL AND METHODS

The method consisted of surveying a limited geographical area (usually the size of a commune) for one week and recording all the species encountered. The searches were non-intensive, limited to a few hours over the week. Observations were opportunistic, during walks, or sometimes a little more targeted when vegetation, litter or the underside of stones were examined. The duration of the trip allows research to be conducted in a wide range of environments with varying

vegetation cover, from open to more closed and more or less disturbed habitats. No trapping techniques were used, nor were more specialized techniques such as beating up vegetation or searching for endogenous using Berlese-type methods. The results obtained are therefore far from exhaustive of all the myrmecofauna present on the site, but they do give an idea of the species that are frequent on the surface and active during the dates we visited the sites. Besides, the inventory allows us to detect discreet, rare or very rare species.

Some specimens were placed in 70° alcohol to be killed and then quickly mounted to be examined using a small binocular magnifying glass, whose 60 × magnification enabled the vast majority of species to be identified. A micrometric eyepiece was used to take measurements that were sometimes essential. Photographs were taken using a camera mounted on a micrometric rail and fitted with a lens suitable for microphotography. This paper presents a list of species arranged in alphabetical order and grouped in their respective subfamily.

LIST OF RECORDED SPECIES

Subfamily Dolichoderinae

Dolichoderus quadripunctatus (LINNÉ, 1791)

Only a few workers observed in the vegetation. *D. quadripunctatus* is an arboreal species, that typically establishes small colonies inside dead branches. The species has been recorded throughout Europe.

Tapinoma magnum MAYR, 1861

Numerous colonies of this *Tapinoma* can be found behind the beach and in hotel gardens on the edge of the pine forest. Colonies can be very extensive, with intense worker activity forming highways between the different nests (figures 1 –2).

T. magnum is a species belonging to the *Tapinoma* complex

Figure 1. Extent of *Tapinoma magnum* colonies in a garden (red arrows). Photo C. GALKOWSKI.

of the *nigerrimum* group recently revised by SEIFERT *et al.* (2017). Species from the *T. nigerrimum* complex are easily distinguished from other *Tapinoma* complexes. The three castes possess morphological characters that allow them to be distinguished from the other species complexes of the genus *Tapinoma* (SEIFERT *et al.*, 2017). All these characteristics are present in the individuals collected at Seget Donji (figures 3–7, table I):

- The workers are highly polymorphic, varying in size from single to double. The width of the head of the largest majors exceeds 1 mm (HW 1.1 mm). Article 2 of the funiculus is visibly longer than article 3 (ratio of length of article

Figure 2. Nest of *Tapinoma magnum*. Photo C. GALKOWSKI.

2 to length of article 3 greater than 1.70). The number of hairs protruding from the anterior margin of the clypeus at the incision is high (between 10 and 15).

- The queens are large (head width 1.38–1.40 mm). The separation of queens from the *simrothi* group is done by calculating a discriminant D(5), the result of which published in SEIFERT *et al.* (2017) contains errors (B. SEIFERT, *pers. comm.*): D(5) < – 1 should be read: *T.* from the *simrothi* group and D(5) > – 1: *T.* from the *nigerrimum* group. All the Croatian gynes measured have D(5) > – 1 and are well placed in the *nigerrimum* group.
- The males are also large (head width 1.1 mm) and the genitalia are characteristic with a very wide basimere.

SEIFERT *et al.* (2017) demonstrate the existence of 4 cryptic species within the *nigerrimum* complex that are difficult to distinguish from one another on the basis of morphological characters alone. Several of these species (*T. magnum*; *T. ibericum* SANTSCHI, 1925 and *T. darioi* SEIFERT, D'EUSTACCHIO, KAUFMANN, CENTORAME & MODICA, 2017), which are of Mediterranean origin, can potentially behave as invasive species when accidentally imported into other territories (LENOIR & GALKOWSKI, 2017; FREITAG & CHERIX, 2019; GOURAUD & KAUFMANN, 2022; LENOIR *et al.*, 2023). BRACKO (2019) reports the presence of *T. magnum* in Slovenia but so far there have been no records from Croatia.

In Greece, *T. magnum* is not native but has been reported as an introduced species originating from the western Mediterranean, where it has established populations on several islands (DEMETRIOU *et al.*, 2023).

Tapinoma magnum have also been analyzed with Cuticular Hydrocarbons (CHs) and COI genes to confirm the data obtained with morphological measures. **Cuticular hydrocarbons:** Dolichoderinae ants from Europe have been analyzed recently via a GC/MS-TQ Agilent by LENOIR *et al.* (2023b). It was shown that the four species of the *nigerrimum* group can be clearly distinguished (figure 8). We used the same method for the ants from Croatia.

To genetically determine the ant species of 6 *Tapinoma* species samples from Croatia, we sequenced the COI gene (655 pb)

Figure 3. Gyne of *Tapinoma magnum* MAYR, 1861 from Seget Donji (scale bar 1 mm). Photo C. GALKOWSKI.

Figure 4. Male of *Tapinoma magnum* MAYR, 1861 from Seget Donji (scale bar 1 mm). Photo C. GALKOWSKI.

Figure 5. Worker of *Tapinoma magnum* MAYR, 1861 from Seget Donji (scale bar 1 mm). Photo C. GALKOWSKI.

Table I. Specimen collection sites for *Tapinoma magnum* in Seget, Croatia. CG: C. GALKOWKI.

Date	Locality	Latitude	Longitude	Altitude	Collector
April 2023, 20th	Seget Vranjica, Croatia	43.510196	16.191596	27m	23-17(CG1)
April 2023, 17th	Seget Donji, Croatia	43.510952	16.208957	5m	23-18 (CG2)
April 2023, 17th	Seget Donji, Croatia	43.514246	16.204274	67m	23-19 (CG3)
April 2023, 20th	Seget Donji, Croatia	43.511682	16.205253	19m	23-20 (CG4)
April 2023, 20th	Seget Donji, Croatia	43.511921	16.208149	18m	23-21 (CG5)
April 2023, 20th	Seget Donji, Croatia	43.511389	16.207146	14m	23-22 (CG6)

Figure 6. Head of worker of *Tapinoma magnum* MAYR, 1861 from Seget Donji (scale bar 0,5 mm). Photo C. GALKOWSKI.

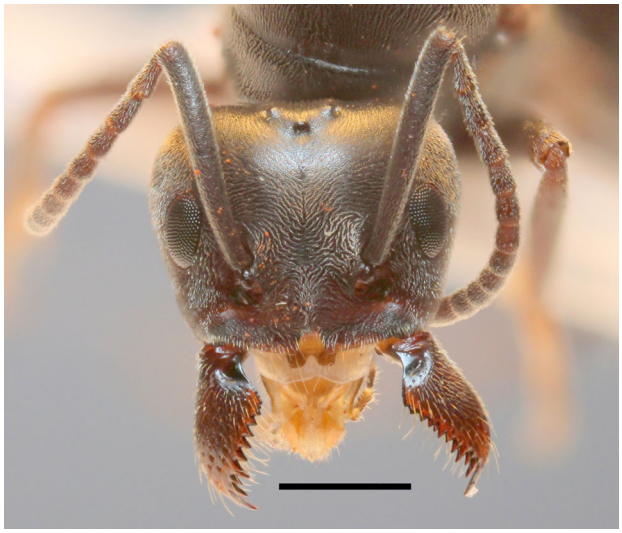

Figure 7. Head of gyne of *Tapinoma magnum* MAYR, 1861 from Seget Donji (scale bar 0,5 mm). Photo C. GALKOWSKI.

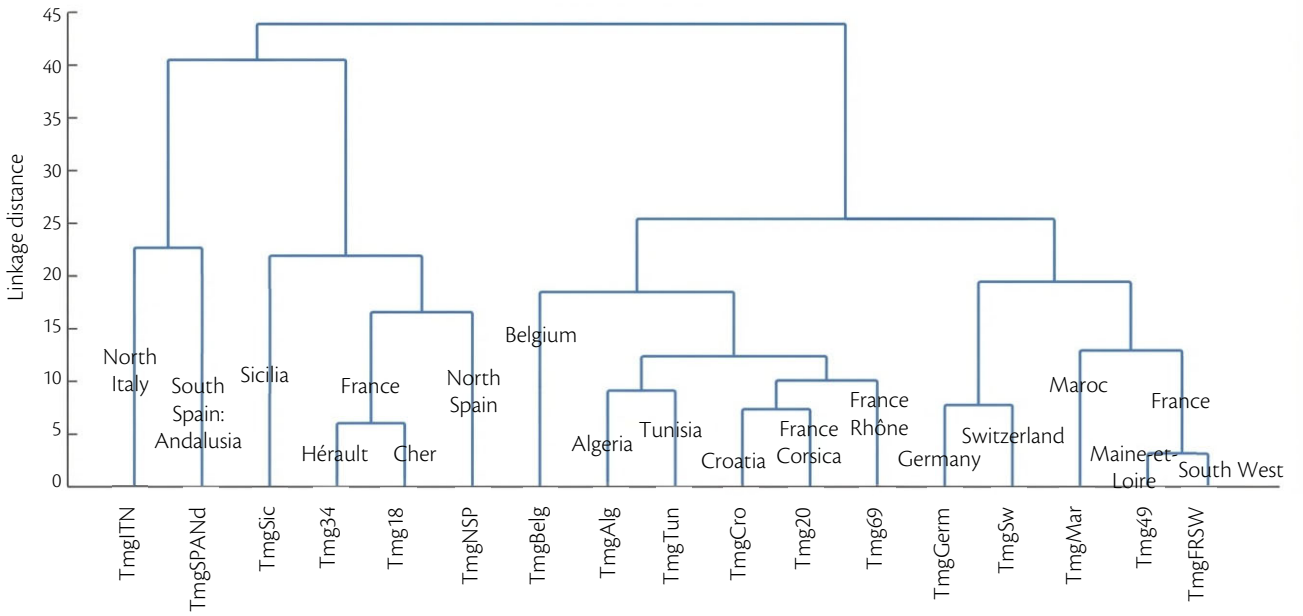

Figure 8. Dendrogram of *T. magnum* with cuticular hydrocarbons. WARD's method, euclidean distances.

and analyzed the sequences as in TOURNEUR *et al.* (2022) (figure 8). *BLAST* results were sorted by percentage identity, and reference sequences showing at least 99.5 % identity with our sequences were used to assign species status. All sequences obtained in this study were submitted to *GenBank*; their accession numbers are OR826840 to OR826845 for the 6 *Tapinoma* samples.

COI analyses revealed that all 6 *Tapinoma* samples in this study belonged to the *Tapinoma magnum* species, with a percentage identity of 99.85 to 100 % and 100 % coverage with *Tapinoma magnum* COI reference sequences from two different and independent studies (*GenBank* Accession MT606393 and KY426608).

Subfamily Formicinae

Camponotus aethiops (LATREILLE, 1798)

Fairly common throughout the site, more abundant in open areas on the limestone hillside. *C. aethiops* is a very common species in European Mediterranean environments.

Camponotus dalmaticus (NYLANDER, 1849)

Only one colony was observed. It was located under a fairly deep stone in the damp valley. A few workers were present in the surrounding vegetation. At the time of observation, the adults were present in the nest. *Camponotus dalmaticus* is a common species in the Balkans.

Camponotus heidrunvogtae SEIFERT, 2019

Some workers found on the edge of the damp valley on the rocks have biometric indices that correspond to this species (length of the scape relatively long compared to the width of the mesosoma and propodeal plate). This species has been recorded throughout the western Balkans.

Camponotus lateralis (OLIVIER, 1791)

A species frequently found in or near vegetation in anthropised areas. *C. lateralis* is an arboreal species that has been reported in all countries around the Mediterranean. In his revision of the *lateralis* group, SEIFERT (2019) distinguishes two morphologies based, among other things, on the amount of hair: morphology 1 with few erect hairs and a pubescence made up of short hairs

and morphology 2 with many erect hairs and a pubescence consisting of long hairs. The specimens present at the station correspond to morphology 1.

Camponotus piceus (LEACH, 1825)

Quite common on the station, colonies are found under stones in the limestone hillside part of the station. At the time of observation, alates were present in the nest. *Camponotus piceus* is a common species throughout the northern Mediterranean basin.

Formica cunicularia LATREILLE, 1798

A few workers have been observed in the town of Senget Donji on pavements. *F. cunicularia* is a very common species in Europe in open environments such as meadows. Its presence in towns is more occasional, near green spaces.

Lasius illyricus ZIMMERMANN, 1935

This species is frequently seen in shaded areas, near buildings, or surveying stumps and dead wood in pine forests and damp valleys. *L. illyricus* is widespread throughout the Balkans. Its ecology is similar to that of *Lasius emarginatus* (OLIVIER, 1792), which is widespread in Western Europe.

Lasius lasioides (EMERY, 1869)

A few workers were found in the same environment as *L. illyricus*, again in vegetation. This species seems to be even more attached to wooded areas. *L. lasioides* is found throughout the Mediterranean basin.

Lepisiota frauenfeldi (MAYR, 1855)

A fairly common species, mainly in anthropised environments, such as city pavements, footpaths and roadsides. The workers are very alert and active at all times of the day, making this species easy to detect. The genus *Lepisiota*, and particularly the species complex to which *L. frauenfeldi* belongs, is particularly confused taxonomically. Several varieties and subspecies of unclear status have been described in the eastern part of the Mediterranean basin and in North Africa. The type of the *L. frauenfeldi* species described by MAYR originates from Sinj, a locality about 30 km north-east of Seget Donji. It is therefore likely that the specimens observed at the station belong to this species.

Lepisiota nigra (DALLA TORRE, 1893)

This species, which is closely related to the previous one, occurs with the same frequency in the same environments. The species *L. nigra* is distributed along the entire Adriatic coast of the Balkans from Italy to Greece. It is also fairly common on Crete.

Plagiolepis pygmaea (LATREILLE, 1798)

Very abundant on the station, mainly in undergrowth or on the edges of wooded plots. The nests, which are very shallow, are easily found by lifting stones. The workers alone are more difficult to detect as this is a very small species, no larger than 2 mm. *P. pygmaea* is a very common species throughout the northern Mediterranean basin.

Prenolepis nitens (MAYR, 1853)

A few colonies have been observed on pavements and vacant lots in towns. This species is very common in the Balkans and Anatolia. Swarming was observed on the morning of April 20th: winged queens and males running and mating on the ground or in low vegetation in the vicinity of the nest. However, some winged moths fly away, but there does not seem to be a massive nuptial flight as in the *Lasius* genus, for example.

Subfamily Myrmicinae

Aphaenogaster balcanica (EMERY, 1898)

The species is common in towns and in anthropised environments such as roadsides and is absent or rarely found elsewhere. This species is the only representative in the station of the *A. testaceopilosa* group as defined by BOER (2013). The species is very common in the Balkans and southern Italy.

Aphaenogaster epirotes (EMERY, 1895)

Numerous colonies found in the pine forest, under stones. The species is common on the station, in shady, damp environments. The species is common along the Adriatic coast and in the southern Balkans.

Aphaenogaster ovaticeps (EMERY, 1898)

A few isolated workers were observed in the pine forest and in the wetter valley. The nests could not be detected. This species appears to be fairly common at the station. *A. ovaticeps* has been recorded all along the Adriatic coast of the Balkans, from Italy in the north to Greece.

Crematogaster scutellaris (OLIVIER, 1792)

An arboreal species frequently found in the different environments of the station. The long columns of workers are particularly visible in low vegetation and shrubs. The species is found throughout the western Mediterranean basin.

Crematogaster sordidula (NYLANDER, 1849)

Several colonies were observed under stones in the pine forest. This small species appears to be fairly well established. *C. sordidula* has been recorded throughout the European part of the Mediterranean basin, as well as in North Africa.

Messor capitatus (LATREILLE, 1798)

Messor ants are harvesting ants whose long columns of workers are clearly visible on the ground, exploiting grasses a few metres from their nest, from which they bring back the seeds. Three species of *Messor* were observed. *M. capitatus* is perhaps the least common at the station. Nests are found, for example, in clearings and paths in the pine forest and on the limestone hillside. *M. capitatus* is a common species in western European Mediterranean environments (Spain, France, Italy, the Adriatic coast of the Balkans).

Messor ibericus SANTSCHI, 1931

More common in anthropised environments, roadsides and hotel gardens. A fairly common species at the station. In some nests, alates have been observed. The species is common in Mediterranean environments from Spain to Greece.

Messor wasmanni KRAUSSE, 1910

In the same environments as *M. capitatus*, but this species is found more frequently. *M. wasmanni* is a northern Mediterranean species, from Corsica to Greece.

Pheidole balcanica SEIFERT, 2016

Several nests found under stones in the pine forest. This species

of the genus *Pheidole* belongs to the *pallidula* group revised by SEIFERT (2016). According to this author, Mediterranean populations of *P. pallidula* (NYLANDER, 1849) can be divided into 4 distinct species. Populations in the southern Balkans and on the Adriatic coast of the Balkans are assimilated to the species *P. balcanica*.

Solenopsis sp.

A few workers stealthily observed under a large stone embedded in the damp valley. Determining the species of the genus *Solenopsis* is difficult, and often requires examination of the adults, which express more discriminating characteristics. Two species of *Solenopsis* have been recorded in Croatia: *Solenopsis fugax* (LATREILLE, 1798) and *Solenopsis wolfi* EMERY, 1915.

Temnothorax affinis (MAYR, 1855)

An arboreal species observed on a single occasion in the vegetation of the wet valley. This species has a very wide distribution in Europe.

Temnothorax brackoi SALATA & BOROWIEC, 2019

A few workers found in the vegetation in the damp valley. *T. brackoi* is an arboreal species nesting in hollow stems in low vegetation. Present in Greece and on the Adriatic coast of the Balkans.

Temnothorax mediterraneus WARD *et al.*, 2014

A few workers observed in vegetation (shrubs) on the seafront. *T. mediterraneus* is an arboreal species belonging to the *angustulus* group and related to *T. algiricus* (FOREL, 1894) found in North Africa. This species is also found in Spain, Corsica, Sardinia, Sicily and southern Italy.

Temnothorax sordidulus (MÜLLER, 1923)

A few workers observed on the rock face in the damp valley. The nests are usually located in stone crevices. *T. sordidulus* has been recorded in Central Europe and the western Balkans.

Tetramorium kephalosi SALATA & BOROWIEC, 2017

Several colonies were observed under stones in the pine forest. This species belongs to the complex of species related to *T. semilaeve* ANDRÉ, 1883. According to SALATA & BOROWIEC (2017), the specimens from Dalmatia can be assimilated to *T. kephalosi* present in Croatia and the southern Balkans.

Subfamily Ponerinae

Ponera testacea EMERY, 1895

A lone worker found under a large stone in the damp valley. *P. testacea* is an endogenous species reported throughout southern Europe, from Spain to Turkey.

DISCUSSION

The inventory revealed the presence of 30 species divided into 16 genera as follows:

- Subfamily Dolichorerinae: 2 genera represented: genus *Dolichoderus* (1 species) and genus *Tapinoma* (1 species)
- Subfamily Formicinae: 6 genera represented: genus *Camponotus* (5 species), genus *Formica* (1 species), genus *Lasius* (2 species), genus *Lepisiota* (2 species), genus *Plagiolepis* (1 species) and genus *Prenolepis* (1 species)
- Subfamily Myrmicinae: 7 genera represented: genus *Aphaenogaster* (3 species), genus *Crematogaster* (2 species), genus *Messor* (3 species), genus *Pheidole* (1 species), genus *Solenopsis* (1 species), genus *Temnothorax* (4 species) and genus *Tetramorium* (1 species)
- Subfamily Ponerinae: 1 genus represented: genus *Ponera* (1 species).

In terms of species, 13 of the 30 species (43 %) have a distribution range limited to the Balkans, with some of them restricted to its Adriatic coast . The other species present have a wider range, extending throughout southern Europe, often beyond the Mediterranean area. The genus *Messor*, which is highly diverse in Mediterranean ecosystems, is well represented here with three species. The genus *Lepisiota*, with two species that are fairly common in the station, is also well diversified in Mediterranean ecosystems, particularly in North Africa. On the other hand, the genera *Formica* and *Lasius*, which are very diverse in cold and temperate Europe, are poorly represented here, with one and two species respectively, which are fairly discreet at the station. This distribution in species categories, genera and sub-families is quite similar to that obtained during an inventory carried out using exactly the same protocol in the municipality of Grasse, in south-eastern France, during one week in August 2010, during which 43 species belonging to 20 genera and 4 sub-families were collected (GALKOWSKI, 2011). Comparisons of these types of inventories can provide a characteristic picture of the organization of the myrmecofauna in Mediterranean ecosystems.

None of the species observed are considered invasive: all the species present here are reported from a station that is part of their known distribution area, with the possible exception of *Tapinoma magnum*. This species is known to be invasive when it establishes itself in a new territory. We lack data to determine whether the population found at Seget Donji is a local population of this species or whether its presence is the result of an introduction. Neither the data concerning cuticular hydrocarbons nor the genetic data obtained from COI sequencing provide sufficient arguments to answer this question. More detailed genetic studies (several genes, including microsatellites) would be necessary.

In his review of the myrmecofauna of Croatia, BRACKO (2006) reports the presence of *T. nigerrimum* in about ten stations in Croatia. These data come from old references (MÜLLER, 1923; ZIMMERMANN, 1930, 1934) and it would be necessary to take back the specimens with the new data resulting from the revision of SEIFERT *et al.* (2017) to specify the identification.
Tapinoma magnum was previously recorded from Elaphiti archipelago, in South Dalmatia in two of the inhabited islands (BUJAN, *unpublished data*). The localities where this species was recorded are anthropogenic, quite similar to the ones in Seget Donji. This includes a dirt trail next to the most popular sandy beach on Lopud Island (42.682506, 17.953205), and the Lopud port (42.691263, 17.941591), with large foraging trails next to the houses. Šipan island is the largest island in the archipelago and we recorded *T. magnum* in the port of Šipanska Luka (42.72735, 17.860065). In all cases, the nests were large with established foraging trails. The workers were always highly polymorphic and extremely aggressive. So, it is likely that this species has a wide distribution across Dalmatian coast which has not been systematically studied yet.

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